Influence of the Passive Anisotropic Properties on Directional Differences in Propagation Following Modification of the Sodium Conductance in Human Atrial Muscle

A Model of Reentry Based on Anisotropic Discontinuous Propagation

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Available models of circus movement reentry in cardiac muscle and of drug action on reentrant arrhythmias are based on continuous medium theory, which depends solely on the membrane ionic conductances to alter propagation. The purpose of this study is to show that the anisotropic passive properties at a microscopic level highly determine the propagation response to modification of the sodium conductance by premature action potentials and by sodium channel-blocking drugs. In young, uniform anisotropic atrial bundles, propagation of progressively earlier premature action potentials continued as a smooth process until propagation ceased simultaneously in all directions. In older, nonuniform anisotropic bundles, however, premature action potentials produced either unidirectional longitudinal conduction block or a dissociated zigzag type of longitudinal conduction (a safer type of propagation, similar to transverse propagation). Directional differences in the velocity of premature action potentials demonstrated that anisotropic propagation was necessary for a reentrant circuit to be contained within an area of 50 mm², even with very short refractory periods. Quinidine produced Wenckebach periodicity, which disappeared after acetylcholine shortened the action potential. Quinidine also produced use-dependent dissociated zigzag longitudinal conduction in the older, nonuniform anisotropic bundles but not in the young, uniform anisotropic bundles. The electrophysiological consequence was that propagation events differed in an age-related manner in response to the same modification of the sodium conductance. The electrical events at microscopic level showed that conditions leading to obliteration of side-to-side electrical coupling between fibers (e.g., aging and chronic hypertrophy) provide a primary mechanism for reentry to occur within very small areas (1–2 mm) due to a variety of propagation phenomena that do not occur in tissues with tight electrical coupling in all directions. (Circulation Research 1988;62:811–832)

Since the demonstration of circus movement reentry in cardiac muscle by Mines in 1913, theories used to account for its initiation by a premature stimulus and to account for the effects of use-dependent drugs on reentrant arrhythmias have considered the passive electrical properties of the reentrant path to play a negligible role, that is, the conduction medium has been assumed to be continuously uniform and isotropic in nature. Models for circus movement reentry based on this assumption include 1) propagation of the impulse around an anatomical obstacle,1,2 2) propagation around two or more veins3 or around an atrioventricular valve,4 3) propagation in atrial pathways that form a circle of atrial muscle with special membrane properties for fast (and safe) conduction5 or propagation through functionally separate α and β paths in the atrioventricular node,6 and 4) propagation around a functional obstacle of refractory tissue (the “leading circle concept” of Allessie et al). All of these models rely on regional differences of the active membrane ionic properties to determine the conduction velocity and excitability of the fibers. For example, in the leading circle concept of Allessie et al, the most widely used model of reentry at present, slow conduction in the reentrant path, occurs because “the head of the circulating wavefront is continuously biting in its own tail of refractoriness.” That is, slow conduction is produced solely by a decrease in $V_{\text{max}}$ secondary to a reduction in the depolarizing sodium current because the fibers are still in the relative refractory period of the previous action potential.

Several years ago we introduced a considerable departure from the above classical theory of reentry by presenting evidence that the anisotropic passive electrical properties of cardiac muscle provide a major additional mechanism for differences in the excitability and safety of propagation of the cardiac action potential.9,10 Fast upstrokes occur with low conduction velocities transverse to the long axis of the fibers, and slower upstrokes occur with high conduction velocities along the long axis of the fibers. This anisotropic mechanism produces a relation between the sodium current and $V_{\text{max}}$ that is diametrically opposite of the classical relation for steady-state propagation in one-dimensional continuous structures where increases in...
the magnitude of the sodium current are associated with increases in $V_{na}$. With anisotropic propagation, increases in $V_{na}$ are associated with decreases in the sodium current.\textsuperscript{11} This anisotropic phenomenon can be accounted for by the effective membrane capacitance being greater with longitudinal propagation (LP) than with transverse propagation (TP), resulting in directional differences in the electrical load at a microscopic size scale.\textsuperscript{11}

This anisotropic mechanism also reverses the usual relation between conduction velocity and safety of propagation. Slow propagation in the transverse direction is safer than more rapid propagation in the longitudinal direction. For example, there are directional differences in the safety of propagating premature action potentials, especially near the stimulus site\textsuperscript{8,12} and at junctions of muscle bundles,\textsuperscript{10} where unidirectional block can occur. Previously, we have used the term “safety factor” to describe this relation. This term is used "to indicate the extent to which the fibre’s ability to be excited and conduct exceeds the minimum,"\textsuperscript{12} and classically it is a description of steady-state propagation, as originally introduced by Rushton\textsuperscript{13} for the analysis of “impulses travelling with constant velocity.” At a microscopic level, however, anisotropic propagation is discontinuous\textsuperscript{8} and often nonuniform; that is, the action potential does not maintain the same shape of the upstroke (or magnitude of inward sodium current) as it propagates from site to site.\textsuperscript{11} These nonuniformities of propagation are widespread in cardiac muscle and depend on the location of electrical boundaries\textsuperscript{14} and the status of side-to-side electrical coupling between small groups of fibers.\textsuperscript{15} Although anisotropic propagation nonuniformities do not fit the conditions to which the safety factor has been applied in the past (uniform continuous conduction), it will be important to extend this concept if possible to anisotropic discontinuous propagation, especially for conduction disturbances due to structural complexities. However, no quantitative analysis or analytical expression is available for the safety factor of this new type of propagation. Further, because of the complexity involved in quantitative measures of the safety factor that are independent of membrane and geometric models,\textsuperscript{16,17} approximate numerical solutions with computer simulations likely will be necessary for descriptions of the safety factors of a variety of anisotropic propagation events. Thus, at present, descriptive interpretations of obvious directional differences and localized changes in conduction are necessary.

The evidence we have obtained thus far indicates that directional differences in the shape of the upstroke affect the kinetics of the membrane ionic channels and the ionic currents. In turn, this alters not only the excitability of the membrane but also the kinetics of use-dependent drug binding to the ionic channels during depolarization.\textsuperscript{11,14} It follows that both the underlying mechanisms of the abnormal propagation events initiating reentry and the mechanisms of drug action on reentrant arrhythmias should involve anisotropy.

Thus, the above evidence suggests that any complete theory or model of circus movement reentry and of drug action on reentrant arrhythmias should include the effects of the anisotropic passive electrical properties of cardiac muscle. A fundamental limitation to the development of such a model at present, however, is that the role of anisotropy as an independent factor in controlling excitability and the safety factor of propagation, as well as influencing use-dependent drug blockade, is almost totally unexplored. Despite considerable information about refractory periods\textsuperscript{18} and changes in conduction velocity along the long axis of the fibers associated with changes in $V_{na}$ minimal information is available for the more complex situation that occurs with simultaneous conduction in different directions in multidimensional cardiac muscle bundles. For example, when propagation slows secondary to decreases in the sodium current that decrease $V_{na}$ in partially depolarized fibers, there is a different lower limit of the conduction velocity in the longitudinal and transverse directions as the take-off potential is reached, at which time propagation ceases.\textsuperscript{11} Thus, the length of a reentrant circuit for a specific refractory period should be determined not only by $V_{na}$ (magnitude of the sodium current) and the absolute refractory period but also by the anisotropic passive properties of the individual muscle bundles that comprise the circus path.

In the present study, the effects of modifying the sodium conductance on propagating depolarization were investigated in preparations with different anisotropic properties (uniform and nonuniform anisotropy*), and directional differences in propagation during premature action potentials and during exposure to use-dependent sodium channel-binding drugs were looked for carefully. The results show prominent age-related differences in the anisotropic propagation responses to these interventions. These differences were, in turn, related to the underlying uniform versus nonuniform anisotropic electrical properties. Very low “effective” conduction velocities (0.03–0.05 m/sec) occurred with the propagation of premature action potentials across the long axis of the fibers in nonuniform anisotropic bundles. The results show that the loss of side-to-side electrical coupling between small groups of fibers at a microscopic level (nonuniform anisotropy) provides a major mechanism for reentry to occur within very small areas (1–2 mm$^2$) in cardiac muscle.

*In uniform anisotropic cardiac muscle, normal action potentials produce an advancing wavefront that is smooth in all directions; the associated extracellular depolarization waveforms are also smooth, indicating tight electrical coupling between groups of fibers in all directions. In the nonuniform anisotropic type, there is tight electrical coupling between cells in the longitudinal direction, but transversely, there are recurrent areas in which side-to-side electrical coupling of adjacent groups of parallel fibers is absent. Thereby, propagation of normal action potentials transverse to the long axis is interrupted such that adjacent groups of fibers are excited in a markedly irregular sequence that results in extracellular waveforms with multiple small deflections.\textsuperscript{9,10}
Materials and Methods

We studied atrial pectinate muscle bundles from 49 patients whose ages varied between 1 and 70 years. Human pectinate muscle bundles were used because of recent evidence that with aging there is a change from uniform to nonuniform anisotropic properties due to uncoupling of the side-to-side electrical connections between small groups of parallel-oriented fibers. 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 (n = 21, ages 41–70), Wolff-Parkinson-White syndrome (n = 18, 8–50 years), and congenital heart disease (n = 10, 1–62 years). Right atrial enlargement was evidenced in the P waves of the electrocardiogram in five patients (ages 15–64) with atrial septal defect with or without Ebstein’s malformation of the tricuspid valve. Two of these patients had a history of atrial flutter and fibrillation (ages 46 and 64); the Wolff-Parkinson-White syndrome patients had arrhythmias due to the accessory pathway at the atroventricular sulcus; and six subjects with coronary disease had ventricular tachyarrhythmias, but none had atrial arrhythmias. Before surgery, 25 patients were on drug therapy; their medications included quinidine, procaine amide, digoxin, verapamil, and propranolol. All drugs were stopped 18–24 hours before surgery. Except possibly for the digitalis preparation, the biological half-life of these drugs was considered to be less than 24 hours (patients on drugs with longer clearance times, such as amiodarone, were not involved in the study). In addition, we considered that within 30 minutes after initiation of vigorous superfusion of the preparation in the bath (vide infra), any residual drug was washed out to such a low level that it did not affect the interpretation of the results.

Successful experiments with measurement of both anisotropic conduction and transmembrane action potentials (or absolute refractory periods) were achieved in 29 of the specimens; 10 were from younger patients (1–28 years), four were from patients of intermediate age (29–42 years), and 15 were from older patients (43–70 years). In 10 preparations, data beyond that presented here were obtained for analyses of the relation between the derivatives of extracellular and transmembrane potentials during LP and TP and for evidence of directional differences in effective membrane capacitance. To make certain that the results were not species-specific, similar experiments were performed in cardiac muscle bundles obtained from adult dogs (weight 15–21 kg). For this study, 12 ventricular papillary muscles were used to evaluate uniform anisotropic bundles and 12 atrial preparations (Bachmann’s bundle and crista terminalis) were used to evaluate nonuniform anisotropic bundles.

Each human specimen was transported to the laboratory in cooled superfusate solution at 2–5°C, pinned to the floor of a rectangular tissue bath (4×5 cm), and maintained at 35°C. The composition of the superfusate was (mM) NaCl 128, KCl 4.69, MgSO4 1.18, NaH2PO4 0.41, NaHCO3 20.1, CaCl2 2.23, and dextrose 11.1. The solution was gassed in a reservoir with a mixture of 95% O2,5% CO2, and perfused through a cannula to produce a high flow rate across the bundle under study to ensure as normal a physiological state as possible. To study anisotropic propagation events on a size scale of 50–500 μm, a combined analysis of transmembrane and extracellular potentials and their derivatives was used. Intracellular potentials were recorded with electrodes made of flexible tungsten wire 50 μm in diameter and insulated except at the tip. A dissecting microscope equipped with a Nikon F250 35-mm camera was used to document each recording position. In five preparations, extracellular metal microelectrodes were used to evaluate propagation events on a size scale of 10–50 μm, as previously described. Each extracellular electrode was connected to an AC-coupled differential amplifier having a frequency response flat between 0.1 and 30,000 Hz. The separate reference electrodes for each extracellular and intracellular electrode were located 7 cm away from the recording area. The individual bundles within each preparation varied in width from 0.6 to 3 mm and in length from 5 to 25 mm.

The mechanical motion of the preparations remained vigorous for 4–9 hours. A pacemaker stimulus was applied to the endocardial surface of each human muscle preparation at 1.5 times threshold (1.5–2.0 times threshold in dog preparations) at a basic cycle length between 250 and 1,000 msec. A small unipolar stimulus electrode 50 μm in diameter was used to initiate excitation at a known site, and the reference for the stimulus was located 7–9 cm distant in the bath. The large distance between the unipolar stimulus electrode and its reference occasionally resulted in a large stimulus artifact with prolonged decay in the extracellular recordings. However, both the amplitude and duration of the artifact were markedly reduced by using short rectangular pulses of constant duration (1 msec) and by moving the reference electrode to different positions in the bath. Additional precautions used to make certain that the stimulus current did not interfere with the recorded waveforms have been previously described. For example, altering the amplitude of the stimulus artifact did not change the shape or time of the extracellular waveforms; that is, the stimulus artifact did not affect the performance of the amplifiers or the accuracy of the measurement of the time differences between waveforms. A PDP-11/44 computer system (Digital Equipment, Maynard, Massachusetts) controlled the pacing rate and synchronized the pacing and premature stimuli with the data recording. The outputs of the recording amplifiers were sampled at rates between 6,600 and 40,000 per second (12-bit samples). The computer stored the data and...
displayed the waveforms on a Tektronix 4014 (Beaverton, Oregon) unit with a persistent screen. The intracellular action potentials were also recorded on a Hewlett-Packard strip recorder (Waltham, Massachusetts) for analysis of the shape and duration of repolarization.

We performed three separate types of measurements that involved the relative and absolute refractory periods for propagation in the longitudinal and transverse directions: 1) Strength-interval curves were determined for LP and TP in uniform and nonuniform anisotropic canine preparations (right ventricular papillary muscle and atrial crista terminalis, respectively) prior to study of the human preparations. The stimulus duration was maintained constant at 1 msec, and premature stimuli were delivered at variable premature intervals following 10 consecutive pulses that occurred at a constant cycle length between 400 and 800 msec. At each premature interval, the minimum current that just produced longitudinal and/or transverse propagation (threshold current) was determined from a monitoring oscilloscope. 2) The absolute refractory period was measured for propagation along the longitudinal and transverse axes of the fibers by delivering a stimulus of 1-msec duration with a stimulus strength between 1.5 and 2.0 times threshold current. The threshold was determined as the minimum stimulus required to initiate propagation on an every-beat basis at a steady-state rate (60–150/min). The premature stimulus was introduced after every 10th control pulse delivered at constant cycle length. The premature interval was shortened in steps of 10 msec until there was no propagation in one or both directions. Then the stimulus interval was changed in 2-msec steps to determine the shortest premature interval that just produced a propagated response in each direction; that is, the absolute refractory period was determined for each direction if there was a unidirectional change in propagation. A propagated response was indicated by the occurrence of typical propagating depolarization extracellular waveforms.\(^\text{14-15}\) Our measurements did not include determination of the shortest time between depolarization waveforms of two consecutive beats, a measurement that involves latency of turn-on of the depolarization currents to initiate propagation as well as the conduction time to the monitoring site. Also, the stimulus strength was restricted to less than 7 mA because large stimuli (e.g., 10–15 mA) in the dog preparations produced ill-defined time boundaries of the absolute refractory period as well as additional problems such as prolonged latency in the onset of depolarization, initiation of propagation at sites away from the stimulus electrode, and (nonreentrant) repetitive firing of the membrane in the region of the stimulus electrode. The longstanding problems due to large stimulus currents in determining the absolute refractory period and threshold have been described in detail by Brooks et al.\(^\text{14}\) 3) To measure the effects on LP and TP of modifying the sodium conductance by changing the takeoff potential, premature action potentials were initiated while extracellular potential waveforms were measured simultaneously at four to eight sites within a single pectinate muscle bundle. In most preparations, the differences in waveform shape and timing produced by premature stimuli were reproducible for as long as 30–40 minutes. This allowed time to move two of the electrodes for measurements at 15–25 additional sites (while checking for reproducible beat-to-beat events) to analyze the pattern of excitation spread within the total bundle. The premature stimulus was delivered at different intervals after every 10th beat, while the basic stimulus rate was maintained between 75 and 300/min. To compare the depolarization shapes of premature transmembrane action potentials during LP and TP, one stimulus electrode was positioned to produce propagation in the longitudinal direction of the fibers and another stimulus electrode was positioned to produce TP at the microelectrode impalement site. Repeated measurements were performed to ensure reproducibility of the results. A similar electrode arrangement was used to measure the effects of two use-dependent sodium channel-blocking drugs, one with slow drug release from the channels during the interval of the rest potential (quinidine gluconate 5–10 \(\mu\)g/ml) and another with fast drug release (lidocaine HCl 5–10 \(\mu\)g/ml).\(^\text{22}\) The end of repolarization was taken as the time of the baseline (rest potential) crossing of a straight line extension of the most rapid component of phase 3 repolarization (i.e., the tail of repolarization near the baseline was not included).

Early premature stimuli were introduced in both uniform and nonuniform bundles in an attempt to initiate reentry. When reentrant propagation did occur, occasionally there were multiple extrasystoles. These runs of extrasystoles interfered with the reproducible induction and measurement of the events during the single premature beat that initiated reentry. Therefore, to study the initiation of reentry (the first beat), the waveforms were sampled continuously during the first 240 msec following the premature stimulus, and the series of regular paced beats was restarted within 300 msec after the premature stimulus to prevent or interrupt runs of extrasystoles. This maneuver greatly facilitated the recording and storage of the reproducible waveforms that occurred during separate premature beats.

After each experiment, the digitally stored waveforms were redisplayed and photographed for initial analysis. Selected waveforms were then transferred to Hewlett-Packard 9000 computer (Fort Collins, Colorado) for automatic plotting of the waveforms and their derivatives as well as for the construction of phase-plane graphs.\(^\text{11,12}\) The transmembrane potential \(V_m\) was obtained numerically by subtracting the extracellular potential waveform \(\Phi\) from the intracellular action potential.\(^\text{8}\) The first time derivatives of \(V_m\) and \(\Phi\) were obtained numerically, and the unsmoothed values were plotted in time steps of 20–150 \(\mu\)sec. Isochrone maps of the spread of excitation of normal and premature action potentials were constructed for bundles with uniform anisotropic properties based on measurements.
from at least 30 sites. In the nonuniform anisotropic bundles, however, the spatial events of transverse propagation were so complex at a microscopic level, especially with premature action potentials, that the isochrone method was abandoned as a reasonable representation of excitation spread across fibers at this small scale in such preparations. In these sequences, the general pattern of excitation spread was estimated from the extracellular waveforms based on recent experimental and theoretical results on the origin of polyphasic waveforms and their derivatives at a microscopic level. The effective conduction velocity was estimated from the isochrone maps where possible or by dividing the distance between two electrodes by the difference in time at which the maximum negative slope of $\Phi$, occurred at each electrode. The anisotropic velocities of the earliest propagated response of premature action potentials in bundles from different groups of subjects were analyzed statistically using one-way analysis of variance (for two groups), and the relative changes in the amplitude of the extracellular waveforms were analyzed using a paired t test. The level of significance was taken as 0.01.

All preparations were placed in Bouin’s fixative after electrical study and routinely processed and embedded in paraffin. Sections were cut at 7 μm and stained by our modification of the picrosirius red technique,24 which stains collagen red and leaves the background colorless. Because this technique alters myoplasmic refractive index, sections were examined while mounted in a mixture of trans-cinnamaldehyde and diethylene glycol monobutyl ether (refractive index 1.60). The preparation was photographed using a green (Kodak Wratten #58) and a didymium filter.

Theory

The purpose of the theoretical analysis was to determine if a simplified cable model could account for the changes found in $V_m$ when use-dependent sodium-channel drug blockade occurred to the point of propagation failure and when the action potential was shortened by the addition of acetylcholine (ACh) in the absence of changes in the resting transmembrane potential. For the purpose of this analysis, therefore, these simulations consider an average description of the tissue in one direction. For this, the problem was simplified as much as possible by using a single transient fast sodium current with the equivalent electrical circuit of a continuous cable.

With propagation in a uniform cable, the time course of $V_m$ is a function of time and space as determined by the cable equation:

$$ I_m = (a/2R) \left( \partial^2 V_m / \partial x^2 \right) = C_m \partial V_m / \partial t + I_{nm} (1) $$

where a is the radius of the cylinder (cm), $R_m$ is the internal resistivity ($\Omega$cm), and $C_m$ is the specific membrane capacitance ($\mu$F/cm$^2$). The ionic current $I_{nm}$ ($\mu$A/cm$^2$) during depolarization was approximated by the fast transient sodium current in parallel with a leakage (repolarizing) current:

$$ I_{nm} = g_{Na} (V_m - V_{Na}) + g_L (V_m - V_L) \quad (2) $$

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

The sodium conductance $g_{Na}$ per unit area was given by:

$$ g_{Na} = \tilde{G}_N m^h \quad (3) $$

where $\tilde{G}_N$ is the maximum sodium conductance, and the dimensionless activation and inactivation variables m and h were considered to follow the kinetics described by Ebihara and Johnson.25 As in recent papers,14-16 voltage-clamp conditions were not simulated (i.e., there were no impressed currents); rather, the natural rest potential was simulated as the starting (take-off) potential at all positions along the cable. For these results, $V_{Na}$ was assigned a value of 33.4 mV to be within the range consistent with observations of changes in sodium concentration in ventricular muscle26 and Purkinje fibers.27 To simulate the effect of use-dependent drug blockade of the sodium channels,28 the take-off potential was maintained at $-80$ mV, and the value of $G_{Na}$ was reduced28-29 in steps from 35 to 9 mS/cm$^2$. $V_m$ was assigned a value of $-80$ mV (i.e., the leakage current was zero at the take-off potential).

When simulating the effects of changes in $G_{Na}$, $g_L$ was maintained constant at 0.05 mS/cm$^2$ (equivalent to a resistance of 20,000 Ωcm$^2$). To mimic the effects of ACh (increasing the conductance of the membrane repolarization currents) on $V_{Na}$, the take-off potential was maintained at the constant value of $-80$ mV and the nonspecific leakage conductance ($g_L$) was varied between 0.05 and 0.5 mS/cm$^2$ at different values of $G_{Na}$ between 21 and 35 mS/cm$^2$. A membrane capacitance of 1.0 μF/cm$^2$ and an internal resistivity of 190 Ωcm were used in a continuous cable with a radius of 5 μm (appropriate for a cardiac cell) to simulate propagation along the long axis of the fibers. The computational procedures for simulating the uniform propagation (no end effects) of $V_m$ during depolarization have been presented in detail in recent papers.14-16

Results

All specimens that responded to electrical stimulation had control resting potentials more negative than $-73$ mV, and the least negative take-off potential that produced a propagated response was $-63$ mV. This allowed us to interpret the mechanism of the most rapid component of the upstroke of the action potential to be the fast sodium current because “slow response” upstrokes due to slow inward currents29 occur when the rest or take-off potential is in the range of $-50$ to $-40$ mV and we never found depolarization to occur at take-off potentials in that range. We also did not find evidence of diastolic depolarization, afterdepolarizations, or triggered activity.29 The propagation responses to modification of the sodium conductance were not
species-specific; that is, similar propagation behavior occurred in dog preparations depending on the uniform versus nonuniform anisotropic properties of the tissue.

**Shape of Action Potential at Different Ages**

Figure 1A shows typical transmembrane action potentials measured at the endocardial surface of bundles that were exposed to brisk superfusion and stimulated at rates between 60 and 80/min. A similar “spike and hump” configuration occurred at all ages. Although a quantitative analysis of the size of the notch after the upstroke was not performed, the qualitative similarities in shape strongly suggest that there were no significant differences in the basic ionic mechanisms that control the shape of the action potential in the bundles from subjects varying between 1 and 70 years of age. Action potentials measured at four to six sites within each of eight bundles failed to reveal differences in the spike and hump shape, although differences in duration (46 msec) occurred at 50% repolarization. However, in bundles located in other regions of the tissue bath that had low superfusion rates\(^1\) or in areas with small stagnant pools of superfusion fluid, the action potentials were shorter in duration and had no separate phases of repolarization (no hump or plateau phase); we considered these action potentials to be influenced by hypoxia (see Figure 32 of Carmeliet and Vereecke\(^2\)).

Figure 1B shows a representative example of the rate dependency of the repolarization shape of the human atrial action potential for bundles from patients of all ages. Little change in the shape or area of repolarization occurred until the stimulus rate was increased beyond 120/min. Further increases in the stimulus rate resulted in the disappearance of the secondary depolarization (hump) with development of a distinct plateau at 150/min, and at higher rates, there were no separate phases of repolarization (Figure 1B, 171/min). There were similar progressive changes in the repolarization shape of the action potential following the onset of infusion of 3.4 \(\times\) 10\(^{-4}\) M ACh HCl, as shown in Figure 1C. Initially, the hump disappeared and was replaced by a distinct plateau phase at a more negative potential; when this subsequently disappeared, there were no separate phases of repolarization (Panel C, 5 minutes) as the action potential continued to shorten in duration.

Gelband et al\(^3\) attributed the different shapes of the action potentials they measured in human pectinate muscles from the anterior wall of the right atrium to be due to two populations of atrial fibers with inherently different membrane ionic properties. They classified atrial fibers as “specialized” or “working” fibers dependent on qualitative differences in the shape of the action potentials; those considered to have a repolarization plateau phase were designated specialized fibers, and those without a plateau phase were designated working fibers. Using this criterion, we failed to find evidence for intrinsically different populations of fibers because the occurrence of a distinct plateau phase of the action potential varied with the background conditions at the time of the measurement. For example, only when the stimulus rate was in the range of 150/min in Figure 1B or during the 1-3-minute interval following the onset of the infusion of ACh (Figure 1C) did a plateau shape occur that was similar to the configuration ascribed to an atrial specialized fiber by Gelband et al.\(^3\) Thus, we concluded that any differences we found in the action potentials or in the propagation behavior of the bundles we studied were not accounted for by the presence of two populations of atrial fibers with intrinsically different membrane ionic properties. Ultimate clarification, however, of the question about populations of atrial fibers with intrinsically different membrane repolarization currents awaits quantitative analysis.

**Age-Related Differences in Anisotropic Propagation Response to Premature Action Potentials**

Extracellular depolarization waveforms and pattern of excitation spread of premature action potentials. In preparations stimulated at a rate of 75/min, progressive shortening of the premature interval below 420 msec produced different types of propagation responses dependent on the presence of uniform versus nonuniform anisotropic propagation properties of tissue. Figure 2A shows the pattern of excitation spread and the associated directional differences in the extracellular waveforms of the normal (left side) and earliest propagated premature action potentials (right side) in a representative uniform anisotropic bundle. As the premature interval was progressively shortened, the waveforms maintained the same general shape with a smooth contour; however, the peak-to-peak amplitude of the extracellular waveforms decreased relatively more with LP than with TP \((p<0.0002)\). The conduction velocity of premature action potentials also decreased relatively more in the longitudinal than in the transverse direction,\(^1\) producing isochrones with a blunted shape (Figure 2A, 345 msec). The shape and even contour of the extracellular waveforms and the isochrones of the premature beats, however, indicated that propagation at a microscopic level continued in all directions as a spatially smooth process with near-synchronous firing of adjacent groups of fibers.

Premature action potentials in the older, nonuniform anisotropic bundles, however, produced two types of propagation response involving an abnormality of longitudinal conduction that did not occur in the young, uniform anisotropic bundles. In the first type of propagation response, progressive shortening of the premature interval resulted in the abrupt occurrence of unidirectional decremental conduction to block in the longitudinal direction (Figure 2B1, positions 3 and 4, 327 msec). With further shortening of the premature interval, slow TP continued in the absence of LP until the absolute refractory period was reached. This experimental result is consistent with our recent simulation results, which show that unidirectional propagation failure can occur at a single low take-off potential if there are directional differences in the effective membrane capacitance.\(^4\) (In Figure 2B1, when the impulse propagating in the transverse direction reached the lateral border of the bundle, propa-
FIGURE 1. Panel A: Representative shape of action potential at low stimulus rates (60-75/min) in bundles from patients of widely different ages. Panel B: Changes in shape of action potential at different stimulus rates. Panel C: Effect of acetylcholine (ACh) on shape and duration of action potential. Time of exposure to ACh is from onset of flow into bath of superfusate with ACh HCl (3.4 × 10⁻⁶ M). Action potentials in Panels B and C were measured in same bundles as action potentials in Panel A for 10- and 62-year-old patients, respectively.
FIGURE 2. Representative propagation responses to premature action potentials in bundles with uniform (A) and nonuniform (B) anisotropic properties. In each panel, normal excitation sequence and a few of the measured extracellular waveforms are shown on the left and those of the premature beat are shown on the right. Interstimulus interval (msec) is shown within boxes above each set of waveforms. Panel A: Isochrones represent time difference of 1 msec. Bundle was from 12-year-old patient. Panel B: Excitation sequences were so complex that it was not possible to construct isochrones. In drawings of normal excitation sequences, elongated open arrow represents narrow region of fast conduction along longitudinal axis of fibers; on the right, elongated triangles represent decremental conduction. "Sawtooth" curves denote irregular course of excitation spread. Bundle in Panel B1 was from a 64-year-old patient, and bundle in Panel B2 was from a 52-year-old patient.
In the older bundles with the second type of propagation response, progressive shortening of the premature interval produced a drastic change in excitation spread along the long axis of the fibers: the large biphasic deflection of the extracellular waveform of longitudinal conduction suddenly changed to a complex waveform with multiple small deflections occurring over an interval of 15–40 msec (Figure 2B2, positions 4 and 5). The initial deflection in the polyphasic waveforms recorded near the stimulus electrode indicated decremental conduction to block within a group of fibers (open arrow at uniphasic positive deflection in Figure 2B2, 360 msec), while excitation occurred later in adjacent fibers. Intracellular-extracellular microelectrode measurements at multiple sites within the narrow 100–200-μm zone of longitudinal conduction confirmed that with early premature beats there was asynchronous firing of adjacent groups of parallel-oriented fibers as depolarization of the premature action potentials progressed longitudinally in a zigzag manner for the length of the bundle. For example, normal action potentials produced synchronous excitation at sites along an axis perpendicular to the long axis of the fibers (V max time difference 0–0.4 msec). On the contrary, early premature action potentials during the same impalements produced marked differences in the time of depolarization of the premature action potentials progressed longitudinally in a zigzag manner for the length of the bundle. For example, normal action potentials produced synchronous excitation at sites along an axis perpendicular to the long axis of the fibers (V max time difference 3–15 msec). Further, different premature intervals produced different durations and shapes of the complex polyphasic extracellular waveforms, a manifestation of changes in the complex sequence of excitation spread at a microscopic level for different degrees of reduction of the depolarizing sodium current. Despite the marked changes in LP produced by early premature action potentials, stable conduction continued in the transverse direction with much less change from normal in the polyphasic waveforms produced by propagation in that direction (Figure 2B2, positions 1–3).

The dissociated zigzag longitudinal propagation (LP-dz) was neither species-specific nor due to a large magnitude of the stimulus current with the fixed duration of 1 msec of the stimulus. This can be seen in the strength-interval curve (Figure 3) measured in a nonuniform anisotropic dog atrial muscle bundle (crista terminalis) at a basic cycle length of 400 msec. At premature intervals between 180 and 200 msec, there was a slight decrease in current strength, as originally shown by Brooks et al. The minimum current necessary to induce propagation increased with further shortening of the premature interval until LP-dz occurred at premature intervals between 150 and 140 msec (while stable but slower transverse propagation continued). Increases in the stimulus strength from the minimum value of 4.3 mA, which just produced propagation at the premature interval of 150 msec, to 7 mA failed to alter the appearance of LP-dz at this interval. That is, the occurrence of LP-dz was due to the effects of the relative refractory period rather than the strength of the stimulus. There was no propagation in either the longitudinal or transverse direction with premature intervals shorter than 140 msec, even with stimulus strengths up to 10 mA. Uniform anisotropic canine ventricular preparations demonstrated similar strength-interval curves with respect to the magnitude of the stimulus current, but LP and TP continued until both stopped simultaneously at the shortest premature interval that resulted in propagation in any direction (absolute refractory period). In the human prepara-

**FIGURE 3.** Strength-interval curve and extracellular waveforms in dog nonuniform anisotropic atrial bundle. Extracellular waveforms were monitored at four sites, two sites along each of the major axes with respect to fiber orientation in crista terminalis. Constant duration pulses (1 msec) were delivered at variable premature intervals after every 10th beat at basic cycle length of 400 msec. Panel A: Each point on curve represents minimum current required to produce longitudinal propagation (LP) and transverse propagation (TP). Vertical solid bar marks absence of propagation in either direction at premature intervals of 138 msec or less, even with current strengths up to 10 mA. Panel B: Waveforms (measured at sites indicated in upper drawing) show development of dissociated zigzag longitudinal propagation (LP-dz) at minimal current strength necessary to induce propagation at short premature intervals (140–150 msec). When current strength was increased to 8 mA at 140–150-msec premature intervals, LP-dz and regular TP persisted (not shown).
tions, the threshold current with steady-state pacing varied between 1.3 and 3.1 mA. Because these threshold values were within the range of those obtained in the dog preparations, a fixed stimulus strength between 1.95 and 4.7 mA (1.5 times threshold) was used for all measurements of premature beats in the human preparations.

Conduction velocity differences due to changes in $V_{na}$ versus anisotropy. In the measurement of the effective conduction velocity of premature action potentials, a decrease in velocity in a given direction was assumed to be caused by a decrease in the depolarizing current (reflected in $V_{na}$) due to the progressively earlier onset of depolarization shifting the take-off potential to a less negative value. Prior measurements of velocity and $V_{na}$ have shown the validity of this assumption as long as excitation spread does not deviate significantly from the original paths of spread in the longitudinal and transverse directions. In one group of nonuniform anisotropic bundles, however, the longitudinal path of excitation spread changed drastically with early premature beats. We, therefore, used extracellular waveforms and their derivatives to simultaneously detect any changes in the sequence of excitation and to measure the effective velocity during LP and TP.

Figure 4A shows the anisotropic velocity profile for the uniform anisotropic bundle of Figure 2A. Both longitudinal and transverse velocities diminished progressively until propagation stopped simultaneously in all directions at the absolute refractory period. With the earliest propagated premature beat, LP was 0.50 of the maximal velocity of normal action potentials and TP was 0.63 of the maximum velocity in the transverse direction. Note that the earliest propagated response along the long axis of the fibers was still twofold faster than normal action potentials propagating in the transverse direction. Figure 4B1 shows the anisotropic velocities in an older, nonuniform anisotropic bundle with the first type of response (longitudinal block) to premature action potentials. In the longitudinal direction, the lowest velocity achieved (before decremental conduction to block occurred) was 0.32 m/sec, a value half that of normal action potentials; however, this minimum velocity of the earliest propagated response in the longitudinal direction was still threefold greater than the highest value of normal action potentials propagating in the transverse direction (0.10 m/sec). Figure 4B2 shows the velocity profile of an older bundle with the second type of response to premature action potentials (dissociated longitudinal conduction). The shift to LP-dz occurred when LP was still at the high value of 0.6 m/sec (0.78 of normal LP); with further shortening of the premature interval, there were step changes in LP-dz until the lowest velocity of LP-dz was 0.22 m/sec (0.35 of normal LP). A decrease in the velocity in the longitudinal direction to less than 0.46 of the normal (maximum) value of LP occurred only when dissociated longitudinal conduction developed.

Table 1 (top) shows the anisotropic velocities of the normal and earliest propagated premature action potentials measured in the different groups of bundles; ratios of the anisotropic velocities are presented below to emphasize age-related differences. In the older, nonuniform bundles that developed longitudinal conduction block, the velocity of the earliest propagated response in the longitudinal direction was greater ($p<0.01$) and in the transverse direction it was less ($p<0.01$) than the corresponding lowest velocities of premature action potentials in the young, uniform anisotropic bundles. The increase in longitudinal velocity and the decrease in effective transverse velocity with increasing age accentuated the directional differences in the velocity of the earliest propagated premature action potentials. For example, the TP/LP velocity ratio of the earliest propagated response in the young preparations was 0.25 and in the older, nonuniform anisotropic preparations that de-
TABLE 1. Anisotropic Effective Conduction Velocities of Normal Action Potentials and Earliest Propagated Premature Action Potentials

<table>
<thead>
<tr>
<th>Anisotropic velocities (m/sec)</th>
<th>Uniform anisotropic properties (n = 9 bundles)</th>
<th>Nonuniform anisotropic properties (patients 43–70 years old)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal LP</td>
<td>Premature response 1 (longitudinal block)</td>
</tr>
<tr>
<td></td>
<td>0.43–0.65 (0.57)</td>
<td>0.58–0.78 (0.70)</td>
</tr>
<tr>
<td></td>
<td>Earliest premature LP</td>
<td>0.25–0.35 (0.31)</td>
</tr>
<tr>
<td></td>
<td>Normal TP</td>
<td>0.09–0.16 (0.12)</td>
</tr>
<tr>
<td></td>
<td>Earliest premature TP</td>
<td>0.06–0.10 (0.077)</td>
</tr>
<tr>
<td></td>
<td>Earliest premature LP-dz</td>
<td>0.55–0.80 (0.68)</td>
</tr>
<tr>
<td></td>
<td>(n = 9 bundles)</td>
<td>0.58–0.43 (0.37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03–0.13 (0.082)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.025–0.08 (0.042)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.55–0.64 (0.57)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.64 (0.51)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.12 (0.082)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.11–0.25 (0.17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25 (0.11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.28</td>
</tr>
</tbody>
</table>

Data obtained at a basic stimulus rate of 75/min. Velocity ratios are of average values in parentheses. LP, longitudinal propagation; TP, transverse propagation; LP-dz, dissociated zigzag longitudinal propagation.

veloped longitudinal conduction failure the ratio was 0.11.

It is possible that the basic stimulus rate influenced the lowest longitudinal and transverse conduction velocities, as well as the propagation behavior, of the premature action potentials. Increases in stimulus frequency modify electrical coupling between cardiac cells and decrease the conduction velocity, especially in the transverse direction, and at high rates, there can be "use dependence" of $V_m$, in the absence of drugs. Therefore, to make certain that the propagation behavior and velocities were not dependent on the background rate, the measurements were repeated at a stimulus frequency of 200/min. The same anisotropic propagation responses to early premature action potentials occurred at low and high background stimulus frequencies (75 and 200/min). With the high stimulus frequency, however, the velocity of the earliest propagated premature responses was slightly lower for both LP (↓ 6–9%) and TP (↓ 10–16%) than at the low stimulus frequency.

The greatest reduction in velocity in a given direction (without dissociation) that was produced by the earliest propagated premature action potential was to a value approximately 0.5 that of the velocity of normal action potentials. The development of dissociated longitudinal conduction with a zigzag course in some of the nonuniform anisotropic bundles, however, resulted in further reduction of the effective velocity to 0.25 of normal in the longitudinal direction (Table 1, bottom).

A hypothesis for dissociated zigzag longitudinal propagation of premature action potentials. Since there is no available model or theory to account for the production of this type of propagation at a microscopic level by premature action potentials, the hypothesis that the shift to dissociated zigzag longitudinal conduction represents a change in the direction of propagation from along the fibers to a more transverse direction, thus resulting in a safer (but slower) form of conduction, was examined. If true, the change in the direction of propagation at a microscopic level should enhance both $V_m$ and the safety factor for propagation, because both are greater during TP than during LP.9,11 The following result, obtained in three older bundles, supports this hypothesis.

Figure 5 shows the typical changes in $V_m$ and in the phase-plane trajectory of $V_{m}$ versus $V_{r}$ of premature beats during both LP and TP at the impalement site (produced by initiating propagation at different locations). A linear ascending limb of the phase-plane loop indicates an exponential rise of the foot of the upstroke, characteristic of continuous propagation, such as occurs in one-dimensional Purkinje strands (e.g.,

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Pressler et al. In our experiment, deviations from a straight-line initial trajectory (nonexponential rise) would denote some type of propagation discontinuity at a microscopic level.

As progressively earlier premature action potentials shifted the take-off potential to less negative values, $V_{\text{max}}$ decreased as expected (although $V_{\text{max}}$ was much greater with TP than LP). At a take-off potential of $-70$ mV (Figure 5A), regular LP continued, but there were minor deviations from linearity in the ascending limb of the phase-plane plot (minor deviations were also perceptible in the normal action potentials of LP); during TP, there was an exponential rise of the initial upstroke. With reduction of the take-off potential from $-70$ to $-66$ mV (Figure 5B), $V_{\text{max}}$ decreased from 44 to 13 V/sec while the extracellular waveforms (not shown) indicated that regular longitudinal conduction persisted. There was a prominent nonlinearity in the ascending limb of the phase-plane trajectory during regular LP, while with TP at the same take-off potential, the ascending limb was linear (Figure 5B2).

When an even shorter premature interval lowered the take-off potential to $-64$ mV, the extracellular waveforms indicated a shift to dissociated zigzag longitudinal conduction (not shown). At this less-negative (lower) take-off potential, $V_{\text{max}}$ continued to decrease with TP (Figure 5C). In the longitudinal direction, however, $V_{\text{max}}$ (of the now LP-dz) increased to a value almost the same as that of TP at the same take-off potential (Figure 5C2); that is, a value greater than that achieved at the more negative take-off potential of $-66$ mV when regular LP was present. This increase in $V_{\text{max}}$ from a lower take-off potential in association with the changes in the extracellular waveforms is consistent with "regular" LP shifting to the transverse direction at a microscopic level when LP-dz developed. Linearity of the ascending limb of the phase-plane plot persisted with TP, but there was marked deviation from a straight line with the dissociated zigzag longitudinal conduction. These differences in the ascending limb of the phase-plane plots of LP-dz and TP in Figure 5C2 suggest that the LP-dz involved additional interactions between small groups of fibers that did not occur with TP.

The dissociated zigzag longitudinal conduction of premature beats at a microscopic level resulted in abnormalities of conduction at a slightly larger scale in three small bundles from older patients, as shown in Figure 6. During the normal excitation sequence (Figure 6A), there was fast LP (0.52 m/sec) along one side of the bundle with large biphasic extracellular waveforms and derivatives with an even contour (sites 1 and 2), while complex multiphasic low-amplitude waveforms occurred with TP at sites 3 and 5—waves characteristic of nonuniform anisotropic muscle. However, at site 4, the $\Phi$ waveform was typical of uniform anisotropy. An early premature stimulus delivered to one side of the bundle induced the following (Figure 6B): slow dissociated zigzag longitudinal conduction (about 0.1 m/sec) occurred along the side of the bundle at sites 1 and 2, while no TP occurred at sites 3, 4, and 5. After the dissociated conduction had progressed longitudinally for a distance of 1 mm from the site of impulse initiation, conduction across the fibers occurred and initiated longitudinal fast conduction back toward the stimulus site along the other side of the small bundle. This was evidenced by the single large biphasic extracellular deflection of LP that replaced the low-amplitude waveforms of normal TP (Figure 6B, positions 3 and 4).

In each of the three bundles that demonstrated the above events (Figure 6), failure of propagation in the transverse direction developed when LP-dz occurred, which seems contrary to our proposal that the safety factor for premature impulses is higher in the transverse...
The common feature in these bundles was the side-by-side juxtaposition of regions in which one region had smooth waveforms with TP (uniform anisotropy) and the adjoining region had polyphasic waveforms (nonuniform anisotropy). The elongated broken line (in the drawing of Figure 6B) where initial TP failure occurred correlated with a regional difference in uniform versus nonuniform anisotropic electrical properties. We considered that the juxtaposed regional differences in anisotropic properties produced associated local discontinuities of effective axial resistance, which previously have been shown to result in localized propagation failure of depressed action potentials (e.g., at branch sites in pectinate muscles). (A full explanation of the TP failure must, however, await two-dimensional simulations of anisotropic propagation.)

Effects of Quinidine and Lidocaine on Multidimensional Propagation in Preparations With Different Anisotropic Properties

Drug effects common to uniform and nonuniform anisotropic bundles. At stimulation frequencies between 120 and 150/min, classical Wenckebach cycles developed in bundles exposed to quinidine in both human (n = 7) and dog (n = 9) preparations with either uniform or nonuniform anisotropic properties. The results of a typical experiment are shown for a uniform anisotropic bundle (dog papillary muscle) in Figure 7. Considerable use-dependent drug block of the sodium channels was first achieved by stimulating the preparation at 100/min with a concentration of 10 μg/ml quinidine gluconate, which produced a decrease in $V_{max}$ during longitudinal propagation from 133 to 72 V/sec. When the stimulus frequency was increased to 120/min, repeated Wenckebach cycles developed with propagation failure (dropped beat) every fifth stimulus. In Figure 7A, the action potential with the earliest excitation time followed the dropped beat, and the next three action potentials occurred progressively later with the increment of prolongation in the local excitation times of LP and TP being greatest with the second beat. Thereafter, the increment of delay was progressively less with each action potential until propagation failure occurred simultaneously in all directions (classical Wenckebach cycles).

The same type of Wenckebach periodicity characterized $V_{max}$ of the transmembrane potential and the maximum negative derivative of the extracellular potential during longitudinal and transverse propagation (Figure 7). The maximum value of each of these variables occurred with the first beat after the pause (dropped beat), the greatest decrease occurred with the second beat of the series of action potentials, and the subsequent decreases in magnitude with each beat occurred with less decrement until propagation failed. The recurrent Wenckebach cycles persisted for 3 minutes but disappeared immediately when the stimulus frequency was returned to the original lower value of 100/min, only to recur when the frequency was

Figure 6. Dissociated longitudinal conduction at two size scales (microscopic and slightly larger) within nonuniform anisotropic bundle in response to premature action potential. Normal excitation sequence and extracellular waveforms are shown on the left (500 msec basic cycle length), and the response to premature action potential on the right (215 msec premature interval). *Location of stimulus site. Preparation was from 66-year-old patient.
**FIGURE 7.** Quinidine-induced Wenckebach periodicity of conduction within single bundle. $V_m$ during longitudinal propagation (LP) (A) and extracellular potential waveforms during longitudinal (B) and transverse propagation (C) LP $\Phi_e$ and TP $\Phi_e$, respectively, were measured simultaneously in dog papillary muscle, which has uniform anisotropic properties. Results shown were obtained after stimulus rate was increased from 100 to 120/min during exposure to quinidine gluconate 10 $\mu$g/ml. (In human pectinate bundles, we were not able to achieve artifact-free intracellular measurement of $V_m$ throughout a complete series of Wenckebach cycles due to marked contraction movement after each dropped beat.)
balance between the kinetics of use-dependent drug blockade during depolarization and drug release kinetics from the sodium channels during diastole. Starmer and Courtney showed that drug blockade can be viewed as a variation in channel population; $g_{Na}^\text{e} = g_{Na} (1 - b)$ where $g_{Na}^\text{e}$ is the sodium conductance during drug blockade and $b$ is the fraction of blocked channels. Thus, the effects of use-dependent drug blockade of the sodium channels on $V_{\text{m}}$ of a uniform propagating action potential (Equation 1) were simulated by altering the value of $G_{Na}$ as an index of the number of available (unblocked) sodium channels, as done in our recent simulations. We consider the computed results based on use-dependent conductance changes, illustrated in Figure 8A, to provide a basis for accounting for the drug-induced Wenckebach events of Figure 7 as follows.

During the initial experimental steady state (before the slight increase in stimulus frequency), considerable blockade of the sodium channels had produced marked reduction in $V_{\text{m}}$ (i.e., a large shift down the $G_{Na}$-$V_{\text{m}}$ curve [Figure 8A]). When the rate was then increased slightly, each subsequent beat further decreased $G_{Na}$ because drug uptake by the ionic channels during depolarization exceeded drug release during the diastolic interval. Because the reduction of $G_{Na}$ was initiated at a very low point on the $G_{Na}$-$V_{\text{m}}$ curve, only a slight increment of drug blockade was necessary to reduce the available sodium channels below the number necessary to sustain propagation. Propagation failure occurred when $G_{Na}$ was reduced below 10 mS/cm². The propagation failure (dropped beat), however, provided a prolonged diastolic interval for drug release from the channels, resulting in an increase in the number of unblocked sodium channels (upward shift in the $G_{Na}$-$V_{\text{m}}$ curve). This resulted in an increase of $V_{\text{m}}$ with the first beat of the next series, with subsequent decreases in $V_{\text{m}}$ occurring with activity to the point of propagation failure again. The absence of Wenckebach cycles during lidocaine exposure can be accounted for by the considerably more rapid release of lidocaine compared with the relatively slow release of quinidine; with lidocaine, drug bound to the channels during depolarization was released during diastole before the onset of the next action potential.

The above indicates that drug-induced Wenckebach periodicity can occur when small changes in the number of available sodium channels shift $V_{\text{m}}$ up and down the $G_{Na}$-$V_{\text{m}}$ curve near the lowest point that will sustain propagation. If this mechanism is the correct one, changes limited solely to the repolarization phase of the action potential should affect the sodium current mechanism during depolarization by producing a shift in the $G_{Na}$-$V_{\text{m}}$ curve in the presence of use-dependent drug blockade of the sodium channels. Thus, experimentally induced changes of repolarization following addition of acetylcholine to bath. Rate of stimulation and take-off potential remained constant. Preparation was dog pectorite bundle.

increased back to 120/min. The normal rest potential showed no change during quinidine exposure, as previously demonstrated by Hondeghem and Katzung,22 even with drug blockade to the point of propagation failure (Figure 7A). During exposure to lidocaine HCl (10 µg/ml), the same shape changes in the intracellular and extracellular potential waveforms and their derivatives occurred with LP and TP when the stimulus frequency was increased, but Wenckebach cycles did not occur with lidocaine. Also, none of the above propagation responses occurred with high frequency stimulation in the absence of drug in any of the preparations, even at stimulus frequencies between 200 and 250/min.

A curve connecting the successive values of $V_{\text{m}}$ max and $-dV_{\text{m}}/dt_{\text{m}}$ during each Wenckebach cycle approximated an exponential decay, which is characteristic of phasic use-dependent ion channel blockade. Therefore, computer simulations (Figure 8A) and further experiments (Figure 8B) were performed to test the following hypothesis: the use-dependent periodic changes (Wenckebach periodicity) of $V_{\text{m}}$ max were due to changes in the number of available (unblocked) sodium channels, which, in turn, were determined by the

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**Figure 8.** Panel A: Theoretical (computed) relation between $V_{\text{m}}$ max and $G_{Na}$ during changes in number of available sodium channels due to drug blockade. Each $V_{\text{m}}$ max value was computed for uniformly propagating action potential in a continuous cable (no boundary effects). $G_{Na}$-$V_{\text{m}}$ curve is presented in form of step changes as corollary of the experimental beat-to-beat changes in $V_{\text{m}}$, as well as to emphasize the relatively greater changes in $V_{\text{m}}$ for same change in $G_{Na}$ at low values of $G_{Na}$. Panel B: Increase in $V_{\text{m}}$ in quinidinized atrial muscle secondary to shortening repolarization phase of atrial action potential following addition of acetylcholine to bath. Rate of stimulation and take-off potential remained constant. Preparation was dog pectinate bundle.

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However, as illustrated in Figure 1C, in most human and dog atrial preparations with normal rest potentials, exposure to ACh was found to result in little or no hyperpolarization of the rest potential, although the action potential shortened markedly, which is consistent with some of the original observations of Hoffman and Suckling.\textsuperscript{41}

The above hypothesis was further tested by exposing five dog atrial pectinate muscles to quinidine followed by the addition of ACh (analyzing only experiments without hyperpolarization of the rest potential). Control exposure to ACh in the absence of quinidine consistently produced a decrease in $V_{\text{max}}$ (e.g., a decrease from 137 to 111 V/sec ($\downarrow$ 19%) during LP [not shown]). Our computer simulations accounted for the ACh-induced decrease in $V_{\text{max}}$ by an increase in the conductance of the repolarization currents; we assumed that ACh increased $g_{L}$ in Equation 2 by increasing the potassium conductance.\textsuperscript{41}

For example, at a rest potential of $-80$ mV, an increase in $g_{L}$ from 0.05 to 0.5 mS/cm\textsuperscript{2} decreased $V_{\text{max}}$ from 192 to 164 V/sec ($\downarrow$ 14.5%) when $G_{N}$ was 35 mS/cm\textsuperscript{2}, and when $G_{N}$ was 21 mS/cm\textsuperscript{2}, the maximum rate of rise decreased from 131 to 101 V/sec ($\downarrow$ 30%).

After washout of the ACh, $V_{\text{max}}$ returned to the control value (133 V/sec during LP), following which quinidine gluconate 7.5 $\mu$g/ml was allowed to equilibrate in the bath. When steady-state conduction was achieved at a basic cycle length of 800 msec (75/min) during quinidine, $V_{\text{max}}$ was 60 V/sec and the action potential duration was 203 msec (Figure 8B1). At this point, ACh was infused, and the action potential shortened to 70 msec with an increase of $V_{\text{max}}$ to 102 V/sec in the absence of hyperpolarization of the rest potential (Figure 8B2). The decrease in the duration of repolarization resulted in an increase in the diastolic interval of 133 msec during which drug release could occur; for example, a 22% increase in the diastolic interval was associated with a 70% increase in $V_{\text{max}}$.

Perhaps equally important was that the reduction in the duration of repolarization reduced the time available for quinidine to bind to the sodium channels (inactive channel blocking), thus increasing the drug recovery interval at the expense of the drug uptake interval. With these changes, it was no longer possible to induce Wenckebach cycles in the presence of the short action potentials, even when the concentration of quinidine gluconate was increased to 10 $\mu$g/ml and the stimulus frequency was increased to 175/min.

We conclude that either "dropped beats" or changes in the ionic conductances and fluxes that shorten the two cutaneous system\textsuperscript{40} during exposure to use-dependent sodium channel–blocking drugs. By increasing the duration of the diastolic interval, drug release from the inactive sodium channels is enhanced, which results in a shift up the $G_{N}$–$V_{\text{max}}$ curve (Figure 8A). This repolarization influence on the sodium-carrying system is an unusual form of use dependency, which generally is considered to involve shortening of the basic cycle length or a change in the rest potential. The phenomenon should be accentuated by drugs with slow release kinetics and diminished by drugs with rapid release kinetics. In two experiments during exposure to lidocaine, using the same protocol, we found only equivocal changes in $V_{\text{max}}$ when ACh shortened the action potential at the same basic cycle length of 800 msec.

Drug effects that were dependent on the anisotropic passive properties. With progressive drug blockade in the uniform anisotropic bundles, the extracellular waveforms and their derivatives during LP and TP decreased in amplitude but maintained the same general shape and an even contour up to the point of simultaneous propagation failure in all directions (Figure 7). This response was the same as that produced by premature action potentials (Figure 2A); propagation in the uniform anisotropic bundles continued as a spatially smooth process without changes in the sequence of excitation spread. On the other hand, when the sodium conductance was decreased by either quinidine or lidocaine in the older, nonuniform anisotropic bundles, LP-dz developed, just as occurred with premature action potentials (Figure 2B). However, during drug exposure, none of these bundles developed unidirectional longitudinal conduction block as occurred with premature action potentials (Figure 2B1). No evidence was found that use-dependent sodium channel–blocking drugs produced further reduction in the lowest velocities achieved during premature action potentials under control conditions. On the contrary, in the presence of quinidine, in three bundles the earliest propagated prematures occurred from more negative take-off potentials and had higher velocities than the earliest premature responses in the absence of quinidine.

Figure 9A shows the use-dependent effects of quinidine gluconate (10 $\mu$g/ml) on the longitudinal conduction velocity and the associated extracellular waveforms in an older preparation. At a stimulus rate of 75/min, quinidine reduced the longitudinal velocity from the control value of 0.40 to 0.25 m/sec (not shown). Following a small increase in frequency to 85/min (Figure 9A, arrow), the velocity decreased exponentially in association with a decrease in the magnitude of the extracellular waveform and its derivative, both of which changed from a single large biphasic deflection with an even contour to a fragmented waveform with an increasing number of small deflections typical of LP-dz. When the stimulus frequency was returned to its original lower value (70/min), the opposite change took place: as the velocity increased, the extracellular waveform also increased in amplitude and returned to the single large biphasic deflection.

After 2 minutes at the higher stimulus rate of 85/min (basic cycle length of 700 msec), recurrent Wencke¬bach cycles developed that were characterized by beat-to-beat progressive changes from regular LP to LP-dz as the velocity decreased with each beat up to the point of propagation failure (Figure 9B). After the pause produced by the dropped beat, the first beat of the next Wenckebach cycle was associated with a
submaximal increase in velocity (still with fragmented waveforms of LP-dz); maximum velocity (and the largest biphasic $\Phi$ waveform of smooth contour) occurred with the second beat of the series, following which there was an approximately exponential decay in velocity with progressive fragmentation and loss of amplitude of the $\Phi$ waveforms (Figure 9B). The failure to achieve the maximum velocity and amplitude of the extracellular waveform immediately after the pause indicated that the drug process affecting the excitation sequence was prolonged. In the young, uniform anisotropic bundles with Wenckebach periodicity, there were no changes in the excitation sequence, and the maximum velocity occurred with the first beat of each Wenckebach cycle.

Role of Anisotropic Propagation in Reentry

Because past reentry models have focused on conduction in the reentrant path in the form of one-dimensional propagation around a barrier, no multidimensional data have been available, such as that of Table 1, to know the actual effective velocities that can occur at a small size scale within the path. Since muscle bundles throughout the atria are inherently anisotropic, one might now consider that propagation within reentrant circuits must involve both LP and TP. Apparently this is not the case. In the recent atrial reentry model of Frame et al., which used the leading circle concept, propagation was interpreted to occur entirely as uniform conduction along the longitudinal axis of the fibers "in a continuous circumferential band of atrial muscle just above the tricuspid valve."

The effective velocities of Table 1 provided a way to calculate whether the low velocities of TP (or LP-dz) are necessary to allow enough time for reentry to occur when premature impulses conduct around a refractory barrier due to a known absolute refractory period (and known minimum length of a reentrant circuit). Therefore, absolute refractory periods were measured under a variety of conditions in bundles stimulated at frequencies between 100 and 300/min. With premature action potentials introduced in the absence of Ach, the shortest absolute refractory period varied between 80 and 110 msec ($n = 17$), and with Ach, it was 75–85 msec ($n = 5$). During exposure to quinidine gluconate (10 $\mu$g/ml), the shortest absolute refractory period was prolonged to between 210 and 400 msec ($n = 6$); following the addition of Ach in the presence of quinidine, it decreased to between 85 and 120 msec ($n = 4$). To calculate the minimum lengths of reentrant paths that can occur in human atrial muscle for the range of the above refractory periods and the range of velocities measured, the data of Table 1 and the simple equation $v = \text{effective velocity} \times \text{time (absolute refractory period)}$ were used. These straightforward calculations demonstrated that for a wide range of refractory periods, even those as short as 80–100 msec, a reentrant path with a length of 25 mm or less would require the low velocities provided only by transverse propagation (in uniform or nonuniform anisotropic muscle) and/or LP-dz in nonuniform anisotropic preparations. The calculations further indicated that in nonuniform anisotropic preparations, reentrant path lengths shorter than 10 mm are possible if the conduction of premature systoles occurs as TP and/or LP-dz. This result is important because the shortest reentrant atrial path (without exposure to cholinergic agents) reported thus far is 23 mm, measured by Smeets et al.\textsuperscript{59} in rabbit atrial muscle.

In our original results of discontinuous propagation, reentrant circuits associated with anisotropic propagation in canine atrial bundles\textsuperscript{8} resembled a rectangle more closely than a circle, the usual shape considered for a reentrant circuit. Therefore, we calculated the minimum area within which reentry could occur if the circuit had the same path length but varied in shape in
the form of a rectangle versus a circle. For a rectangular circuit in which the length was four times that of the width (the ratio of the normal LP and TP velocities was 4 : 1), the area was 0.5 that of a circle. For a rectangular circuit in which the length was nine times that of the width (the ratio of LP and TP velocities of early premature systoles was 9 : 1), the area was reduced further to 0.28 of the area of a circular circuit. Based on a rectangular circuit and the measured velocities of Table 1, the calculations showed that the theoretical minimum area within which reentry can occur in nonuniform anisotropic bundles is as small as 2-4 mm², a value much lower than the minimum area of 25-50 mm² thus far reported. 14,44,45 Therefore, we stimulated bundles at frequencies between 170 and 200/min (to shorten the refractory period) and introduced premature pulses in an attempt to produce reentry within single atrial bundles.

As expected, reentry was never encountered within the young bundles; in the uniform anisotropic bundles, unidirectional block did not occur to set the stage for reentrant propagation. However, reentry occurred in three older bundles with markedly nonuniform anisotropic properties, as shown in Figure 10. The markedly polyphasic extracellular waveforms with normal transverse propagation (Figure 10, 350 msec) indicate sparse side-to-side electrical coupling between small groups of cells, 15 consistent with the presence of extensive collagenous septa separating small groups of fibers in this bundle (Figure 11). At a premature interval of 233 msec, there were additional multiple deflections of the polyphasic extracellular waveform of TP (positions 1 and 2), while in the longitudinal direction, decremental conduction to block occurred near site 5 (evidenced by the uniphasic positive deflection 9). Progressive reduction of the premature interval resulted in further delay in the time between the multiple deflections of TP, which led to reentry in the longitudinal path proximal to the location of the initial conduction block (Figure 10, bottom). The multiple small deflections of the reentrant waveforms (Figure 10, bottom, sites 3 and 4) were typical of dissociated zigzag longitudinal conduction, now progressing in a retrograde direction toward the site where the impulse was initiated.

The reentrant waveforms were quite reproducible for as long as 40 minutes, which provided time for the measurements at multiple sites beyond those shown, for example, at seven additional sites within the area shown for the reentrant circuit (Figure 10, bottom right, area within solid line) and at 16 sites outside the circuit (darkened area). This demonstrated that the maximum width of the circuit was 0.6 mm and the maximum length was 2.6 mm, an area on the endocardial surface of 1.6 mm². None of the muscle outside this area participated in the initiation of reentry because the time of local excitation in those areas occurred after that of the sites that were in the same region but located within the perimeter of the reentrant circuit. Conduction of the premature impulse did not occur in the region marked A on the other side of the stimulus electrode. However, the details of the complex spread of excitation at a microscopic level (< 200 μm) were impossible to map with either intracellular or extracellular electrodes during reentry. Some of the small deflections that occurred in the polyphasic waveforms of TP or LP-dz could have originated as much as 100 μm from the monitoring site on the endocardial surface and to a depth of at least 50 μm below the surface. 13 Thus, the actual path of propagation that initiated reentry may have been contained within a two-dimensional area that was smaller than that indicated by the drawing shown in Figure 10 (bottom right). Also, the real path of excitation spread between small groups of fibers at a microscopic size scale in nonuniform anisotropic muscle 16 has a very irregular and complex shape (zigzag microscopic spread), thus producing a longer reentrant path length than that represented by the smooth line marking the direction of propagation along the maximum perimeter of the circuit in Figure 10.
Intracellular measurements were made during the regular paced beats (the basic cycle length remained 350 msec) at multiple sites in the bundle of Figure 10. This demonstrated that the action potentials in the region of the reentrant circuit (region B) were 4–5 msec shorter at 50% repolarization than those in the region on the other side of the stimulus site (region A), a result we found in both human and dog atrial bundles. This is exactly opposite to the prediction of the leading circle concept, which requires the unidirectional block and the initial reentrant excitation wave to occur in the region of longer, not shorter, action potentials (refractory periods).

The question arises as to whether the reentry that occurred within areas as small as 1–2 mm² was due to reflection rather than due to circus movement with anisotropic propagation. If the reentry was due to reflection, a reflected action potential would have to arise at or distal to the site where longitudinal block was identified. Also, a reflected reentrant action potential would have to propagate in a retrograde fashion along the longitudinal axis of the same fibers. Thus, TP would have no influence on the initiation of reflection reentry, but conduction in the transverse direction near the site of origin would be necessary for circus movement reentry. To distinguish between these two possibilities when reentry was encountered in three bundles, a second stimulus electrode was positioned in the initial area of TP. When both sites were stimulated simultaneously, reentrant propagation disappeared. That is, the transverse excitation waves initiated at each stimulus electrode collided and extinguished TP, while longitudinal decremental conduction to block now occurred from both stimulus sites; the initial LP was never found to continue past the site of block. Subsequently, reentrant beats occurred only when the stimulus at the second (transverse) site was omitted and, contrariwise, no reentry occurred when TP was functionally interrupted by the second stimulus. From this, we conclude that the microreentry we measured was on the basis of circus movement reentry due to anisotropic propagation rather than reentry due to reflection.

**Discussion**

The major conclusion from the results is that the anisotropic passive properties at a microscopic level highly determine the propagation behavior of individual cardiac muscle bundles when the sodium conductance is modified. A significant consequence, due to progressive electrical uncoupling of the side-to-side connections between small groups of fibers with increasing age and chronic hypertrophy, is that propagation events differ in age- and condition-related manners in response to the same modification of the sodium conductance. For example, the results show that progressive obliteration of side-to-side electrical connections between fibers results in more marked nonuniform anisotropic properties, which provides a primary mechanism for reentry to occur in progressively smaller areas. This is due to the resultant lower effective conduction velocities of premature action potentials propagating in the transverse direction, as well as the development of LP-dz of premature action potentials, which did not occur in uniform anisotropic muscle.

The preparation encountered with the most markedly nonuniform anisotropic electrical properties was from a 62-year-old patient with a markedly enlarged (hypertrophied) right atrium. There were a large number of small deflections in the extracellular waveforms.

**FIGURE 11.** Collagenous septa in bundle analyzed in Figure 10. Septa (gray to black) in this longitudinal section are often both thick and long and thus isolate adjacent fibers and groups of fibers (white). Bar = 200 μm.
potentials either in the transverse direction or as channels. reentry within regions less than 50 mm². The basis for role in determining the velocities required to initiate
reentry in the region of shorter, rather than longer, action potentials (Figure 10) is attributable to the
anisotropic passive properties rather than the active membrane ionic properties.

We conclude that the consistent appearance of reentry in the region of shorter, rather than longer, action potentials (Figure 10) is attributable to the following: anisotropic discontinuous propagation provides a mechanism for the initial unidirectional

Implications for Reentry Based on Anisotropy
Measurement of the actual conduction velocities throughout reentrant circuits simply has not been technically possible in the past. Therefore, estimates of the average velocity through reentrant paths have been obtained from calculations based on the length of the path and the time required to traverse the circuit. Our measurements within individual muscle bundles demonstrated that anisotropic propagation plays a dominant role in determining the velocities required to initiate reentry within regions less than 50 mm². The basis for this conclusion is that conduction block and the route of reentry within anisotropic muscle bundles depend heavily on interactions between the depolarizing current and the local and downstream effective membrane capacitance to be discharged, a factor determined by anisotropic structural complexity as well as by the kinetics of the membrane ionic channels.
Arrhythmias and Proarrhythmia Effects of
strongly suggest that the ACh-induced shortening of
simply its increased availability due to ACh-induced
direct effect on the sodium-carrying system40 than
possible to initiate reentrant beats. Johnson and
V m without making the resting membrane potential
secondary effect by decreasing the number of sodium
excitation first in the area of initial block (the area of
shortest action potentials), the returning slow propa-
gation (TP or LP-dz) can turn on the sodium current and
propagation can continue. In regions of longer action
potentials, latent reentrant excitation waves are extin-
guished during the absolute refractory period of the
yet-to-be-repolarized tissues.

**Implications for Drug Therapy of Reentrant Arrhythmias and Proarrhythmia Effects of Use-Dependent Sodium Channel–Blocking Drugs**

In the older preparations with nonuniform anisotro-
pic properties, TP remained stable with use-dependent
drug blockade, but LP shifted to a slow zigzag
dissociated type of conduction, similar to TP. This
strongly suggests that when sodium channel–blocking
drugs reduce the sodium current to near-threshold
levels, these tissues switch to a safer type of disorga-
nized longitudinal conduction before propagation fails.
A major implication of this drug-induced shift is that
it results in a sudden and marked decrease in the
effective conduction velocity in the longitudinal di-
rection. This could enhance reentry within small
regions (Figure 6) in nonuniform anisotropic bundles
and contribute to the well-known clinical phenomenon
that antiarrhythmic drugs actually augment tachy-
arrhythmias in certain patients, most commonly older
patients with ischemic heart disease.50,51

Quinidine, but not lidocaine, produced rhythm
abnormalities characteristic of the Wenckebach phe-
nomenon in bundles from patients of all ages. During
quinidine exposure, V m increased after a pause
(dropped beat in the Wenckebach cycles) and after the
duration of the action potential was decreased by ACh.
Further, we were not able to initiate reentry in any of
the bundles during quinidine exposure, which
increased the minimum absolute refractory periods to a
range of 210-400 msec (without quinidine, the range
was 80-110 msec). When the action potential duration
(and absolute refractory period) was markedly short-
ened by adding ACH to the quinidinizated preparation,
the Wenckebach cycles disappeared, and in the older
nonuniform anisotropic preparations, it then became
possible to initiate reentrant beats. Johnson and
Robertson63 first demonstrated that ACH can increase
V m without making the resting membrane potential
more negative. They proposed that ACH had a more
direct effect on the sodium-carrying system64 than
simply its increased availability due to ACH-induced
hyperpolarization of the rest potential. Our results
strongly suggest that the ACH-induced shortening of the
repolarization phase of the action potential produced a
secondary effect by decreasing the number of sodium
channels blocked by quinidine. That is, increased drug
release from the sodium channels occurred due to an
increase in the diastolic interval, an effect similar to the
insertion of a brief pause into a series of action potentials
(Wenckebach periodicity).

We conclude that 1) the type of anisotropic passive
properties and the number of available sodium chan-
nels (degree of drug blockade) interact to determine
the propagation response and 2) that spatial nonuniformi-
ties in drug binding to the channel site occur due to
anisotropic differences in the shape of the upstroke11
and spatial nonuniformities of drug release from the
sodium channel occur because of known regional
differences in the duration of the repolarization phase
of atrial action potentials.53

**Acknowledgments**

We thank Dr. James E. Lowe for providing the atrial
specimens removed at surgery. Also, we gratefully
acknowledge Dr. C. Frank Starmer and Dr. E.A.
Johnson for their helpful discussions and thank Dr.
Joseph D. Sloan for carrying out the computer simu-
lations.

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KEY WORDS: anisotropic reentry model · sodium channel drug blockade · anisotropic drug effects · fiber uncoupling
Influence of the passive anisotropic properties on directional differences in propagation following modification of the sodium conductance in human atrial muscle. A model of reentry based on anisotropic discontinuous propagation.

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doi: 10.1161/01.RES.62.4.811

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