Mini-review

Altering the topology of gap junctions a major therapeutic target for atrial fibrillation

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Nicole's question about the role of cardiac structure in atrial fibrillation likely will become the central issue in achieving an overall solution for tachyarrhythmias, most of which are reentrant in nature. It is well known that most tachyarrhythmias begin with an early premature beat. After the wavefront spreads from the premature site, the spatially inhomogeneous properties of the cellular network alter the excitation wave sufficiently to create an asymmetry of conduction, and circus movement reentry ensues [1]. To formulate the best possible answer to Nicole's question, we shall focus on the underlying causes of the conduction disturbances that create reentrant circuits, while mechanisms that trigger premature beats will not be considered. A framework of mechanisms derived solely from the sarcolemmal ionic current channels, which represents the classical approach based on the assumption that cardiac muscle behaves electrically as a continuous syncytium, is quite limited when applied to clinical arrhythmias. Recent electrophysiologic and clinical evidence suggests an expanded picture that points to an adaptive structural mechanism—the distribution of gap junctions associated with the development of microfibrosis [1]—as the major cause of most forms of atrial fibrillation. This new evidence supports the presence of an integrated feedback mechanism involving interactions between the microscopic myocardial architecture and the sarcolemmal ionic currents [2]. In the end, all of the evidence produces a picture that generates the following hypothesis: rather than continuing to focus on drugs designed to target sarcolemmal ionic current channels, as done with conventional pharmacological antiarrhythmic therapy, identification of new molecular and/or genetic targets that alter the distribution of gap junctions will produce better therapeutic results.

1. Establishing a framework of mechanisms

The essential knowledge needed for this analysis is that atrial fibrillation is a reentrant process. Here, we are on firm ground based on the recent documentation of re-entrant circuits during atrial fibrillation by Allessie et al. [3] and by Cox and Boineau [4]. That information allows the investigation to proceed by generalizing the analysis of atrial fibrillation to the requirement for initiating any reentrant circuit — an asymmetry of the safety factor of conduction is necessary so that unidirectional conduction block occurs in one pathway while conduction (usually slow) is maintained in a second pathway [1]. The conduction disturbances known to fulfill this role in initiating cardiac reentry are the following: (1) decremental conduction; (2) functional longitudinal dissociation due to differences in the effective refractory period; (3) unidirectional and bidirectional conduction block due to spatial differences in the effective refractory period; and (4) slow conduction.

Presumably all of these conduction disturbances are involved in atrial fibrillation. Therefore, we need to simplify the problem with a first-principles approach based on the two components of any propagating excitation wave: the "source" and the "sink". Stable propagation of the excitation wave occurs when the sarcolemmal membrane of each cell can, once its permeability mechanism is turned on, supply enough excitatory current (the "source") to depolarize itself and to supply the necessary current to charge the capacitance of the neighboring tissue (the "sink") that has yet to depolarize. The downstream capacitance and membrane resistance produce an electrical "load" on each patch of activated sarcolemmal membrane. When multiple cells are connected together by gap

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junctions, the electrical load a "source" cell sees is related to the details of the architecture of the multicellular network [2]. The term "safety factor" has been used to compare the maximum current a cell can supply with the current required to bring it to threshold [1]. Conceptually, the safety factor is easy to understand, although a generally applicable mathematical description of the safety factor has not been developed for cardiac muscle. Consequently, qualitative descriptions of the safety factor have provided the major applications of the concept to cardiac conduction phenomena.

The safety factor of an excitation wave therefore can be altered only in two ways: by altering the magnitude of the sarcolemmal excitatory currents (the "source") or by altering the myocardial architecture (the "sink"). From this point on, a detective's approach is needed to discern all of the "source" and "sink" mechanisms of conduction disturbances that can initiate reentry, and then identify which ones are involved in atrial fibrillation. This strategy merely follows the adage of Sherlock Holmes; "Eliminate all other factors, and the one which remains must be the truth." [5] However, such a strategy must be accompanied by the following caveat—from a scientific point of view what is presently known as the "truth" or a "fact" often changes as new factors become known in the future [6].

To implement this strategy, we begin by noting that until 1981 it was considered that the microscopic architecture of the myocardium behaves electrically as a continuous medium. Consequently, conduction disturbances leading to reentrant arrhythmias were considered to be due solely to alterations or differences in the sarcolemmal ionic channel properties. However, rather than being electrically continuous, cardiac muscle was shown to be discontinuous in nature due to repetitive discontinuities of axial resistance produced by the distribution of the gap junctions which, in turn, alters the activity of the ionic channel currents [7]. In such structures spatial differences in the effective refractory period, which produce the conduction disturbances required to initiate reentry, are created by two phenomena. One phenomenon is the traditional region-to-region variation in the duration of action potentials. This nonuniformity produces changes in the source of the excitation wave by altering the magnitude of the excitatory currents secondary to variations in the takeoff potential as premature impulses propagate into regions that are at different stages of repolarization of the previous action potential [1]. The other more recently observed phenomenon occurs in structures that contain discontinuities of axial resistance, which alter the "sink" of the excitation wave. This nonuniformity produces changes in the electrical load of each cell due to microscopic resistive discontinuities created by the connections between cells (gap junctions) [7–10]. The microscopic variations in electrical load, in turn, have feedback effects that alter the excitatory currents of the sarcolemmal membrane [2,7].

Based on the foregoing, recent evidence indicates that the myocardial architecture becomes proarrhythmic when its electrical properties change from "uniform anisotropy" to those of "nonuniform anisotropy" (Fig. 1) [1,8,10]. This electrical permutation is due to a change in the distribution of gap junctions as a structural response of the myocardium to many diseases, as well as to aging [9]. The process consists of a loss of side-to-side electrical connections between cells in association with an increase in collagen in the contiguous extracellular matrix (microfibrosis) [1,8,9].

1.1. An initial framework limited to sarcolemmal ionic current channels

It has long been held that all conduction disturbances that initiate re-entry are due to spatial inhomogeneities created by regional differences in the sarcolemmal ionic currents. This premise has been used widely in the analysis of cardiac conduction disturbances based on the assumption that the myocardium behaves as a continuous syncytium. That is, the multicellular architecture of cardiac muscle plays a passive or inert role from an electrophysiological standpoint. This assumption has been considered valid because at a macroscopic size scale extending over many cell lengths the results of experimental measurements appear consistent with an electrically continuous medium [1,2]. In such a medium, the safety factor is determined solely by varying the magnitude of the net depolarizing currents of the ionic channels of the sarcolemmal membrane. Regions with the greatest magnitude of depolarizing current produce the largest values of the maximum rate of rise of the action potential (dV/dt_{max}), along with the fastest and safest conduction [7]. Consequently, the propagation of cardiac action potentials has been analyzed experimentally and in numerical models by focusing on the sarcolemmal ionic currents, assuming that the influence of the multicellular structure is the averaged effect of many cells in an electrically continuous syncytium.

Based on this assumption, one of the most fundamental precepts of cardiac electrophysiology is that spatial differences in the effective refractory period are caused only by regional variations in the sarcolemmal ionic currents that produce spatial differences in the depolarization and repolarization phases of transmembrane action potentials [1]. This precept led to the expectation by many scientists that altering the sarcolemmal ionic channel currents in isolated single cells would provide information that could be directly extrapolated to predict the behavior of action potentials propagating in multicellular networks. As a result, the therapy of atrial fibrillation has focused on the use of drugs that directly or indirectly alter the intrinsic properties on the sarcolemmal ionic channels.

It is true that the greatest successes of modern cardiac electrophysiologic research are discoveries about the sarcolemmal ionic current channels. Molecular biology, for example, has been immensely successful in isolating specific molecules that serve as targets for drugs and genetic manipulation. The achievements have been so overwhelming that for many scientists the aim of cardiac electrophysiology research is identified with the "molecular dissection" of the sarcolemmal ionic current channels. This aim is exemplified by a new therapeutic framework put forth by the "Sicilian Gambit" [11], which focuses on molecular targets for drug therapy of cardiac tachyarrhythmias. The Sicilian Gambit provides a framework of antiarrhyth-

mic drugs based on the manner in which each drug alters the activity of specific ionic channels. In turn, knowledge about the effects of altered ionic channel currents is based primarily on the results of drug-induced changes in isolated single cells.

In contrast to the remarkable advances in the science of sarcolemmal ionic channels, the management of atrial fibrillation is far from satisfactory [12]. Additionally, clinical trials have shown that selective drugs which suppress ventricular premature beats create life-threatening events [13] in some patients by inducing proarrhythmic behavior of the impulses propagating through the ventricle. While these trial results create obvious important therapeutic problems for clinicians, they also create a perplexing problem for basic scientists—most available information focuses on electrophysiologic changes that occur when the activity of sarcolemmal ionic current channels is altered, yet clinical evidence now suggests there is some additional factor (at present, a literal *mystery* factor) that is important in reentrant arrhythmias. In fact, recent theoretical [14] and in vitro studies [15] have revealed a multicellular proarrhythmic property derived from the antiarrhythmic effects of drugs on a single cell. The present dilemma focuses on the following question: what new mechanisms should be targeted for better pharmacological therapy? To obtain clues as to how to resolve this dilemma, we may be wise to follow the advice of Hercule Poirot, the famous Belgian detective, who counseled his assistant, Captain Hastings, that to solve a mystery one's attention should not be limited to the smallest possible level: "*Mon ami*,... But it is the romantic idea that all important clues must be infinitesimal!" [16].

1.2. Expanded framework to include the topology of the gap junctions

Accordingly, our investigation will shift from the molecular size scale of ionic channels to propagation of the impulse in multicellular networks at the size scale discernible with the light microscope. Here, we find that instead of multidimensional propagation in cardiac muscle being continuous in nature, at a cellular level it is quite discontinuous in nature [1,2,7]. In a continuous medium dV/dt_{max} does not change when the observation site or

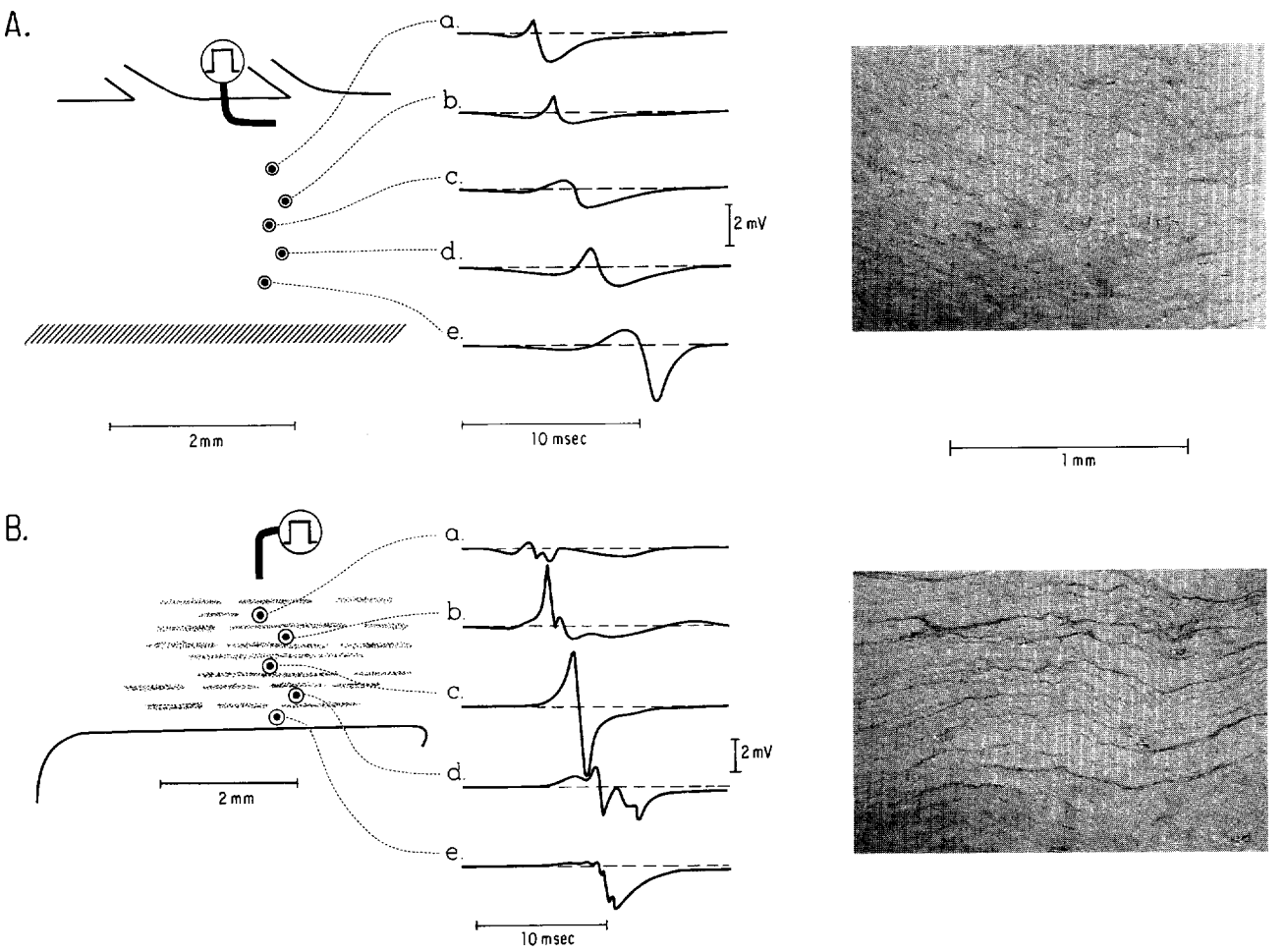

Fig. 1. Relation between extracellular waveforms during transverse propagation and the underlying microscopic architecture of atrial bundles. The two preparations were from the same atrium of a puppy 1 week after birth. Panel A = crista terminalis; panel B = limbus of the fossa ovalis. Smooth waveforms with a single deflection occurred with uniform anisotropy (A) and irregular multiphasic waveforms occurred with nonuniform anisotropy (B). The associated microscopic structure is shown on the right. In the newborn crista with uniform anisotropy, collagenous tissue is distributed among the myocytes in a scattered and irregular manner (panel A). In the limbus with nonuniform anisotropy, collagenous tissue is organized into fine connective tissue septa (microfibrosis), which appear as wavy horizontal lines that separate individual myocytes and groups of myocytes along the long axis of the fibers (panel B). By adulthood many areas of the atria, including the crista, show a change from uniform to nonuniform anisotropic properties with the development of connective tissue septa and multiphasic extracellular waveforms similar to those shown in panel B. Reproduced from Ref. 8 by permission of the American Heart Association, Inc.

the direction of conduction is altered. However, recent action potential measurements in anisotropic cardiac bundles show that the values of dV/dt_{max} vary considerably from site to site for a given direction of conduction, and at the same site dV/dt_{max} has multiple values when conduction approaches a given site from multiple directions [2]. Further, fast upstrokes of propagating action potentials occur with low velocities in a direction across cells, and slower upstrokes occur with high propagation velocities along the long axis of the cells [7]. The significance of these variations in dV/dt_{max} is that they reflect microscopic variations in electrical load due to the anisotropic distribution of the cellular connections [2]. The lower the value of dV/dt_{max}, the greater the load, and vice versa.

It quickly became apparent that there are two classes of anisotropic electrical properties of cardiac muscle—uniform and nonuniform anisotropy (Fig. 1) [8]. Smooth extracellular waveforms seen during transverse propagation, a characteristic of uniform anisotropic properties with tight (low resistance) electrical coupling between cells in all directions, occur in atrial bundles from children [1,9]. A change from uniform to nonuniform anisotropic cellular coupling occurs normally in different atrial muscle bundles at different ages: (1) rapid changes occur with growth and development during the first several months following birth [8], and (2) slow changes occur later in life with aging, or with abnormalities such as hypertrophy [9,10]. The appearance of multiphasic extracellular waveforms during transverse propagation in adult atrial bundles correlates histologically with the appearance of fine longitudinally-oriented collagenous septa that surround individual cells and small groups of cells [9,10]. This microfibrosis (Fig. 1B) provides a histological marker for identifying tissues with the special electrical properties of "fine" nonuniform anisotropy [1].

The effective refractory period of a pathway is determined by the shortest premature interval at which a conducted impulse occurs. Since measurements of the effective refractory period involve propagating impulses, there are two factors that can alter the effective refractory period: (1) spatial differences in action potential duration; and (2) spatial differences in loading due to the myocardial architecture. According to the classical concept, any difference in the refractory periods of two areas is caused by cellular repolarization differences. Fig. 2A, however, shows in human atrial bundles that there is another mechanism

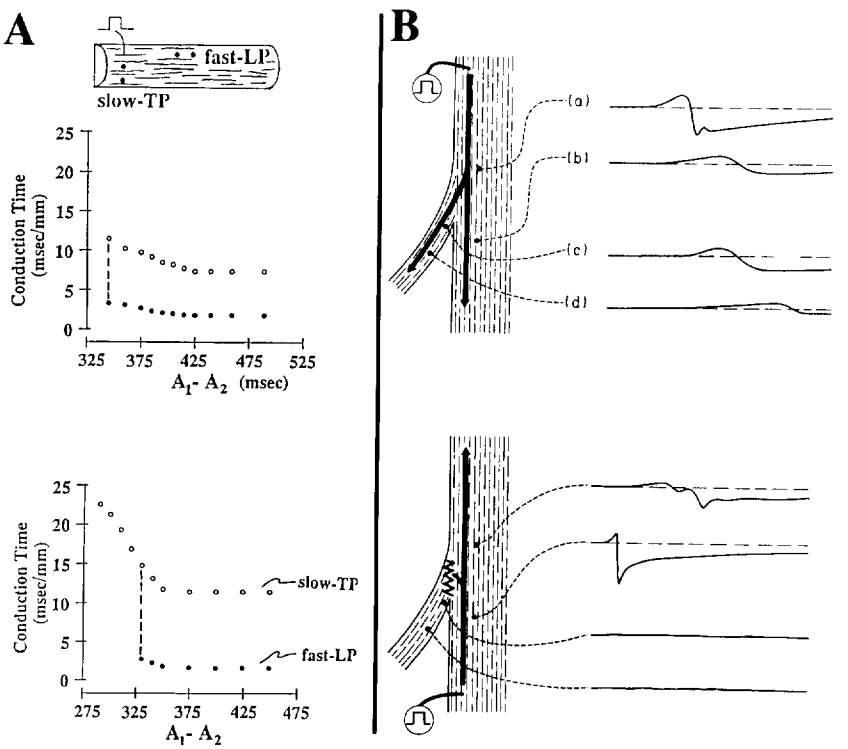

Fig. 2. Nonuniform anisotropy as a mechanism of spatial differences in the effective refractory period (panel A), and direction-dependent conduction block at a branch site (panel B). *Panel A*: Anisotropic conduction time curves were obtained in a uniform anisotropic pectinate muscle of a 12-year-old child (top) and in a nonuniform anisotropic pectinate muscle of a 62-year-old male (bottom). Premature action potentials were introduced at variable intervals (A_1–A_2) after every 10th stimulus at a basic cycle length of 800 ms. Measurements of fast longitudinal propagation (LP) and slow transverse propagation (TP) were obtained as illustrated in the drawing. Conduction times in ms/mm were obtained by measuring the time of conduction of the peak negative derivative of the extracellular waveform between the two electrodes of each pair; the conduction times were then normalized to a distance of 1 mm. In the uniform anisotropic bundle (top) no discontinuity occurred in the conduction curves: i.e., the effective refractory periods of LP and TP were the same. In the nonuniform anisotropic bundle (bottom), a discontinuity occurred due to failure of fast LP at a longer refractory period than that of slow TP; the difference in the effective refractory periods of LP and TP was 45 ms. *Panel B*: The waveforms were recorded at an extracellular potassium concentration of 9.0 mEq/l in a canine preparation in which a small branch was formed by the origin of an atrial pectinate from the larger crista terminalis. The direction of the fiber orientation (confirmed histologically) is indicated by the broken lines. The pattern of propagation is indicated by the solid lines with arrows. Conduction block at the localized site occurred only when the excitation wave approached the branch site from below. When the excitation wave approached the branch site from above, there was no change in the direction of conduction with respect to the underlying fiber orientation at the branch site. However, when propagation approached the branch site from below, the pectinate bundle formed an acute angle with the crista and produced an abrupt change in the direction of propagation with respect to the alignment of the fibers at that localized site. Panel A is reproduced from Ref. 1 by permission of Futura Publishing Co., and panel B is reproduced from Ref. 8 by permission of the American Heart Association, Inc.

for the spatial asymmetry of effective refractory periods. In young atrial bundles with uniform anisotropic properties (Fig. 2A, top), reducing the premature interval (A_1–A_2) to the absolute refractory period resulted in simultaneous conduction failure in all directions. However, in the older nonuniform anisotropic bundles (Fig. 2A, bottom), as the A_1–A_2 interval was decreased, conduction failed in the longitudinal direction while transverse propagation continued with further shortening of the premature interval. The conduction curves shown are representative of those that have been previously reported in detail [1,10]. These differences in effective refractory period (Fig. 2A, bottom) occurred in the absence of spatial differences in the action potential duration, thus confirming that differences in the effective periods are due not only to differences in the membrane ionic properties but to loading due to nonuniform anisotropic properties of the myocardial architecture.

These directional differences in the safety factor led to the prediction and experimental confirmation of anisotropic reentry within a single atrial bundle [7,10]. Subsequently all of the conduction disturbances known to lead to reentry initiated from a single stimulus site were shown to be produced by nonuniform anisotropic structural properties [1,8,10]. Traditionally, areas of slow conduction (< 0.1 m/s) have been considered to be due to "depressed" membrane properties that produce slow upstroke action potentials such as occur with slow conduction in the atrioventricular node. In nonuniform anisotropic human atrial bundles, we found effective conduction velocities as low as 0.03 m/s (lower than values of the normal AV node) in the presence of normal, fast upstroke action potentials [10].

At the larger macroscopic level abrupt changes in the distribution of gap junctions, such as occur at sites where atrial muscle bundles branch or join other bundles, localized slowing of conduction occurs. In atrial bundles with nonuniform anisotropic properties, we found localized unidirectional block at these sites, the block occurring when the wavefront was required to abruptly change direction with respect to the orientation of the underlying cells (Fig. 2B, bottom). Within reentrant circuits in canine atrial preparations with nonuniform anisotropic properties [17] in vitro measurements demonstrated that these sites of localized block and local conduction delay considerably reduce the size of atrial reentrant circuits, along with a reduction of the overall wavelength of reentry [3]. These sites, as well as nonuniform anisotropic conduction within atrial bundles, likely provide an underlying structural mechanism for recent observations that regions of high curvature of a wavefront are associated with slow conduction and block [18].

2. The link between the topology of gap junctions, microfibrosis, and anisotropic conduction

Clinically, the association of atrial fibrillation and a chronic increase in atrial size is a long-known relationship. In animals of different sizes and ages, Moore et al. [19] found it difficult to initiate and maintain atrial fibrillation in the younger small animals, but atrial fibrillation could be readily induced in the older large animals. The electrophysiological explanation for this relationship has been that an atrium of larger size provides a larger area within which a reentrant circuit can develop. These authors concluded that the observed difference is more likely a function of atrial mass than of age.

When rheumatic heart disease was common in Western countries, it was a standard clinical observation that atrial fibrillation was more likely to occur when the left atrium became chronically enlarged. However, Probst et al. [20] found that the duration of mitral stenosis was the major factor, with a stepwise increase in the incidence of atrial fibrillation with age, noting that enlarged atria of similar size early in the disease had a low incidence of fibrillation. At present, rheumatic heart disease has decreased markedly in Western countries, and now it is not a major factor in atrial fibrillation in these countries. However, the emerging major cause of atrial fibrillation is aging, with the highest incidence in subjects over 70 years of age, and these septuagenarians usually have atria of normal size (at least before they develop atrial fibrillation). This clinical development has introduced an additional unresolved problem —how can the multiple atrial reentrant circuits of atrial fibrillation, as documented by Allessie et al. [3] and by Cox and Boineau [4], develop in a normal-sized heart? The occurrence of reentry within small areas has defied explanation based solely on regional differences in sarcolemmal repolarization currents in a continuous isotropic structure, which requires a relatively large area [1]. However, studies of anisotropic reentry in human atrial bundles have now revealed that reentry can be initiated in quite small areas that exhibit normal action potentials [7,10].

What is the mechanism for reducing the spatial requirements for reentry to occur in such small areas? It is now clear that as the side-to-side electrical connections between human atrial fibers become less frequent, the effective conduction velocity across groups of cells (transverse propagation) decreases [1,9,10]. In this relationship, we have noted that an increase in microscopic collagenous septa is correlated with a decrease in side-to-side electrical coupling between fibers, as evidenced by an increase in notching of the extracellular potential waveforms during transverse propagation [9,10]. In atrial pectinate bundles from children, we found the mean effective transverse conduction velocity to be 0.12 m/s [10]. These bundles had smooth extracellular waveforms with little notching, indicating there is extensive side-to-side coupling between fibers (uniform anisotropy). Histologically, the pectinate muscles from children contained collagenous septa that were short and rod-shaped, and the collagenous bundles did not completely surround fiber groups, thus allowing for a plethora of side-to-side sarcolemmal contact sites. However, in larger pectinate muscle bundles from adults older than 50 years, the mean effective transverse velocity was only 0.085 m/s ($P < 0.01$) [10]. During transverse propagation in these older bundles, the extracellular waveforms had multiple notches, indicating sparse side-to-side connections between fibers for at least several cell lengths (nonuniform anisotropy). Histologically, elongated prominent collagenous septa were present, and the septa often completely surrounded cell groups, thereby electrically isolating cells from their transverse neighbors [9,10].

The presence of microscopic collagenous septa therefore mark areas where there cannot be side-to-side cell contact, thus defining areas where there are barriers to the transfer of currents from the interior of one cell to that of its neighbors. From an electrophysiological view, it should be emphasized that in using collagenous septa as markers of microscopic "insulated" side-to-side electrical barriers, the principal feature is the distribution of the gap junctions rather than the connective tissue *per se*. The extracellular matrix associated with microfibrosis is highly conductive due to the gels formed by the strongly hydrophilic glycosaminoglycans, with their high density of negative charges which attract clouds of cations, as well as due to spaces provided by the weave arrangement of the fibrillar collagen [21].

3. Which non-uniformities apply to atrial fibrillation?

In contrast to arrhythmias associated with acute changes in the ventricle following myocardial infarction, the foregoing illustrates that the underlying substrate for atrial fibrillation in most patients does not develop acutely. That is, the evidence suggests that the underlying mechanism develops over considerable time, reflecting adaptive structural changes that result in the appearance of nonuniform anisotropic electrical properties. This feature is consistent with clinical observations that atrial fibrillation occurs most commonly with increasing age in patients with hypertension and coronary artery disease, or with aging in otherwise healthy people [22]. If correct, the temporal requirement for developing an arrhythmic substrate emphasizes the challenge of developing experimental animal models of atrial fibrillation to mimic the human condition. In the absence of direct experimental data to identify underlying mechanisms of atrial fibrillation in patients, the following evidence is presented to "identify" the role of the nonuniform anisotropic distribution of gap junctions.

1. In our in vitro measurements of conduction in human atrial muscle bundles, we found nonuniform anisotropic properties in right atrial bundles from older subjects but not in those from children and young adults. Decreases in the transverse conduction velocity occurred with increases in the size of the bundles in association with increasing age, and very slow conduction (< 0.07 m/s) occurred only in the presence of nonuniform anisotropic bundles from patients over 60 years of age [10]. Additionally, premature impulses produced spatial differences in the effective refractory periods, as well as reentry within small areas, in the atrial bundles from these subjects over 60 years of age. However, directional differences in the effective refractory period and reentry could not be induced in the young bundles that had no microfibrosis [10]. These in vitro experimental results are in keeping with the clinically well-known increase in the incidence of atrial fibrillation with aging. It is interesting to note that Michelucci et al. [23] found in atria of normal subjects that the maximum dispersion of the effective refractory period increased with age. Such increases are usually considered to reflect spatial differences in action potential duration. However, as illustrated in Fig. 2A, there are similar spatial increases due to an age-related increase in nonuniform anisotropy; e.g., the magnitudes of the spatial differences in effective refractory period in Fig. 2A (bottom) are comparable to the values of dispersion found by Michelucci et al. [23] in subjects 62 years of age.

2. Changes in heart rate have different effects on the vulnerable period during which unidirectional block occurs. The different effects depend upon which of the two basic mechanisms produces the spatial variation of the effective refractory period (ERP). When ERP differences are due to spatial differences in action potential duration, increases in rate decrease the vulnerable period because the action potential durations become more similar from region to region (more similar return of excitability) [24]. When ERP differences are due to nonuniform anisotropy, however, increases in rate do not alter the spatial differences in effective refractory period because cellular loading due to the myocardial architecture is not rate-dependent. The differences are illustrated by the following. In intact canine atria Han et al. [24] found the spatial dispersion of the return of excitability to decrease from 56 ms at a heart rate of 100/min to 24 ms at a heart rate of 300/min. However, there was no rate dependence of the spatial difference in effective refractory periods within single nonuniform anisotropic atrial bundles: i.e., the 45 ms directional difference in effective refractory periods of Fig. 2A (bottom) did not change when the basic stimulus rate was increased from 75 to 240/min.

3. In the past, when a large number of patients with rheumatic heart disease were seen at our Medical Center, we encountered many children and young adults who had severe rheumatic heart disease with markedly enlarged atria, yet it was exceedingly unusual to encounter atrial fibrillation. In adults who had documented rheumatic heart disease for many years with enlarged atria of similar or smaller size, however, atrial fibrillation was a common occurrence. This experience is consistent with the results of Probst et al. [20] who found that age is an etiological factor in the production of atrial fibrillation in patients with mitral stenosis. We suggest that the presence of enlarged atria over many years accelerates the underlying alterations in gap junction distribution leading to an arrhythmic substrate derived from nonuniform anisotropy.

4. In the CAST study [13] the proarrhythmic effects of drugs occurred primarily in patients with coronary heart disease who had underlying structural changes in the ventricle that involved fibrosis, which has been shown in humans after ventricular infarction to involve changes in the topology of the ventricular gap junctions [25]. In children, other than marked use dependency due to high heart rates, the drugs used in the CAST study rarely produce major problems, especially life-threatening events. Again, this points to the association of an absence of nonuniform anisotropy in children (not proarrhythmic) and the presence of structural cardiac changes in adults (proarrhythmic).

4. A new therapeutic target for atrial fibrillation

Rather than a fixed pattern of cellular interconnections, the evidence accumulated leads directly to the concept that

the microscopic myocardial architecture is temporally dynamic with a time constant of the order of many months (infants) [26] to many years (older adults) [9,10]. However, electrical loading of cells due to the distribution of gap junctions and the associated feedback effects on the sarcolemmal ionic currents [2] are complicated notions. They involve relationships that are not derivable by direct experimental quantitative measurement, as done in studies of single cell sarcolemmal ionic currents under conditions that require an absence of electrical load on the membrane. Until quite recently, details of the distribution of cardiac gap junctions and of the irregular geometry and arrangement of cardiac myocytes have not been included in the experimental and theoretical analysis of cardiac conduction [2]. In contrast, there have been remarkable advances in the science of gap junctions in heart disease [25,27] and in the regulation of reactive and reparative myocardial fibrosis [21].

Due to the close association between collagenous septa and the distribution of side-to-side electrical connections between fibers, we have recently suggested the following hypothesis: the genetic expression of collagenous septa and of side-to-side electrical connections between cardiac fibers are under similar developmental and regional control [28]. A fundamental question is whether the loss of side-to-side electrical connections between cells is causally related to the development of increased collagen in the contiguous extracellular matrix (microfibrosis), and whether both events are brought about by mechanical forces perceived by cells and the extracellular matrix [29]. These considerations raise major questions about pharmacological interventions that may alter the distribution of the intercellular connections. With regard to fibrosis, it is now clear that interventions which alter the renin–angiotensin–aldosterone system can not only prevent but also reverse myocardial fibrosis: e.g., the use of selective ACE inhibitors [21]. The reader is referred to an excellent review of this subject by Weber et al. [21] for the responses of fibroblast collagen synthesis to hormonally mediated events that accompany raised plasma concentrations of angiotensin II and/or aldosterone, as well as to interventions that prevent or cause fibrous tissue to regress.

Even if therapy is developed to produce regression of atrial microfibrosis, a final question will have to be resolved. Will the cells realign to achieve side-by-side cell contact [30] and, if so, will they reestablish side-to-side electrical coupling between the previously separated cells? In primary liver cultures, Fujita et al. [31] showed that the extracellular matrix glycosaminoglycans and dermatin sulfate proteoglycans induce gap junction synthesis and can regulate tissue-specific gene expression.

5. Conclusions

It is unclear whether there are significant changes in the intrinsic sarcolemmal channel properties over long periods of time prior to the onset of atrial fibrillation. Following the adage of Sherlock Holmes that that which remains must be the "truth", we therefore conclude that the loss of side-to-side electrical coupling between cells (nonuniform anisotropy) plays a central role in the genesis of atrial fibrillation. This conclusion offers an important paradigm to serve as a future guide to discover as-yet-unknown factors that alter atrial muscle to produce the substrate which eventually leads to atrial fibrillation. At present, however, there is little known about how the ionic channel events of individual cells are altered through loading effects as excitation waves encounter varying distributions of the gap junctions [2]. The feedback effects of microscopic myocardial architecture on the sarcolemmal ionic currents through electrical load provide an important new area of integrative investigation to achieve a picture of the mechanisms that interact to produce the conduction disturbances that initiate reentrant arrhythmias. At this point, Nicole is presented a new major challenge—that of exploring ways to alter the distribution of gap junctions in nonuniform anisotropic atrial muscle as a way to reduce the arrhythmogenic potential of the myocardial architecture in the treatment or even prevention of atrial fibrillation in her patients.

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