Frequency-Dependent Breakdown of Wave Propagation Into Fibrillatory Conduction Across the Pectinate Muscle Network in the Isolated Sheep Right Atrium

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Abstract—Atrial fibrillation (AF) may result from stationary reentry in the left atrium (LA), with fibrillatory conduction toward the right atrium (RA). We hypothesize that periodic input to the RA at an exceedingly high frequency results in disorganized wave propagation, compatible with fibrillatory conduction. Simultaneous endocardial and epicardial optical mapping (di-4-ANEPPS) was performed in isolated, coronary-perfused sheep RA. Rhythmic pacing of Bachmann’s bundle allowed well-controlled and realistic conditions for LA-driven RA. Pacing at increasingly higher frequencies (2.0 to 6.0 Hz) led to increasing delays in activation distal to major branching sites of the crista terminalis and pectinate bundles, culminating in spatially distributed intermittent blockade at or above 6.5 Hz. At this “breakdown frequency,” the direction of RA propagation became completely variable from beat to beat and thus transformed into fibrillatory conduction. Such frequency-dependent changes were independent of action potential duration. Rather, the spatial boundaries between proximal and distal frequencies correlated well with branch sites of the pectinate musculature. Thus, there exists a breakdown frequency in the sheep RA below which activity is periodic throughout the atrium and above which it is fibrillation-like. The data are consistent with the ideas that during AF, high-frequency activation initiated in the LA undergoes fibrillatory conduction toward the RA, and that sink-to-source effect at branch points of the crista terminalis and pectinate muscles is important in determining the complexity of the arrhythmia. (Circ Res. 2002;90:1173-1180.)

Key Words: fibrillatory conduction ■ atrial flutter ■ atrial fibrillation ■ Fourier analysis

During atrial fibrillation (AF), excitation frequency in the left atrium (LA) may be higher than in the right atrium (RA).1–5 and LA activation patterns may be spatially and temporally periodic.6–8 Moreover, a consistent LA-to-RA dominant frequency (DF) gradient correlates with impulse propagation from LA to RA9,10 across interatrial pathways, such as Bachmann’s bundle (BB) and the inferoposterior pathway along the anterior wall of the coronary sinus.11 Thus, at least in some cases, AF results from activation by relatively stationary high-frequency sources of impulses, which undergo complex spatially distributed intermittent block patterns, imposed by the presence of functional and/or anatomical obstacles in their path.

We sought to study rigorously and in detail the response of the RA to incoming high-frequency excitation from the LA. Specifically, we used an isolated, coronary-perfused RA preparation to quantify the frequency-dependent nature of the propagation of wavefronts entering the RA from BB9 and the basis of fibrillatory conduction. We hypothesize that the bundle-like structure of the normal RA, with its complicated network of pectinate muscles, is a substrate for frequency-dependent conduction delay and block. Our preparation provides realistic and well-controlled conditions for testing such a conjecture. By pacing BB at varying frequencies, we demonstrate that 1:1 propagation across the crista terminalis and the grid of pectinate muscles yields to intermittency. When the input frequency is critically high, the response patterns are so complex as to result in fibrillatory conduction. These results enhance the understanding of the response of the RA to input frequency typical of that of the LA during AF (>7 Hz).

Materials and Methods

Isolated RA Preparation

Experimental protocols conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Young sheep (n=6; purchased from Cornell University, Ithaca, NY) were heparinized (500 IU, IV) and subsequently anesthetized with sodium pentobarbital (35 mg/kg IV). Hearts were excised and the right coronary artery cannulated.12 Nonperfused areas were removed, and the isolated preparation (including the RA, right ventricle, and BB) was placed on a frame within a transparent chamber. Preparations were then continuously perfused with a standard warm (36 to 38°C)
oxygenated (95% O₂, 5% CO₂) Tyrode’s solution (composition in mmol/L: NaCl 130; KCl 4.0; CaCl₂ 1.8; MgCl₂ 1.0; NaHCO₃ 24.0; NaH₂PO₄ 1.2; glucose 5.5; and albumin 0.04 g/L; pH 7.4) at 80 mm Hg and superfused with the same solution at 40 mL/min. The isolated RA preparation is illustrated in Figure 1. The left panel shows the epicardial view, with the appendage (RAA) at the bottom, and the superior vena cava (SVC) on top. Point J indicates the junction between BB and the crista terminalis (CT). The endocardium (right) is characterized by an intricate network of pectinate muscles (PMs, outlined for clarity) that branch out of the CT and tricuspid valve (TV) rim toward the RAA and across the cavity.

Unless otherwise stated, diacetyl-monoxime (DAM; 15 mmol/L) was perfused to abolish contraction. A bolus injection of 5 to 10 mL of the potentiometric dye di-4-ANEPPS (15 μmol/L) dissolved in DMSO enabled imaging of membrane potential. A pacing electrode was placed on the epicardium of BB to simulate LA-to-RA propagation (see Figure 1, left panel). Brief pulses, 5-ms duration and twice diastolic threshold, were delivered at constant rates, varying from 2 Hz up to the fastest rate that allowed 1:1 capture of the tissue in the immediate vicinity of the electrode. The white arrows in Figure 1 indicate the general direction of propagation during pacing of BB at a frequency of about 2 Hz. This setup enabled us to precisely control both the site and the rate of the input into the RA and to mimic conditions of AF maintained by a stationary high-frequency rotor in the LA, with fibrillatory conduction toward the RA.6,13 Fibrillatory conduction was defined as fibrillation-like activity that terminated immediately after pacing. Episodes showing self-sustained activity were excluded from analysis.

High-Resolution Optical Mapping

Excitation light (520 nm) was shone on the preparation and 2 synchronized CCD cameras were used to map fluorescence at 640 nm from the same area (∼3×3 cm²) on both epicardium and endocardium.12,13 Frames with 64×64 pixels were acquired at a rate of 600 frames/sec for 2 to 5 sec. Background subtraction and spatial filtering resulted in an effective resolution of 0.4 to 0.8 mm, depending on magnification. A true image of the preparation under the same condition provided the precise anatomical structure.

Data Analysis

Isochrone maps: Using a threshold technique, the time of activation for each pixel was determined without interpolation and color-coded.14 Dominant frequency maps: Using fast Fourier transform (FFT) analysis at each color-coded pixel,7 the frequency with highest power (resolution, 0.2 to 0.5 Hz) was considered the DF. Action potential duration (APD) maps: APD maps were created at 75% repolarization (APD₇₅) as described previously.13 Directionality analysis: Activation times for each pixel were measured at 50% of the action potential amplitude with a parabolic best-fit of the mid upstroke. Direction of propagation was obtained from a vector bilinear best fit for a sliding 5×5-pixel window (∼2.5×2.5 mm²) of activation times. Velocities below 0.06 m/sec were discarded from this analysis. Histograms of all the activation directions yielded the predominant direction (PD) of propagation. The recurrence of the predominant direction (RPD) in a pixel was measured from such histograms as the ratio of the number of activations in the PD to the total number of activations.

Statistics

Multiple comparisons within and across hearts were carried out using analysis of variance (ANOVA). A value of P<0.05 was considered statistically significant. For cross-correlation, we used simple linear regression r².

Results

Branching and Frequency-Dependent Propagation Delay

The endocardial isochrone maps of individual beats shown in Figure 2 were taken from a representative experiment during stimulation of BB at varying frequencies (J indicates the first...
endocardial site to be activated). Each color map is superimposed on a sketch of the endocardial anatomical structure. At 2.0 and 5.0 Hz, the total activation time in the optical field was less than 30 ms. Impulses propagated at a relatively high and uniform velocity (red to orange) downstream from J along the CT and into the PM branches. At higher pacing rates, the pattern became increasingly nonuniform. At 6.3 Hz, the impulse was delayed at the junction between CT and the first PM of the grid (red to green); in addition, a secondary wavefront seemed to travel from the J region directly into the bundle grid. Although 1:1 propagation was preserved, total activation time increased to \( \approx 50 \text{ ms} \). At 6.7 Hz, activation time across the sharp line of conduction slowing became exceedingly long (red to green and red to blue and purple), and there was an abrupt change in the propagation pattern. In this case, the primary activation front traveled very slowly from the CT through the PM in the center of the field and reached the TV at about 90 ms. Meanwhile, at \( \approx 70 \text{ ms} \), an impulse emerged as a breakthrough (asterisk) and activated adjacent tissues upward and to the right, stopping abruptly near the CT (a sharp transition from blue to red). Total conduction time was \( \approx 120 \text{ ms} \). Similar results were obtained in all experiments, although the specific anatomical structure and sites of conduction delay varied from one preparation to another.

To understand the mechanisms of the complex pattern of propagation illustrated in Figure 2, we carried out a detailed analysis of the activation delays at equidistant pixel locations along the CT and PM at pacing rates between 2.0 to 6.7 Hz. Figure 3A shows a picture of the preparation to indicate the pixel locations; in Figures 3B and 3C, the top graphs show the conduction delay at each location. The superimposed optical action potentials in Figures 3B and 3C were taken, respectively, from pixels c1 to c5 on the CT and p1 to p6 on the PM in the center of the recording field. At frequencies between 2.0 and 5.9 Hz, conduction was relatively uniform across all pixels. However, at higher frequencies, activation time increased abruptly at specific locations of augmented structural complexity. For example, pixels c3 to c5 were located at the region at which the CT underwent extensive branching into the PM region (see Figure 3A). Thus, at c4, the conduction delay increased abruptly from 5 ms at 6.3 Hz to 18 ms at 6.7 Hz. Similarly, at c5, conduction delay jumped from 11 ms at 6.3 Hz to 24 ms at 6.7 Hz.

At the higher frequencies, the delays along the PM were much longer and exceedingly nonuniform. As shown in Figure 3C, at 2 Hz the delays between p1 and p6 were relatively short (total, \( \approx 10 \text{ ms} \)) and increased linearly across all pixels. Similarly at 5.0 and 5.9 Hz, the delay between p1 and p6 increased gradually to 18 and 21 ms, respectively. However, at the pacing frequency of 6.3 Hz, the action potential upstrokes in p4 and p5 became humped and there were disproportionately long step delays in p6 (red arrows). This pattern reflected the development of decremental conduction at p4 and p5, with p6 activation through a different pathway. This is more evident at 6.7 Hz (see also Figure 2) in that conduction block occurs in the p5 to p6 direction, with p6 being activated much later from a different source. On close scrutiny of the anatomical structure of the preparation (see Figure 3A), it is clear that pixels p3 and p4 reside in an area with many small branches. Also, between p5 and p6, there is a connection to a thick PM emerging from above the TV rim. Thus, this complex structure may be a substrate for sink-to-source mismatch and low safety for propagation. Impor-

antly, only major branch sites (with discontinuities in the scale \( >1 \text{ mm} \)) exhibited marked frequency-dependent increase in activation time (see Discussion).

Breakdown of the DF Response

During experimentially induced AF in the sheep heart, stationary reentrant sources in the LA usually generate impulses at frequencies \( >7 \text{ Hz} \). Yet the RA is never activated at such high frequencies during the arrhythmia, which suggests that there must be a critical frequency at which the 1:1 input/output relation between the LA and RA breaks down. As shown by the DF maps presented in Figure 4A (see Materials and Methods for technical details), rhythmic stimulation of BB at 5.0 Hz resulted in 1:1 activation of the entire right atrium, and thus, the output frequency was also 5.0 Hz. However, at 7.7 Hz, activation was no longer 1:1. Instead, a heterogeneous distribution of DF domains was established by guest on July 10, 2017 http://circres.ahajournals.org/ Downloaded from
both in the endocardium and the epicardium, with frequencies ranging between 3.5 and 7.7 Hz. Composite data from all 5 experiments are presented in Figure 4B. The DFs measured on the endocardium, are plotted as a function of the pacing frequency. Clearly, below 6.7 Hz, the response DF showed no dispersion in any of the experiments, which meant that activation in all regions was 1:1. Above the breakdown frequency of 6.7 Hz, there was a large DF dispersion manifested as multiple domains whose individual frequencies were either equal or lower than the pacing frequency.

To validate the optical mapping data and ensure that the breakdown frequency was not an artifact induced by the presence of the potentiometric dye and/or the electromechanical uncoupler (DAM), we performed 5 additional control experiments in the isolated Langendorff-perfused heart, in which the integrity of both atria was preserved. We paced BB at varying cycle lengths and used 7 to 12 bipolar electrodes to record the response in various epicardial sites of the RA, in the absence of dye or DAM. Figure 4C shows sample recordings from 5 sites (see Figure 1) in one such control experiment. The left traces with their power spectra show that rhythmic stimulation of BB at 6.0 Hz (cycle length 167 ms) resulted in a 1:1 activation pattern at all sites (although a DF of 12.0 Hz is seen in the distant CT site, its basic frequency is 6.0 Hz). However, a completely different picture emerged when the pacing rate was increased to 8.0 Hz (cycle length 125 ms). While the DF at the right atrial end of BB was 8.1 Hz, the more distant RA regions responded with a typical fibrillatory-like activity with DFs of 6.3, 5.6, and 4.1 Hz. In Figure 4D, we summarize the degree of intermittent blockade in the 5 whole-heart experiments. We counted the number of electrodes in each experiment that had DF equal to or smaller than the pacing rate. Clearly, the incidence of intermittent blockade was zero for pacing rates \( \leq 6 \) Hz. At 6.5 Hz blockade appeared at about 8% of recording sites and this number increased sharply \((P<10^{-6})\) up to about 70% at 8 Hz, above which the incidence of intermittence remained stable.

Overall, these data demonstrate that, regardless of the experimental conditions, stimulation of BB at frequencies below about 6.5 Hz results in 1:1 activation of the entire preparation. Above that breakdown frequency the response patterns and spatial distribution of DFs become highly complex and indistinguishable from those demonstrated during AF.

**Intermittent Block and DF Distribution**

From the foregoing, it is clear that pacing BB at rates below or equal to \( \approx 6.5 \) to 6.7 Hz resulted in frequency-dependent delays at specific branch sites. Such delays converted to intermittent block at higher input rates. Figure 5 shows an example of intermittent blockade at 7.7 Hz in the same preparation as in Figures 2 and 3. Figure 5A shows a picture of the preparation. In Figures 5B and 5C, time-space plots were used to highlight, respectively, the activation sequence along the CT (broken red line a-b in Figure 5A) and at the PM in the center of the field (broken red line c–d in Figure 5A). White bands in each plot show the local activation times at pixels located between a and b and between c and d for each consecutive impulse at 2 different BB pacing frequencies. At 5.0 Hz, the activity along both bundles proceeded uninterrupted in a 1:1 manner, and therefore, the DF of the response at all sites was 5.0 Hz. However, at 7.7 Hz, propagation was not uniform and intermittent block patterns occurred at fixed locations (solid red lines). This resulted in distinct excitation DFs depending on the position with respect to a branch site; the areas closer to the J point on the CT (from which the primary wave emerges in Figure 3), showed a DF similar to the pacing rate (7.7 Hz), whereas the distant regions showed a lower DF of 5.5 Hz. In each case, the location of block was near a major branch (solid lines intersecting the dashed lines in Figure 5A), suggesting that branching imposed a sink-to-source mismatch, which reduced the ability of the impulse to activate distal tissue at the higher frequencies.

**Figure 4.** Breakdown frequency. A and B, Endocardial and epicardial DF maps of same isolated RA preparation as in Figures 2 and 3 paced at 5.0 and 7.7 Hz. Note appearance of heterogeneous DF domains at 7.7 Hz. B, Response DFs vs the pacing rate \((n=5)\). Each symbol represents 1 experiment. Pacing BB at rates below \( \approx 6.7 \) Hz, results in 1:1 activation. At higher rates, the number of domains increases but the DFs decrease. C and D, Langendorff-perfused hearts. C, 5 representative bipolar electrograms from various RA sites with their respective FFTs during pacing at 6.0 Hz (left) and 8.0 Hz (right). Recycling sites and labels are defined in Figure 1. D, Incidence of intermittent blockade as a function of pacing rate. Vertical axis denotes the average number of electrograms \((\text{mean} \pm \text{SE}, n=5 \text{ hearts, except at } 9 \text{ Hz, where } n=2)\) having a DF value that was lower than the pacing rate.
Role of Action Potential Duration Dispersion

Although the results of Figure 5 suggest that sink-to-source mismatch at or near branch sites underlies the DF dispersion in space, heterogeneity in the intrinsic refractoriness along the propagation trajectory may also play a role. Therefore, we determined the spatial distribution of APD during slow pacing and compared it with the distribution of the DFs at fast pacing. Figure 6 shows APD (panel A) and DF (panel B) maps from one sample experiment. Note that the areas of long APD are actually those with the highest DFs, contrary to what one might expect if dispersion of refractoriness were to determine the DF. In fact all five hearts showed no significant correlation between APD and DF distributions (\(R^2/0.05\), 0.018\(\leq/0.021, n=5\)), demonstrating that APD dispersion at 3.3 Hz is a poor predictor of DF, and suggesting that dispersion of refractoriness does not underlie DF heterogeneity in AF.

Activation Frequency and Direction of Propagation

Pacing BB at exceedingly high rates results in unidirectional block at branch sites along major RA bundles (see Figure 5). Thus, it is reasonable to expect blocks to occur asynchronously at multiple sites, which should result in highly nonuniform activation. We sought to quantify such nonuniformity by measuring beat-to-beat changes in direction of propagation and correlating them to the changes in the organization of DFs (see Figure 4). Figure 7 shows an example of directionality analysis. The top panels present isochrone maps for 15 consecutive beats taken from a conglomerate of 25 pixels (see asterisk in Figure 8) in the PM region, with the black arrow indicating the direction of propagation during each beat. At 3.3 Hz (Figure 7A), the wave front moved in the same direction every beat. As shown by the histogram in the bottom left panel, in this group of pixels, the predominant direction (PD) was 285\(^\circ\) (arbitrary reference) and its recurrence (RPD) was 69%. At 10 Hz (Figure 7B), directionality changed on a beat-to-beat basis, the PD shifted to 120\(^\circ\) and its RPD dropped to 14%. In Figure 8, we summarize the directionality analysis. Figure 8A shows RPD response maps for 3 different pacing rates (see Materials and Methods). As the rate increased from 3.3, to 7.1, and to 10 Hz, the mean RPD of the selected preparation dropped from 70\(\pm/17\), to 64\(\pm/21\), and to 39\(\pm/12\), respectively. Figure 8B presents the frequency response of the mean RPD values in the endocardial and epicardial maps of 5 preparations. On both surfaces, the RPD is sharply reduced at pacing frequency above 6.7 Hz (\(P/0.01\)). The similarity of that frequency to the breakdown frequency in Figure 4 demonstrates that the increase in the DF dispersion is related to the increase in the complexity of propagation, as indicated by the reduction in the mean RPD.

Discussion

Major Results

The most important new result of this study is the demonstration of a breakdown frequency (6.5 to 6.7 Hz), at
which input from BB transforms the RA response from one characterized by a single DF throughout the mapped area (1:1 propagation), to another in which multiple DFs distribute in well-demarcated domains (fibrillatory conduction). The mechanism of breakdown is intermittent block at or near branching sites of the CT and major PM. The complex DF distribution could not be attributed to spatial differences in intrinsic APD. On the other hand, we found that intermittent block results in a significant loss of consistency in the beat-to-beat direction of wave front propagation, which provides a direct explanation for the difficulty in finding an origin of the activation during fibrillatory conduction.

Role of the RA Structure

The effect of the sink-to-source mismatch in branching sites of bundles have led several investigators to focus on the role of the subendocardial anatomical structure of the atria in AF. Spach et al. showed that structural discontinuities of a scale >1 mm in the muscle structure play an important role in the establishment of unidirectional block and the initiation of reentry. Schuessler et al. demonstrated increasing discordant activation of the epicardium and endocardium with increased excitation frequency, up to ~5.9 Hz, particularly in those regions in which the 3-D anatomy of the atrium was most heterogeneous. Gray et al. showed that the CT and the PMs were sites of preferential propagation whose frequency dependence (cycle lengths above 150 ms) enabled disparity between endocardial and epicardial activation with local block at branch. Subsequently, Wu et al. used isolated canine RA in the presence of acetylcholine to conclude that the PM forms a substrate for conduction block, allowing stationary reentry with increased organization of the overall atrial activity. Altogether, the above studies support the importance of the anatomic structure to the frequency dependence of impulse conduction and the idea of a common mechanism for atrial flutter and fibrillation. Our results expand significantly on such studies and demonstrate in addition that it is possible to obtain fibrillation-like patterns of activity even in response to periodic input, provided that the rate of that input is greater than ~6.5 to 6.7 Hz. Analysis of the most prominent activation delays and intermittent block demonstrated that they occurred at sites of extensive branching. However, the resolution of our recording system did not allow the detailed determination of the roles played by other factors, including fiber organization within the bundles, distributions of cell-to-cell coupling, and geometry of the branching tissue. As such, the precise location of block with regard to branching requires further investigation.

Role of APD and Dispersion of Refractoriness

Spatial dispersion in APD and refractoriness, measured at relatively slow stimulation rates, are usually invoked to
explain complex wave propagation during AF.\textsuperscript{28} Wang et al\textsuperscript{29} found that at a cycle length of 250 ms in dogs susceptible to sustained AF, the dispersion of refractoriness in the RA epicardium was 19±3 ms, with the longest refractoriness being ≈120 ms at the FW. Satoh and Zipes\textsuperscript{30} reported that refractoriness was shortest at the lower portion of the CT relative to the SVC. However, none of the above seemingly conflicting studies measured refractoriness in the PM region and their data are therefore difficult to compare with our results. In the present study, we constructed high-resolution APD maps at a cycle length of 300 ms (3.3 Hz; Figure 6) to assess indirectly the degree of spatial dispersion of refractoriness.\textsuperscript{18} In agreement with the results of Feng et al.,\textsuperscript{31} our data also indicate that the CT has the longest APD during pacing at slow rate (3.3 Hz). Spach et al\textsuperscript{12} also reported that, at 1.7 Hz, APD in the CT is longer than in the PM. In single cells, Yamashita et al\textsuperscript{13} showed that APD in the rabbit CT was longer than in the PM at 1 Hz. In our experiments, however, the CT consistently shows the largest DF when BB is paced at a rate comparable to the frequency of the LA during AF (>7 Hz). Thus, the distribution of APD seems different from the distribution of DF domains, which leads us to suggest that dispersion of refractoriness at normal frequencies is a poor predictor of the spatial distribution of intermittent block patterns that characterize AF.\textsuperscript{9}

### Atrial Flutter and Fibrillation

The differentiation between atrial flutter and fibrillation in patients is usually based on the periodicity of the ECG signals, which is typically reduced with increasing rate. Although the upper limit of human atrial flutter rate varies considerably among investigators, its lower value relative to fibrillation is well documented.\textsuperscript{34–36} The periodic pattern of 1:1 propagation seen here at frequencies just below 6.7 Hz bears electrophysiological similarity to atrial flutter. Thus, the results provide mechanistic support for differentiation between flutter- and fibrillation-like activities based on spectral analysis of frequencies and demonstrate, for the first time that, in the sheep RA, there exist a “breakdown frequency” of about 6.5 to 6.7 Hz below which the activity is periodic and above which it is fibrillation-like. Finally, it is important to note that the relevance of our results to the behavior of regions other than the sheep RA (eg, the LA), or to other species, including man, or even to diseased hearts,\textsuperscript{37} remains to be determined. In the case of the LA, there is substantial evidence that, during AF, activation frequencies are much higher than in the RA.\textsuperscript{6,7,9,36} Therefore, one might expect that the breakdown frequency in the LA should be higher than that demonstrated for the RA.

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