Left-to-Right Gradient of Atrial Frequencies During Acute Atrial Fibrillation in the Isolated Sheep Heart

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Background—Recent studies demonstrated spatiotemporal organization in atrial fibrillation (AF). We hypothesized that waves emanating from sources in the left atrium (LA) undergo fragmentation, resulting in left-to-right frequency gradient. Our objective was to characterize impulse propagation across Bachmann’s bundle (BB) and the inferoposterior pathway (IPP) during AF.

Methods and Results—In 13 Langendorff-perfused sheep hearts, AF was induced in the presence of acetylcholine (ACh). Fast Fourier transform of optical and bipolar electrode recordings was performed. Frequency-dependent changes in the left-to-right dominant frequency (DF) gradient were studied by perfusing D600 (2 μmol/L) and by increasing ACh concentration from 0.2 to 0.5 μmol/L. BB and IPP were subsequently ablated. At baseline, a left-to-right decrease in DFs occurred along BB and IPP, resulting in an LA–right atrium (RA) frequency gradient of 5.7±1.4 Hz. Left-to-right impulse propagation was present in 81±5% and 80±10% of cases along BB and IPP, respectively. D600 decreased the highest LA frequency from 19.7±4.4 to 16.2±3.9 Hz (P<0.01) and raised RA DF from 8.6±2.0 to 10.7±1.8 Hz (P<0.05). An increase in ACh concentration increased the LA-RA frequency gradient from 4.9±1.8 to 8.9±1.8 Hz (P<0.05). Ablation of BB and IPP decreased RA DF from 10.9±1.2 to 9.0±1.5 Hz (P<0.01) without affecting LA DF (16.8±1.5 versus 16.9±1.8 Hz, P=NS).

Conclusions—Left-to-right impulse propagation and frequency-dependent changes in the LA-RA frequency gradient during AF strongly support the hypothesis that this arrhythmia is the result of high-frequency periodic sources in the LA, with fibrillatory conduction away from such sources. (Circulation. 2001;103:2631-2636.)

Key Words: arrhythmia ■ fibrillation ■ Fourier analysis ■ mapping

It is generally accepted that atrial fibrillation (AF) is a random arrhythmia.1–3 However, recent studies have shown various degrees of organization in AF.4,5 In addition, studies from our laboratory demonstrated spatiotemporal organization during AF, mainly in the left atrium (LA).6–8 Also, animal and human studies have shown that activity during AF is more rapid in the LA than in the right atrium (RA).9–12 These studies suggest that high-frequency sources in the LA act as triggers and/or drivers for some types of AF.

One would expect that impulses emanating from a high-frequency source should be subject to spatially distributed intermittent blockade imposed by the presence of functional and anatomic obstacles in their path. We therefore hypothesized that impulses emanating from sources in the LA produce local activation at progressively lower frequencies as they propagate away from these sources, resulting in a frequency gradient between the LA and RA. In such a case, interatrial pathways such as Bachmann’s bundle (BB) and the inferoposterior pathway (IPP), which runs along the coronary sinus,13,14 would be expected to act as preferential routes for left-to-right propagation of fibrillatory impulses. Here, we provide further evidence that LA sources drive AF in the Langendorff-perfused sheep heart and demonstrate for the first time that fibrillatory conduction away from such sources results in a left-to-right frequency gradient.

Methods

Langendorff-Perfused Sheep Heart Preparation

Sheep (weight range 18 to 25 kg) were purchased from an authorized breeder and treated according to NIH guidelines. Animals were anesthetized with sodium pentobarbital (35 mg/kg). The heart was removed, connected to a Langendorff apparatus, and perfused at 200 mL/min with warm (36°C to 38°C), buffered HEPES-Tyrode’s solution (composition in mmol/L: HEPES 15; NaCl 148; KCl 5.4; CaCl2 1.8; MgCl2 1.0; NaHCO3 5.8; NaH2PO4 0.4; glucose 5.5; and albumin 0.04 g/L), and bubbled with 95% O2-5% CO2.6,8

High-Resolution Optical Mapping

High-resolution optical mapping has been described elsewhere in detail.6 Briefly, we recorded potentiometric dye fluorescence simultaneously from 40 000 sites on the RA free wall (3.5×3.5 cm²) and 40 000 sites on the LA appendage (3×4 cm) using 2 synchronized cameras (Cohu 6500) at a sampling interval of 8.33 ms. Background fluorescence was subtracted from each frame. Low-pass spatial filtering (weighted average of 15 neighboring pixels) was applied to improve the signals, resulting in a spatial resolution of <0.5 mm.

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Isochrone Maps and Pseudoelectrocardiograms
Isochrone maps were generated from optical recordings by analysis of each pixel value over time.8,9,15 Pseudoelectrocardiograms (pseudo-EGs) were constructed from optical recordings by integration of the transmembrane fluorescence signal over the entire mapped region.5,8

Electrode-Based Mapping
Epicardial and endocardial electrogroms were obtained with bipolar electrodes located at the following sites: BB (6 electrodes), IPP (4 electrodes), RA free wall (2 electrodes), pulmonary vein region (1 electrode), base of the LA appendage (1 electrode), and left ventricle (1 electrode). A decapolar catheter with 13 mm between each bipolar pair and 2.5-mm interelectrode distance was inserted up to 3.9 cm in the coronary sinus to record from the IPP. The left and right sides of BB were mapped with 6 thin silver bipolar electrodes, separated by 10 mm, with 1.5-mm interelectrode distance. Similar electrodes were used for all other sites. Electrogroms filtered between 0.3 and 500 Hz were recorded with a 16-channel amplification system (model MMP100WSW; Biopac). A biatrial electrogram (EG) was recorded as the difference between 2 epicardial leads, one on the RA and the other on the LA.

Signal Analysis
Spectral analysis was performed by fast Fourier transform (FFT) on optical and bipolar electrode recordings. The content in the 0.4- to 60-Hz band was analyzed. In each FFT, total power varied according to the intensity of fluorescence signal. As such, the magnitude of various peaks was not compared from one FFT to another. Rather, the relative amplitudes of peaks in each FFT were compared, and the frequency of the peak with the largest amplitude was assigned to be the dominant frequency (DF). Optical recordings were acquired at 120 Hz (8.33 ms) for 400 frames (3.2 seconds). Bipolar EGs were acquired at 1000 Hz for 10 seconds and filtered (band pass 0.5 to 500 Hz), providing a spectral resolution of 0.1 Hz over the range of 0.4 to 60 Hz.

DF Maps
Movies of AF yielded pixel-by-pixel time series. For each pixel, the power-spectrum density was estimated and the DF determined. These DFs were presented as a map that described their spatial distribution.7,16 The estimation of the power spectrum was obtained by the method of Welch.16,17 Briefly, the time series were subdivided into 2 partially overlapping segments. The power spectra of each 2.13-second-long segment were obtained via FFT, and these were averaged and normalized to their total power. The outcome of this procedure was removal of the inconsistent variability, enhanced distribution.7,16 The estimation of the power spectrum was obtained from 13 experiments.

Procedures and Protocols
AF was induced by burst pacing (10 Hz) in the presence of 0.1 to 0.6 μmol/L acetylsalicylic acid (ACh). Subsequently, the heart was stained with fluorescent dye (5-mL bolus injection of 20 μmol/L Di-4-ANEPPS).

Recordings and Frequency Analysis
We obtained 10-second tracings from the bipolar EGs. Simultaneously, we acquired 4-second movies of the RA and LA. Later, pseudo-EGs and DF maps were constructed from optical recordings.

Frequency-Dependent Changes in LA-RA Gradient
Slowing of AF Frequency
Metoxxyverapamil (D600) has been shown to decrease the frequency of ventricular fibrillation.18 We used it here to decrease AF frequency. In 4 hearts, AF was induced in the absence of D600, and baseline recordings were obtained, after which D600 (2 μmol/L) was added to the perfusate; after a period of 10 minutes, recordings were obtained again.

Increase of AF Frequency
We determined the effect of an increase in source frequency on the LA-to-RA frequency gradient in 4 hearts by increasing the ACh concentration from 0.2 to 0.5 μmol/L and obtaining recordings at each stage.

Attenuation of Interatrial Communication
In 5 hearts, BB and the IPP were transected during AF by electrocautery. Ablation was performed on the left side of the septum from the epicardial surface. AF recordings were obtained at baseline and after ablation. AF was terminated by ACh washout, after which the LA and RA were paced at a cycle length of 400 ms and recordings obtained from the opposite atrium.

Statistical Analysis
Frequency values are shown as mean±SD. Statistical comparisons were performed with Student’s t test, paired or unpaired when appropriate, and MANOVA with Tukey’s honest significant difference test. P<0.05 was considered statistically significant.

Results
As in our previous work,6,8 AF was defined on the basis of an irregular biatrial EG and optical recordings showing complex activation patterns. Altogether, 48 episodes of AF were analyzed from 13 experiments.

Frequency Distribution and Gradient in AF
In Figure 1, we show the distribution of EGs with their corresponding FFTs in the LA and RA and the left and right sides of BB from an episode of AF. The optical EG from the LA (Figure 1A) shows rapid activity, with a DF of 18.8 Hz. In Figure 1B, the recording on the left end of BB shows a DF of 18.7 Hz. Moving further to the right, the DF at the right end of BB (Figure 1C) is 14.5 Hz, and finally, the RA (Figure 1D) shows a DF of 9.8 Hz. Figure 1E shows DF maps of the LA and RA in which the colored areas represent DF domains, together with their corresponding values (in Hz). It is clear that the LA is being activated at a higher frequency (18.8 Hz), with a left-to-right decrement in DFs. These data confirm and expand previous results from this laboratory demonstrating higher AF frequencies in the LA than in the RA.6,8

In Figure 2, we present data from 20 episodes of AF for which we plotted the mean DFs at different locations along BB and IPP. At each location, the DF from each episode was normalized to the LA DF in that same episode. The highest frequency ratios were always seen in the LA and decreased as one proceeded to the right, with the lowest ratio on the RA free wall. The mean gradient, calculated as the difference between the mean LA and RA DFs, was 5.7±1.4 Hz.

Spatially Distributed Intermittence
In Figure 2, a sharp decrease in the DFs was observed between the right end of the interatrial pathways and the RA free wall. As shown in Figure 3, optical data from the RA in Figure 1 provided us with clues regarding a possible mechanism. In Figure 3A, pixel 1 was located at the terminal end of the right side of BB, and pixels 2 and 3 were from the RA free wall. Figure 3B shows the optical signals from these sites along with their respective FFTs. The DFs were 14.1, 9.8, and 7.5 Hz at pixels 1, 2, and 3, respectively. Importantly, pixel 2 showed a smaller but significant peak at 14.1 Hz, revealing that this site was being activated at 14.1 Hz for a substantial
amount of time, which suggested that the activity at site 1 was linked to the activity at site 2.\textsuperscript{16} Similarly, activity at site 3 was linked to activity at site 2 by the presence of a significant peak at 9.8 Hz. Thus, the respective power spectra showed 2 major components, one corresponding to the frequency of the input signal and the other corresponding to the frequency of the output.

Moreover, the frequency ratios of 14.1:9.8 and 9.8:7.5 correspond approximately to 3:2 and 4:3 left-to-right activation ratios, which suggests the occurrence of intermittent block distributed in space.\textsuperscript{16} Similar results were obtained in 85% of episodes. Possibly, the complex atrial architecture of the network of pectinate musculature may have been the substrate for fibrillatory propagation on the RA free wall.\textsuperscript{8,15,16}

**Directionality of Interatrial Propagation**

We sought further support for our hypothesis by monitoring the direction of conduction along BB and IPP over a distance covered by a minimum of 3 consecutive electrodes, ie, 2 cm over BB and 2.6 cm over IPP. Figure 4A is an example of left-to-right propagation along BB. In Figure 4B, quantification of this finding revealed that it occurred in 81±5% and 80±10% of the analyzed activations along BB and IPP, respectively. On the other hand, right-to-left propagation occurred in a significantly smaller percentage of cases.

**Alteration in LA-RA Gradient**

In previous studies,\textsuperscript{6,8} we demonstrated a high degree of spatiotemporal periodicity during AF. In addition, we showed that reentry forms the basis of this spatiotemporal periodic activity and that the cycle length of sources in the LA determines the dominant peak in the frequency spectra in this experimental model of AF. These data, together with data from the present study, suggest that in this model, AF results from impulses generated at high frequency by sources in the LA. Such impulses propagate along interatrial pathways to activate the RA in a spatially complex manner. Because the conditions are those of fibrillatory propagation, one would expect the existence of LA-RA frequency gradients that would be directly related to the source frequency (highest local frequency observed in the AF episode), with greater degrees of intermittent block at faster rates. To this end, we used D600 to decrease the frequencies (n=4). Verapamil analogs have been shown to increase ventricular fibrillation organization that is accompanied by a decrease in the ventricular fibrillation DF.\textsuperscript{18} In another set of experiments, we increased ACh concentration to increase the AF frequency (n=4). As shown in Figure 5A, D600 decreased the fastest LA frequency from 19.7±4.4 to 16.2±3.9 Hz (P<0.01) and paradoxically increased RA DF from 8.6±2.0 to 10.7±1.8 Hz (P<0.01).

**Figure 1.** Left-to-right decrement of DFs. A, Optical EG from LA with its corresponding FFT; B through D, Electrode recording and FFT from (B) left end of BB, (C) right end of BB, and (D) RA. E, DF maps of epicardial surfaces of LA and RA, with values of DFs along BB and IPP. Areas of frequency maps indicate optical mapping field. Small areas in red (1 in LA and 2 in RA) have frequency value of 60 Hz and represent noise artifact.

**Figure 2.** Left-to-right frequency gradient along BB in A and IPP in B. DFs were normalized to LA DF at different locations, shown along abscissa. BB indicates left of BB; BBS, BB at septum; BBR, right of BB; IPPPL, left of IPP; and IPPPR, right of IPP.
Hz ($P<0.05$). In contrast, increasing the ACh concentration from 0.2 to 0.5 μmol/L increased the LA-RA frequency gradient from $4.9\pm1.8$ to $8.9\pm1.8$ Hz ($P=0.02$), as shown in Figure 5B.

**Reduction of Interatrial Communication**

In an additional 5 experiments, BB and IPP were severed by an electrocautery. These 2 pathways are not the only means of interatrial communication, and thus, such a procedure is expected to reduce rather than abolish interatrial communication. Once stable AF was induced, cuts were made on the left side of the septum, as shown in Figure 6A. After ablation, both atria continued to fibrillate (Figure 6B). Yet, RA DFs decreased from $10.9\pm1.2$ to $9.0\pm1.5$ Hz ($P<0.01$), whereas LA DFs remained unchanged ($16.8\pm1.5$ versus $16.9\pm1.8$ Hz, $P=NS$). In all experiments, we also confirmed that these 2 pathways were not the only ones for interatrial electrical continuity. AF was terminated by ACh washout, after which the LA and RA were paced at a cycle length of 400 ms and recordings were obtained from the opposite atrium. In all cases, electrical continuity between the 2 atria persisted after ablation.

**Discussion**

The most important findings of this study are as follows: (1) during AF in the isolated sheep heart, there is an LA-RA frequency gradient; (2) there is an overwhelming predominance of left-to-right propagation of impulses across BB and the IPP; (3) the LA-RA gradient is directly related to the fastest AF frequency; and (4) reduction of interatrial communication by cutting BB and the IPP reduces RA frequency, with a resulting increase in the left-to-right gradient. These results are a natural extension of our previous work demonstrating vortex-like reentry around minuscule cores with high-frequency periodic activity in the posterior LA and complex activation of the RA. The overall results strongly support our general hypothesis that in this experimental model, high-frequency periodic sources located in the LA drive AF. Fibrillation results from rapidly successive wavefronts emanating from these sources that propagate through both atria and interact with anatomic and/or functional obstacles, leading to fragmentation and wavelet formation.

**Organization and Hierarchy of Frequencies in AF**

According to the multiple wavelet hypothesis, AF is characterized by multiple wavelets that move randomly throughout the atria. However, recent studies have shown organization in AF and others have shown shorter cycle lengths in the LA than in the RA. Moreover, studies from our laboratory demonstrated spatiotemporal organization in AF. Altogether, the data support our contention that AF is deterministic and that, at least in some cases, high-frequency periodic sources that maintain AF may be localized in the LA.
Data presented in this study show that LA frequencies were always higher than those of the RA. These findings are in agreement with previous results from our laboratory, as well as with other studies. To better understand the mechanisms underlying the hierarchy of frequencies, we studied impulse propagation along 2 interatrial pathways, BB and IPP. We demonstrated a left-to-right directionality of impulse propagation along these 2 interatrial pathways that was accompanied by a left-to-right frequency decrease. As suggested by the data in Figure 3, the highly heterogeneous atria, with areas of varying refractoriness and complex anatomic structure, provide the appropriate substrate for the occurrence of spatially distributed intermittent block patterns that establish the LA-RA frequency gradients and result in fibrillatory conduction.

**Frequency Dependence of LA-RA Gradient**

The mere presence of left-to-right frequency gradient between the 2 atria does not prove that LA drives AF in our model. In fact, Schuessler et al found that in the canine isolated RA with high ACh concentrations, sustained AF was the result of a single reentrant source in the inferior wall of the RA. In addition, one could argue that such a finding could be the result of a gradient of refractoriness between the atria, limiting the rise of RA frequencies. Many previous studies demonstrated a direct relationship between AF cycle lengths and local refractory periods. However, such a scenario, even if present, does not exclude the dependency of the RA on the LA, as demonstrated by the ACh and D600 experiments (Figure 5). Increasing the ACh concentration resulted in an increase in the LA frequencies, with a resultant increase in the frequency gradient. More interestingly, D600 decreased LA frequency and paradoxically increased RA frequency. Thus, it becomes evident that the LA-RA frequency gradient and RA frequency are determined by the LA frequency. Such a result is incompatible with the multiple wavelet hypothesis with an independent RA and could only be explained by a frequency-dependent change in fibrillatory propagation away from an LA source, allowing a greater number of waves to reach the RA at lower source frequencies. In fact, a higher source frequency would result in intermittent blockade because of sink-to-source mismatch between the interatrial pathways and the thicker RA. When the source frequency was reduced, RA DF increased, which indicates lesser mismatch at lower frequencies and explains the experimental findings with D600.

**Role of Interatrial Pathways in AF**

BB and the IPP are well-established routes of interatrial electrical communication. Although the 2 sides of the interatrial septum have been shown to be electrically insulated, interatrial continuity is present on the superior and inferior aspects of the fossa ovalis, regions that remained intact after both BB and IPP were cut. The presence of alternative routes of interatrial communication other than these 2 pathways was also substantiated by impulse propagation from one atrium to the other during postablation pacing of the BB and IPP (data not shown).

We have shown that during AF, BB and IPP are routes for impulse trafficking from the LA to the RA. The increase in the LA-RA frequency gradient after ablation on the LA side of these pathways provides strong support for our contention that LA sources drive AF in our model. After ablation, AF persisted, with no change in LA frequency but with a significant decrease in RA frequency and a consequent increase in the LA-RA.
gradient. This can be explained by the persistence of alternative routes of interatrial communication, as discussed above, and by the fact that a reduced interatrial communication established a larger sink-to-source mismatch at its boundary with the RA and resulted in a higher degree of blockade. Therefore, a reduction in interatrial communication causes greater degrees of LA-RA intermitten
cesse, with a resultant decrease in overall RA frequency.

Clinical Implications

These data improve our understanding of the mechanisms of AF and provide important clues for the understanding of previous clinical studies. Using precordial and esophageal recordings, Pehrson et al found a spatial dispersion in cycle lengths in patients with chronic AF. Although that dispersion was inconsistent among patients, it suggested an intraindividual frequency distribution. Other studies demonstrated a greater rate of success of LA radiofrequency (RF) ablation (60% to 80%) than of RA ablation to eliminate AF. In a recent study by Roithinger et al, single RA and LA RF ablation caused prolongation of AF cycle length in the ablated atrium. Most importantly, LA ablation increased the cycle length on the RA, whereas RA ablation had no appreciable effect on LA AF cycle length, supporting our idea that the LA is the primary driver during AF, at least in some cases. Our results, together with those of Roithinger et al, open potentially exciting new possibilities for targeting LA sources for therapies, whether ablative, electrical, pharmacological, or hybrid.

Technical Considerations

Certain technical issues warrant discussion in relation to our results. First, this study is limited by the small number of bipolar electrodes. However, such a deficiency was compensated by the high spatial resolution of our optical mapping. Second, optical recordings were acquired at 120 Hz for ~3 to 4 seconds, with a resolution of 0.25 to 0.5 Hz. Previous studies have shown that such a resolution is sufficient in the case of AF. Third, the studies were performed in an acute animal model of AF under artificial conditions. The relevance of these data to human AF remains to be studied. Finally, the limitations resulting from the use of voltage-sensitive dye have been discussed in detail elsewhere.

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