Stable Microreentrant Sources as a Mechanism of Atrial Fibrillation in the Isolated Sheep Heart

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Background—Atrial fibrillation (AF) has traditionally been described as aperiodic or random. Yet, ongoing sources of high-frequency periodic activity have recently been suggested to underlie AF in the sheep heart. Our objective was to use a combination of optical and bipolar electrode recordings to identify sites of periodic activity during AF and elucidate their mechanism.

Methods and Results—AF was induced by rapid pacing in the presence of 0.1 to 0.5 μmol/L acetylcholine in 7 Langendorff-perfused sheep hearts. We used simultaneous optical mapping of the right and left atria (RA and LA) and frequency sampling of optical and bipolar electrode recordings (including a roving electrode) to identify sites having the highest dominant frequency (DF). Rotors were identified from optical recordings, and their rotation period, core area, and perimeter were measured. In all, 35 AF episodes were analyzed. Mean LA and RA DFs were 14.7 ± 3.8 and 10.3 ± 2.1 Hz, respectively. Spatiotemporal periodicity was seen in the LA during all episodes. In 5 of 7 experiments, a single site having periodic activity at the highest DF was localized. The highest DF was most often (80%) localized to the posterior LA, near or at the pulmonary vein ostium. Rotors (n = 14) were localized on the LA. The mean core perimeter and area were 10.4 ± 2.8 mm and 3.8 ± 2.8 mm², respectively.

Conclusions—Frequency sampling allows rapid identification of discrete sites of high-frequency periodic activity during AF. Stable microreentrant sources are the most likely underlying mechanism of AF in this model. (Circulation. 2000;101:194-199.)

Key Words: atrium • arrhythmia • mapping • imaging • Fourier analysis

Reentry is the most widely accepted mechanism of atrial fibrillation (AF), but the exact electrophysiological bases of AF initiation and maintenance are still not fully understood. We recently demonstrated spatiotemporal periodicity during AF in the isolated sheep heart,1,2 i.e., when runs of sequential wave fronts with temporal periodicity originated at the same site and had similar spatial patterns of propagation. Only in a small number of cases were the sources of periodic activity during AF seen as rotors and breakthroughs on the epicardial surface of the left atrial (LA) appendage (LAA). In the majority of episodes (70%), periodic activity originated from outside the optical mapping field of view.

We hypothesized that periodic activity during AF in the isolated Langendorff-perfused sheep heart in the presence of acetylcholine (ACh) results from a single source or a small number of stable sources localized primarily to the LA. Alternatively, as suggested by recent studies, repetitive activation may be localized to the septum3 or the pulmonary veins (PVs).3,4 Fibrillation as seen on the ECG results from rapidly successive wave fronts emanating from these sources that propagate through both atria and interact with anatomic and/or functional obstacles, leading to fragmentation and wavelet formation.2

We have extended our initial observations in an effort to establish the mechanism of the stable periodicity underlying AF activity in the LA, particularly in cases in which the periodic sources were outside the field of view.1 Our first objective was to localize such periodic activity using a combination of optical and multiple bipolar recordings. To this aim, we devised a novel technique of frequency sampling, with offline and online spectral analyses of optical and bipolar electrode recordings, as a stepwise method to rapidly identify regions having the highest dominant frequency (DF). Subsequently, we identified the source of that DF by isolating specific sites having periodic activity at that frequency. Our final objective was to elucidate the mechanism and determine the path length of the reentrant sources.

Methods

Langendorff-Perfused Sheep Heart Preparation

Young sheep (18 to 25 kg) were anesthetized with sodium pentobarbital (35 mg/kg). The heart was removed and placed in cold cardioplegic solution for transportation, then connected to a Langendorff apparatus.1 The coronary arteries were continuously perfused at 200 mL/min via a cannula in the aortic root with warm (36°C to 38°C) Tyrode’s solution buffered to a pH of 7.4 and bubbled with 95% O₂/5% CO₂. We ensured that the heart was in...
sinus rhythm and contracting forcefully at the initiation of the experiment. In most cases, ventricular fibrillation occurred on rewarming shortly after the heart was connected to the Langendorff apparatus.

**High-Resolution Optical Mapping**

The video imaging approach used for these studies has been described elsewhere in detail. Briefly, we recorded potentiometric dye fluorescence simultaneously from 20,000 sites on the right atrial (RA) free wall and 10,000 sites on the LAA. We used 2 cameras (Cohu 6500) at a sampling interval of 8.33 ms. The areas of the mapped regions were 3 × 5 cm of the RA free wall and 3.5 × 3.5 cm of the LAA. To reveal the signal, 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 an effective spatial resolution of <0.5 mm.

**Isochrone Maps and Pseudoelectrograms**

Isochrone maps were generated from optical recordings by analysis of each pixel value over time. Pseudoelectrograms were constructed from optical recordings by integrating the transmembrane fluorescence signal over the entire mapped region.

**Electrode-Based Mapping**

Epicardial and endocardial surface electrograms were obtained with 16 silver bipolar electrodes (interpolated distance=1 mm) located at selected sites and a single custom-made roving bipolar electrode. For further details, see Procedures and Protocols below. Electrograms were filtered between 0.3 and 500 Hz, recorded with a 16-channel amplification system (model MMP100WSW; Biopac), and stored on a PC computer.

**Signal Analysis**

Spectral analysis was performed with fast Fourier transforms (FFTs) on bipolar electrode signals, pseudoelectrograms, and single-pixel optical recordings. Content in the 0.4- to 60-Hz band was analyzed, and the relative amplitudes of peaks in each FFT were compared to determine the dominant peak. Optical recordings were acquired at 120 Hz (8.33 ms) for 400 frames (3.3 seconds), providing a spectral resolution of 0.3 Hz. The bipolar electrograms were acquired at 1000 Hz for 10 seconds and filtered (bandpass 0.5 Hz to 500 Hz), providing a spectral resolution of 0.1 Hz over the range of 0.5 to 60 Hz.

**Measurement of Path Length of Reentrant Sources**

Reentrant wave fronts were identified from the optical recordings, after which their rotation period, core area, and perimeter were measured by tracing the trajectory of the pivoting point, which is located near the tip of the vortex where the front and tail of the rotating wave meet. The gray level values for each site were binarized by use of a cutoff value of 50% of maximum to classify regions as either active (>50%) or repolarized (<50%). The resulting binary images were then sequentially subtracted, resulting in movies containing only wave fronts and wave tails, which meet at the pivoting point.

**Procedures and Protocols**

At selected sites, 14 bipoles were placed (Figure 1) over the epicardium and endocardium of both atria, as follows: Bachmann’s bundle left and right (n=1 each); RA free wall (n=1); region around the PV ostium (in the sheep heart, the PVs come together to a common ostium that opens into the posterior wall of the LA) (n=6); base of the LAA (n=1); left septum (n=2); and right septum (n=2). A bariatral electrogram was recorded as the difference between 14 local electrograms, biatrial electrogram, and pseudoelectrograms of each atrial optical recording. Thereafter, the site having the highest DF was determined.

**Roving Bipole**

We then used the roving electrode to carefully explore the atrial endocardium/epicardium in the vicinity of the highest DF site. The specific site of origin of the periodic source/activity was determined by the following criteria: (1) a periodic source/activity, defined as spatiotemporal periodicity,1 was maintained during this AF episode, and (2) its FFT revealed a single peak.

**Statistical Analysis**

Correlation of frequencies was performed by simple linear regression analysis (Statview 4.53, Abacus Concepts). Slopes are presented with 95% CIs. Correlation coefficients ($R^2$) are also presented with associated probability values.

**Results**

As in our previous work, AF was defined on the basis of an irregular bariatral electrogram and optical recordings showing complex activation patterns. From 7 experiments, 35 episodes of AF were analyzed.

**Periodic Activity During AF**

Figure 2A shows 3 sequential 8.3-ms isochrone maps of activation recorded from the LA over a period of 201 ms. During that interval, the LA was repetitively activated by the same spatially oriented wave (upper left). Such periodic activity, defined as spatiotemporal periodicity,1 was maintained during this AF episode for 30 minutes. The cycle length of the periodic waves was 67 ms (14.7 Hz). Figure 2B shows 2 isochrone maps of the RA corresponding in time to those in A. The RA activation patterns, in contrast to those of
the LA, were slower (RA DF=8.5 Hz) and changed from beat to beat with no periodic activity. Spatiotemporal periodicity over periods of 20 to 30 minutes was seen in the LA in 80% of episodes but only in 20% of RA episodes.

**Frequency Sampling of AF**

In Figure 3, we present bipolar and optical recordings along with their corresponding FFTs from an AF episode in which we localized a site having high-frequency periodic activity (see Figure 1 for location of recording sites). The biatrial electrogram, pseudoelectrograms of the RA and LA, and bipolar recordings from the base of the LAA show disorganized activity, with DF peaks at 7.5, 10.3, 13.3, and 19.7 Hz, respectively. Activity from a site located in the groove between the LAA and PV ostium was also irregular, with a DF of 21.3 Hz. These recordings suggested that the source of the fastest activity was present in the posterior LA. To localize periodic activity at the fastest frequency (21.3 Hz, \(\approx 47\) ms), we then recorded electrograms at many sites in the posterior LA using the roving electrode. In contrast to those shown above, the electrogram at the bottom is very rapid and periodic. Its FFT shows 2 narrow peaks: DF at 21.3 Hz and its harmonic at 42.6 Hz. This signal was obtained when the roving electrode was positioned on the endocardial surface of the PV ostium at the 3 o’clock position. With the roving electrode, the periodic site was located within 10 minutes, during which time the frequency content of the fastest sites remained stable. Clearly, high-frequency periodic activity at this site during AF induced during ACh perfusion strongly suggests the presence of a stable reentrant source near or at that site. However, simultaneous recordings at sites located closely around the PV ostium revealed aperiodic activity and wide-band FFTs at some sites (not shown), establishing that anatomic reentry around the PV ostium was not the mechanism underlying the periodic activity. As in the example presented above, in 5 of 7 experiments, a single site having periodic activity at the highest DF was localized. Specific locations of such sites were PV ostium (\(n=3\)), base of LAA (\(n=1\)), and left side of Bachmann’s bundle (\(n=1\)).

**Mechanism Underlying Periodic Activity**

In Figure 4, we present data from another AF episode, in which a site of high-frequency periodic activity was localized. The biatrial and RA free wall electrograms were irregular, with DFs of 8.2 and 6.9 Hz, respectively. Signals from the region inferior to and from the PV ostium were also irregular, with multiple peaks on their FFTs. Activity recorded from the groove between the PV ostium and LAA
showed more rapid activity, with a DF at 14.7 Hz. The
electrogram at the bottom, recorded from the base of the
LAA, was rapid and regular, and its FFT showed a dominant
peak at 14.7 Hz, suggesting that a stable source might have
been present at that site. Examination of the LA optical
movies established the mechanism underlying AF in this
episode. In Figure 5A, the isochrone map of optical activity
from the LAA shows a vortex rotating clockwise at a period
of 67 ms, \( \approx 14.7 \) Hz; the vortex persisted for the entire length
of the episode (25 minutes). The fact that the frequency of
this source was equal to the highest DF recorded from all sites
(optical and bipolar electrodes) provides direct evidence that
it was the mechanism underlying the maintenance of this AF
episode. In actuality, single-pixel recordings at 3 separate
locations (Figure 5B) demonstrate that the entire LAA was
being activated at 14.7 Hz. Moreover, all 3 sites show
identical activation sequences and FFTs, even though only 2
of 3 sites were an integral part of the reentrant circuit. Finally,
the electrode that recorded the periodic activity (bottom trace
in Figure 4) was located at the base of the LAA \( \approx 1 \) cm away
from the rotor. The FFT of this signal showed a single peak
at a frequency (14.7 Hz) identical to that of the rotor,
indicating that the activity emanating from the rotor propa-
gated to that site in a 1:1 manner.

**Dispersion of Frequencies During AF**

The data presented thus far support our general hypothesis
that the sustained activity of a single or a small number of
stable sources localized primarily to the LA maintains AF.
The question remains, however, as to what causes disorga-
nized activity in AF. One possibility is that such activity is
secondary to fibrillatory conduction, ie, progressive breakup
of high-frequency waves as they propagate away from a
periodic source. One way of measuring dispersion is to
calculate the SD of the DFs measured at all recording sites in
each episode (all bipoles \( n = 15 \) and optical pseudoelectro-
grams) and plot it against the highest DF among all the sites.
Figure 6 demonstrates that there is a direct relationship
between the highest DF and the SD and thereby the disper-
sion of frequencies. In other words, the faster the reentrant
source, the greater would be the degree of fibrillatory con-
duction toward more distal sites.

**Spatial Distribution of Frequencies During AF**

It is our contention that sites having the highest DF (irrespec-
tive of whether activity at the site is periodic) are located
closer to the source of the highest DF than sites that have
lower DFs. In 66% of all AF episodes, \( \geq 2 \) sites had the
highest DF. As shown in Figure 7, in the 35 AF episodes
analyzed, the region surrounding the PV ostium had the
highest DF in 80% of AF episodes. The percentages of other
sites having the highest DF in a particular AF episode were as
follows: groove between the LAA and the PV ostium, 60.6%;
posterior LA region inferior to the PV ostium, 48.2%; base
of the LAA, 45.7%; LAA (optical), 45.7%; left septum, 42.9%;
Bachmann’s bundle (left), 26.4%; and RA free wall, 4%.

**Figure 5.** Microreentrant source of AF. A, Isochrone map of
optical activity from LAA, which shows a vortex rotating clock-
wise. B, Optical signals and corresponding FFTs from sites
marked 1 to 3 on isochrone map.

**Figure 6.** Highest DF for an AF episode is plotted against SD
(ie, dispersion of frequencies) of that episode.

**Figure 7.** Spatial distribution of highest DFs (HDF) expressed as
% of AF episodes (\( n = 35 \)). IPV indicates region inferior to PV
ostium; BLAA, base of LAA; LA Opt, LA pseudoelectrogram; L
Sept, left septum; and BBL, left side of Bachmann’s bundle.
Other abbreviations as in previous figures.
Frequency Sampling
During AF, intracardiac electrograms are rarely discrete and thus are difficult to interpret in the time domain. Frequency analysis has been used extensively to study VF,7,9 but only few studies have reported its use during AF.1,10 Using a limited number of closely spaced bipolar electrodes, we demonstrated a wide distribution of frequencies and readily identified sites with the highest DF. Subsequently, with a roving electrode, we could rapidly localize sites having periodic activity at the highest DF. However, as illustrated in Figure 5, spectral analysis is unable to differentiate between a signal recorded directly from a source of periodic activity (sites 1 and 2) and a site having 1:1 propagation from it (site 3). The utility of frequency sampling therefore lies in being able to rapidly localize areas that are critical for AF maintenance, i.e., sites of fastest periodic activity that most likely contain or are closely linked to the sources underlying AF maintenance. The approach allowed us to demonstrate that LA frequencies were higher than RA frequencies, which is in accordance with previous reports in various models of AF, including human AF.1,11,12 The highest DFs were localized most often (80%) to the posterior LA, including the PV region, strongly suggesting that this region contained high-frequency periodic sources and is therefore critical for AF maintenance.

Mechanism(s) of AF: Multiple Wavelets Versus Mother Rotors
The electrophysiological bases of AF remain unclear, and it is likely that the arrhythmia is the result of >1 mechanism. Two major hypotheses prevail: (1) “multiple wavelets” and (2) a single reentrant source giving rise to “fibrillatory conduction.” In a computer model of multiple wavelets by Moe et al.,13 mother waves were found to give rise to independent daughter wavelets, and the model predicted the need for a minimum of 23 to 40 coexisting wavelets for arrhythmia sustenance. The study by Allessie et al.14 showed that 4 to 6 wavelets coexisted during AF in the dog heart in the presence of ACh. Yet, to the best of our knowledge, no rigorous quantitative tests have been done to determine whether this number was critical and whether the arrhythmia terminates when the number of coexisting wavelets decreases to <5 or 6.

Incomplete reentry and multiple unstable reentrant circuits have been described in various settings during AF.3,14–17 However, since the multiple-wavelet hypothesis was first tested by Allessie et al.14 few studies have revisited the idea, originally put forth by Lewis18 and later by Scherf,19 that a single high-frequency source of stable reentry may be an underlying mechanism of AF. The work of Schuessler et al.20 in an isolated canine right atrial preparation is important in this regard. These authors found that with increasing concentrations of ACh, activation patterns characterized by multiple reentrant circuits converted to a single, relatively stable, high-frequency reentrant circuit that resulted in fibrillatory conduction. Our results are in agreement with the data of Schuessler et al.20 They support the hypothesis that a single or a small number of sources of stable ongoing reentrant activity are the mechanism underlying AF. Moreover, our results strongly suggest that functional reentry in the form of spiral

Microreentrant Sources
The presence of high-frequency periodic activity during AF suggested to us that the responsible reentrant sources rotated along extremely small trajectories/paths, measurement of which requires high spatial resolution that fortunately is provided by our optical mapping system. In almost 46% of all AF episodes (Figure 7), the highest frequencies were seen on the left atrial optical recordings. Hence, we examined all optical recordings of those episodes to examine the characteristics of reentrant sources and determine how their activity relates to fibrillatory activity in the corresponding atrium. Vortices (n=14) undergoing complete rotations were detected only in the LA. The mean rotation period was 68.6±8.9 ms (=14.7 Hz). Moreover, the rotation periods of individual vortices (Figure 8A) correlated strongly with the inverse DF (optical pseudoelectrogram) of the corresponding episode of AF (R=0.84, R=0.91). This result strongly suggests that the periodicity of rotating spiral waves is the main contributor to the DF of the optically mapped regions. Measurement of dimensions of the core of these rotating waves (Figure 8B) revealed minuscule cores; the mean core perimeter and area were 10.4±2.8 mm and 3.8±2.8 mm², respectively.

Discussion
We have introduced a novel technique of frequency sampling to characterize the spatial distribution of excitation frequencies during AF. Most importantly, the technique allows accurate identification of localized sites of periodic activity during the arrhythmia. Overall, the results support our hypothesis that stable localized sources are responsible for AF maintenance in the isolated sheep heart. In addition, using high-resolution video imaging, we demonstrated that such sources correspond to vortex-like reentry around minuscule cores (microreentry). The small path length of these sources explains their very high frequencies and the large dispersion of frequencies observed throughout the atria during AF.
waves rotating around microreentrant circuits of ∼1 cm is the most likely underlying mechanism of AF in our model. First, because of the continuous presence of ACh, it seems unlikely that, if focal activity was present, it was the result of pacemaker or triggered activity. We favor purely functional reentry or anatomic reentry with a functional component2,21 (ie, rotors anchored to structural discontinuities) as the 2 possible underlying mechanisms of AF in this model. Second, we have demonstrated that rotors contribute to the frequency content of AF, as shown by the strong correlation (R = 0.91) between the periodicity of individual rotors with the inverse DF of the optical pseudo-ECG of the corresponding AF episode.

Definite proof for the above-described mechanism is lacking for the majority of AF episodes whose sources lie outside the field of view of our imaging system. Nevertheless, on the basis of the presence of ACh during the experiments and the temporal stability of DFs in both the optically mapped regions and the area mapped with bipolar electrodes, it seems reasonable to extrapolate our findings from the optically mapped regions to regions such as the posterior LA. We therefore submit that similar rotors, ie, either a single rotor as shown in Figure 5 or a small number of rotors, having similar core sizes and rotation periods, are also responsible for high-frequency periodic activity at sites, such as the posterior LA region, that are outside the field of view of our optical mapping system.

Limitations
Several limitations need to be considered. First, the work presented here was carried out in an animal model of acute AF under the artificial conditions of isolation and crystalloid perfusion. Second, our model is essentially a model of cholinergic AF, because ACh was continuously perfused. As such, the relevance of these data to human AF remains to be determined. Third, this study is limited by the number of bipolar electrodes used. However, it must be considered that in addition to fixed bipoles, we also used a roving bipole that helped to record signals from many sites. Most importantly, bipolar electrodes were used in conjunction with optical mapping, which allowed high-resolution mapping from a significant portion of both atria. Finally, the limitations of this technique resulting from the usage of a voltage-sensitive dye and a mechanical uncoupler have been discussed repeatedly and in detail elsewhere.

Conclusions
Frequency sampling allows rapid identification of discrete sites of high-frequency periodic activity during AF. Stable microreentrant sources are the most likely underlying mechanism of AF in this model.

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