Methods for Determining the Refractory Period and Excitable Gap During Persistent Atrial Fibrillation in the Goat

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Background—Recently, the temporal excitable gap during atrial fibrillation (AF) has been identified as a vulnerable parameter for cardioversion of AF. In this study, we evaluated 5 methods to measure the refractory period (RP_{AF}) and the excitable period (EP_{AF}) during persistent AF.

Methods and Results—in 11 goats instrumented with 83 epicardial atrial electrodes, persistent AF (43±34 days) was induced with a median AF cycle length (CL) of 98±14 ms. To measure RP_{AF}, premature stimuli were applied to the center of the electrode array on the right or left atrium. The RP_{AF} measured by mapping of premature stimuli was 70±12 ms ("gold standard"). The RP_{AF} determined during entrainment of AF was 77±17 ms (R^2=0.88, P<0.01). Statistical analysis of the effects of synchronized stimuli (each coupling interval ×100) on the AFCL histogram yielded an RP_{AF} of 70±13 ms (R^2=0.94, P<0.01). A further simplification was to apply slow fixed-rate pacing (1 Hz) during AF. For each stimulus (n=250 to 500), the paced AFCL was plotted against its coupling interval, and capture was determined by statistical shortening of the AFCL (RP_{AF} 71±17 ms, R^2=0.84, P<0.01). The 5th percentile of the AFCL histogram as an index of RP_{AF} was 77±12 ms (R^2=0.90, P<0.01).

Conclusions—During persistent AF with an AFCL of 98±14 ms, the RP_{AF} determined by mapping of synchronized premature stimuli (gold standard) was 70±12 ms, with an excitable period of 28±8 ms. Although the indirect methods to measure RP_{AF} all correlated well with the gold standard, slow fixed-rate pacing seems to be the most attractive technique because of the ease of acquiring the data and the clear graphic result. (Circulation. 2001;104:957-962.)

Key Words: fibrillation ■ electrophysiology ■ excitation

It was recently suggested that the temporal excitable gap during atrial fibrillation (AF) is a critical determinant for perpetuation and termination of AF.1,2 In the presence of a short excitable gap, fibrillation waves are more likely to die out by encountering refractory tissue. Conversely, a short excitable gap may also promote the formation of new wavelets because of an increase in the likelihood of intra-atrial conduction block. Thus far, determination of the excitability gap has been limited to the recording of monophasic action potentials.3,4 Recently, the refractory period during persistent AF (RP_{AF}) has been measured directly by programmed electrical stimulation.5 Cardioversion by class I drugs was found to be associated with a 2- to 3-fold widening of the temporal excitable gap.2

The aim of the present study was to develop clinical tools for the measurement of the RP_{AF} and temporal excitable gap during persistent AF. Five methods were evaluated in a goat model of persistent AF using mapping as "gold standard."

Methods

The goat model of AF was described previously.6 In 11 goats (51±8 kg), 2 plaques (3.5×2.5 cm, 50 electrodes, distance 4 mm) were sutured to the free wall of the right atrium (RA) and left atrium (LA). One strip (6×1 cm, 23 electrodes, distance 6 to 10 mm) was pulled along Bachmann’s bundle and sutured to both atrial appendages. The leads were tunneled subcutaneously to the neck and exteriorized by three 30-pin connectors. A subcutaneous silver plate served as indifferent electrode. After the goats had recovered from surgery, persistent AF was produced by a fibrillation pacemaker.6 After amplification (×300) and filtering (1 to 500 Hz), all unipolar atrial electrograms were stored on tape. Local activation times were determined automatically by a custom-made algorithm detecting the maximal negative slope of the fibrillation electrograms. The goats were studied after 43±34 days of AF at a median AF cycle length (CL) of 98±14 ms. A central pair of electrodes on the RA (n=7) or LA (n=4) was used for stimulation. Biphasic stimuli of 2 ms were generated by a constant-current stimulator equipped with an amplifier recording a bipolar electrogram from the pacing electrodes (Medtronic SF3111). The minimal current required for regional entrainment of AF was taken as the threshold for stimulation.7

Methods to Measure RP_{AF}

Figure 1 illustrates the 5 methods to measure RP_{AF}. Mapping was used as the gold standard. Single premature stimuli of 4× threshold were synchronized to the fibrillation waves at the pacing site. Capture was verified by activation maps around the pacing site. In
Mapping

A second method to measure RP AF is to apply premature stimuli during regional entrainment of AF with an interval equal to the median AFCL. The S1-S2 interval was changed in steps of 2 ms. Capture was determined by the latency and morphology of an electrogram recorded 4 mm from the pacing site. In case of capture, the premature electrogram showed a morphology and latency similar to that during entrainment. The longest S1-S2 interval that failed to capture the atria was taken as the RP during entrainment of AF.

A third method to measure RP AF is to determine the statistical effect of synchronized premature stimuli on the AFCL histogram. Each coupling interval was repeated 100 times. The shortest coupling interval (classes of 5 ms) resulting in a significant shortening of AFCL (Kolmogorov-Smirnov 1-sample test) was defined as the RP AF. Because this method was developed during evaluation of the other techniques, it was applied only in the last 6 of 11 goats.

A fifth method to determine RP AF is to determine the 5th percentile of the AFCL histogram, constructed from 100 consecutive AF cycles from a unipolar RA or LA electrogram.

Excitable Gap During AF

The term "excitable gap" refers to both the excitable tissue between fibrillation waves (spatial excitable gap) and the time window of excitability during the AF interval (temporal excitable gap). We used the term excitable period (EP AF) to indicate the temporal excitable gap. With all 5 methods, EP AF could be directly measured from the histogram of the interval between the stimulus and the first response (S-AF). Whereas during subthreshold pacing, the S-AF intervals were equally distributed, pacing at 4× threshold caused a gap in the S-AF histogram. The longest S-AF interval occurring less frequently than expected (95% CI) was taken as the EP AF.

Results

Mapping of the Refractory Period During AF

Figure 2 shows an example of the RP AF determined by mapping. In the upper panel, a stimulus was applied 70 ms after the pacing site was activated. The activation map shows that the stimulus did not capture the atrium. A stimulus with a slightly longer interval of 75 ms (lower panel) resulted in capture, as evidenced by radial spread of activation from the pacing site. When stimuli with the same coupling interval were repeated 20×, however, sometimes capture occurred and sometimes not. In Figure 3, we plotted the percentage of capture at different coupling intervals. At short coupling intervals (<65 ms), the atrium was never captured. Prolonging the interval from 65 to 90 ms resulted in a progressive increase in the probability of capture. A coupling interval >90 ms resulted in 100% capture. The S-shaped curve shows that the RP AF is not a deterministic but a probabilistic variable. We arbitrarily defined the RP AF as the shortest coupling interval that captured the atrium 20% of the time. This value represents the shortest of a wider range of refractory periods during AF. In all goats, the RP AF measured in this way was 70±12 ms. The temporal variation in RP AF (5% to 95% capture) was 19±4 ms.

Refractory Period During Entrainment of AF

Figure 4 shows an example of the RP during entrainment of AF. Entrainment was performed at the LA with an S1-S2...
interval of 110 ms (median AFCL 113 ms). The unipolar electrogram recorded next to the pacing site shows 8 entrained beats followed by a single premature stimulus (S₂). During entrainment, the activation maps revealed radial spread of activation. An S₂ stimulus of 90 ms did not capture the atrium, whereas an S₂ stimulus with a coupling interval of 92 ms did capture the atrium (radial spread). For determination of the RPₐf, capture was verified by the morphology of the unipolar electrogram next to the pacing site. In case of no capture, the electrogram showed a clear R wave and a variable time interval between the stimulus and the next activation. In case of capture by the premature stimulus, the latency and electrogram morphology were similar to those during entrainment of AF. The RP during entrainment, defined as the longest S₁-S₂ interval that did not capture the atrium, was 77±17 ms (6 goats).

Measurement of the RPₐf by Synchronized Stimuli
The response to synchronized stimuli was determined statistically by applying a premature stimulus 100 times. For each coupling interval, a histogram of the paced AFCL was reconstructed from a neighboring electrogram. In Figure 5, an example is given of a premature stimulus with a coupling interval of 50 ms. The control AFCL histogram (sampled 1 second before each premature stimulus) shows a normal distribution with a median AFCL of 80 ms. In contrast, the paced AFCL histogram showed a bimodal distribution. Because of capture, 39% of the AF cycles were now ≤60 ms. The Wilcoxon 2-sample test revealed a significant difference between the 2 histograms (P<0.05). Premature stimuli with a coupling interval of ≤50 ms did not result in statistically different AFCL histograms. In 11 goats, the RPₐf measured by the shortest coupling interval yielding a statistically different AFCL was 70±13 ms. This value represents the shortest of a range of refractory periods during AF.

Measurement of the RPₐf by Fixed-Rate Pacing
An alternative way to determine RPₐf is to stimulate the fibrillating atria at a slow fixed rate of 1 Hz, resulting in a series of randomly applied premature stimuli. In Figure 6, the
AFCL at an electrode close to the pacing site is plotted for all coupling intervals during 4 minutes of fixed-rate pacing \((n = 240)\). Two populations of data points can be clearly distinguished. At shorter coupling intervals, AFCL shows the normal variation (median 88 ms). At longer coupling intervals, the normal variation in AFCL was lost, and because of capture, the paced AFCL was now determined by the coupling interval of the stimulus. At intermediate coupling intervals (48 to 69 ms), only part of the stimuli captured the atrium, illustrating the temporal variation in RPAF (shaded column). In all goats, the range of temporal variation of the RP AF was 24 to 67 ms. The shortest coupling interval producing a significant shortening of AFCL, as determined by the Kolmogorov-Smirnov test, was 71 to 17 ms. This method was applied only in the last 6 of 11 goats, because it was developed during evaluation of the other techniques in the first 5 goats.

With fixed-rate pacing, EP_AF can be directly visualized (Figure 7). During 16 to 17 minutes of 1 Hz pacing, all poststimulus intervals \((n = 1000)\) were plotted in a histogram. During subthreshold pacing, the S-AF intervals were equally distributed, with a mean incidence of 10.7 ± 2.9 (95% CI between 4.9 and 16.5). When stimulus strength was set at 4× threshold, the S-AF intervals were no longer equally distributed. Now, a high incidence \((n = 368)\) of short S-AF intervals was observed, representing the latency between stimulus and response during capture. At the same time, the incidence of S-AF intervals between 5 and 30 ms markedly decreased. These intervals disappeared because they were shortened by capture of the premature stimuli. The distribution of S-AF intervals did not change during pacing. The upper limit of the excitable period was determined by the longest S-AF interval that occurred less frequently than the 95% CI. In 6 goats, EP_AF measured in this way was 27 ± 4 ms. This value represents the longest of a range of excitable periods during AF. Because of variation in AFCL and RP_AF, during many cycles EP_AF will actually be shorter. This is illustrated in Figure 7 by the atrial responses that still occurred during the measured excitable period.

### p5 AFCL Value as an Index of the RP_AF

In 11 goats, the median AFCL measured at the RA or LA was 98 ± 14 ms. The 95th and 5th percentiles of the AFCL were 120 ± 16 and 77 ± 12 ms. The 5th percentile (p5) value was used as an index of RP_AF. This is based on the assumption that the shortest AF cycle lengths have no or only a very short excitable period.

### Comparison of the Different Measurements of the RP_AF

In Table 1, the values of RPAF as obtained by the 5 methods are listed for all goats. The RP_AF measured by mapping is

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**TABLE 1. RP_AF Values Obtained by Different Methods**

<table>
<thead>
<tr>
<th>Goat</th>
<th>Site</th>
<th>AFCL (Median)</th>
<th>Refractory Period During AF, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mapping</td>
</tr>
<tr>
<td>1</td>
<td>RA</td>
<td>105</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>RA</td>
<td>107</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>LA</td>
<td>88</td>
<td>55</td>
</tr>
<tr>
<td>4</td>
<td>RA</td>
<td>88</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>LA</td>
<td>93</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>LA</td>
<td>86</td>
<td>65</td>
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<tr>
<td>7</td>
<td>RA</td>
<td>90</td>
<td>70</td>
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<tr>
<td>8</td>
<td>RA</td>
<td>127</td>
<td>95</td>
</tr>
<tr>
<td>9</td>
<td>LA</td>
<td>103</td>
<td>75</td>
</tr>
<tr>
<td>10</td>
<td>RA</td>
<td>113</td>
<td>80</td>
</tr>
<tr>
<td>11</td>
<td>RA</td>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>98 (73)</td>
<td>(77)</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>14 (15)</td>
<td>(17)</td>
</tr>
</tbody>
</table>

*R² = 0.88* 0.94* 0.84* 0.90*

Numbers in parentheses indicate mean and SD of goats 6 through 11; *R², correlation of the RP_AF measured by the different techniques compared with mapping.

*P<0.01.
considered the gold standard. All methods yielded an $R_{AF}$ between 70 and 80 ms. During entrainment, the RP was $4 \pm 5$ ms longer than the $R_{AF}$ determined by mapping ($77 \pm 17$ versus $73 \pm 15$ ms, $P=0.12$). The correlation between the values obtained by entrainment and mapping was 0.88 ($P<0.01$). The $R_{AF}$ determined by synchronized stimuli yielded the same values as obtained by mapping ($70 \pm 13$ versus $70 \pm 12$; $R=0.94$; $P<0.01$). The $R_{AF}$ measured by fixed-rate pacing also gave a similar result ($71 \pm 17$ versus $73 \pm 15$ ms; $R=0.84$; $P<0.01$). The 5th percentile of the AFCL histogram was $7 \pm 4$ ms longer than $R_{AF}$ measured by mapping ($77 \pm 12$ versus $70 \pm 12$ ms, $P<0.05$), with a correlation coefficient of 0.90 ($P<0.01$). Although the study was not designed to compare measurements in RA and LA, AFCL and $R_{AF}$ were consistently longer and showed more variation in the RA ($P=0.33$ to 0.47).

### Discussion

**Refractory Period During Atrial Fibrillation**

The present study shows that the refractory period during AF exhibits a considerable temporal variation. Measurement of $R_{AF}$ by mapping or fixed-rate pacing revealed a time window of 20 to 25 ms during which premature stimuli sometimes captured the atrium and sometimes not (Figures 3 and 6). This probabilistic nature of $R_{AF}$ is probably a result of beat-to-beat variations in AFCL and direction of propagation. Also, fragmentation of fibrillation waves and electrotonic modulation of the action potential by dissociated neighboring wavelets may cause temporal variation in $R_{AF}$. The $R_{AF}$ measured by mapping was defined as the coupling interval resulting in $>20\%$ capture. The other methods also determined the shortest refractory period during AF. The values obtained by the 5 methods ranged between $70 \pm 12$ and $77 \pm 17$ ms (70% to 80% of median AFCL). Although these measurements of the refractory period ignore the existing temporal variation, they may be useful to explore the effects of changes in $R_{AF}$ on perpetuation and termination of AF. In addition, they might be of value to evaluate the spatial variation in refractory periods during AF. The observed greater variability in AFCL and $R_{AF}$ in the right atrium may be associated with a more complex geometry, allowing 3D propagation during AF.$^{10}$

### Comparison of Different Techniques to Measure $R_{AF}$

The different methods to measure RPAF all have certain advantages and disadvantages (Table 2). Mapping of premature stimuli is the most reliable technique and can be regarded as the gold standard. Because of its complexity and invasive nature, however, it can be used only under exceptional circumstances. Measurement of the refractory period during entrainment of AF has the advantage that it uses the conventional extra-stimulus technique ($S_1$-$S_2$) that can be applied during paroxysmal AF. The somewhat longer values obtained during entrainment are probably a result of electrotonic prolongation of the action potential at the pacing site.$^{9}$ Conversely, entrainment of AF is not always feasible during type III AF.$^{11-13}$ Statistical comparison of the AFCL histogram during synchronized premature stimuli is a highly objective way to determine the $R_{AF}$. It is a rather complex technique, however, requiring specific expertise. The easiest way to pace the atria during AF is slow fixed-rate pacing. The required equipment is limited to a standard pacemaker, a multipolar catheter, and software for data analysis. The data acquisition time is $\approx 15$ minutes, during which no intervention by the electrophysiologist is needed. This method produces a clear graphic output from which both $R_{AF}$ and $E_{AF}$ can be directly read out. It was applied only in the last 6 of 11 goats, because the technique was developed in the first 5 goats. Although the p5 AFCL is even more simple, its reliability is questionable. During administration of class I drugs, the $R_{AF}$ became considerably shorter than the p5 AFCL.$^{2}$

**Excitable Period During Atrial Fibrillation**

In the present study, $E_{AF}$ calculated as the difference between AFCL (98\pm14 ms) and $R_{AF}$ (70\pm12 ms), was 28\pm8 ms. The $E_{AF}$ measured directly during fixed-rate pacing was 27\pm4 ms (Figure 7). $E_{AF}$ however, must also show a considerable temporal variation. In our experiments, the p5 and p95 AFCL were 77\pm12 and 120\pm16 ms. This variation in AFCL (43\pm8 ms) cannot be completely explained by the variability in $R_{AF}$ (19\pm4 ms). This implies that at short AF cycles, the excitable period may become as short as 7 ms, whereas during long cycles, the excitable period might be as long as 50 ms. This beat-to-beat variability in $E_{AF}$ may play a role in perpetuation of AF.

The excitable period during AF might be explained by the different types of reentry during AF.$^{1}$ In case an impulse circulates around an anatomic obstacle, $E_{AF}$ is determined by the difference between the conduction time around the obstacle and the refractory period within the reentrant circuit.$^{14}$ In case of functional reentry, $E_{AF}$ is caused by the

### Table 2: Comparison of Different Techniques to Measure the $R_{AF}$

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Equipment</th>
<th>Time, min</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mapping</td>
<td>High-density mapping</td>
<td>60</td>
<td>“Gold standard”</td>
<td>Only in exceptional circumstances</td>
</tr>
<tr>
<td>Entrainment</td>
<td>Programmed electrical stimulation</td>
<td>15</td>
<td>Conventional $S_1$-$S_2$ pacing</td>
<td>Not always possible</td>
</tr>
<tr>
<td></td>
<td>Multipolar catheter</td>
<td></td>
<td>Also in paroxysmal AF</td>
<td></td>
</tr>
<tr>
<td>Synchronized Stimuli</td>
<td>Stimuli synchronized to pacing site</td>
<td>30</td>
<td>Statistical determination of $R_{AF}$</td>
<td>Complex</td>
</tr>
<tr>
<td></td>
<td>Multipolar catheter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fixed-Rate Pacing</td>
<td>Fixed-rate pacemaker</td>
<td>15</td>
<td>Easy to use</td>
<td>Takes 15 minutes</td>
</tr>
<tr>
<td></td>
<td>Multipolar catheter</td>
<td></td>
<td>Direct measurement of $E_{AF}$</td>
<td></td>
</tr>
<tr>
<td>p5AFCL</td>
<td>Recording of atrial electrogram</td>
<td>3</td>
<td>No pacing required</td>
<td>Reliability questionable</td>
</tr>
</tbody>
</table>

This method produces a clear graphic output from which both $R_{AF}$ and $E_{AF}$ can be directly read out. It was applied only in the last 6 of 11 goats, because the technique was developed in the first 5 goats. Although the p5 AFCL is even more simple, its reliability is questionable. During administration of class I drugs, the $R_{AF}$ became considerably shorter than the p5 AFCL.$^{2}$
curvature of the circulating wavefront at pivot points. Because of the high curvature, the excitatory current generated by the turning wavefront may not be enough to make a rapid 180° turn,15–17 and the resulting conduction delay creates an excitable period in the returning limb of the turning wavefront.18 In addition, when functional reentrant circuits are drifting through the myocardium, the excitable gap will be shortened or lengthened by the Doppler effect.19,20 In case of random reentry, an excitable period will arise at areas remote from the site of reentry because of the anterograde and retrograde conduction time to the site of reentry.1 Conversely, the excitable period will be shortened by epicardial breakthrough of wave fronts propagating in one of the pectinate muscles.10 The resulting short circuit of epicardial reentry may play an important role in perpetuation of AF, not only because of the 3D nature of the reentrant process but also by narrowing its excitable gap.

We recently suggested that EP_{AF} might be a critical determinant for termination of AF. Cardioversion of persistent AF by class I drugs was associated with a dose-dependent widening of EP_{AF}.2 We speculated that widening of the EP_{AF} decreases the number of head-tail interactions between fibrillation waves, resulting in less dissociation and a higher degree of organization of the multiple wavelets. Cardioversion of AF would occur if multiple wavelets would fuse into a single wave front whose circulating pathway is ultimately interrupted. The possibility cannot be excluded, however, that under other circumstances, cardioversion of AF might be due to closure of the excitable period. Measurement of RP_{AF} and EP_{AF} during pharmacological cardioversion of AF by the methods developed in the present study may further elucidate the significance of the excitable period for termination of AF.

Limitations

In the present study, epicardial mapping was used to determine RP_{AF}. Because no endocardial recordings were made, the 3D structure of the atrial wall, which might play a role in the activation during AF,10 was not taken into account. Also, the study was not designed to determine differences in AFCL, RP_{AF}, and EP_{AF} at different atrial sites. Variation in atrial architecture and underlying geometric discontinuities may cause spatial variability in the RP and EP during AF. Future clinical studies measuring RP_{AF} with the use of a catheter will be needed to elucidate differences between RA and LA. Another limitation is that measurements of RP_{AF} were not compared with monophasic action potential recordings. Therefore, it remains unknown whether the EP_{AF} is due to a diastolic interval between the successive action potentials during fibrillation. Because in the present study, RP_{AF} was measured by chronically implanted epicardial electrodes, clinical application of these methods must await validation by use of multipolar endocardial catheters.

Acknowledgment

Dr Duyschaever is a recipient of a Research Fellowship from the Scientific Research Fund, Flanders.

References

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Circulation. 2001;104:957-962
doi: 10.1161/hc3401.093156

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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