Role of Progressive Widening of the Temporal Excitable Gap for Perpetuation of Atrial Fibrillation in the Goat

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Background: Previous studies suggest that a short temporal excitable gap exists between the fibrillation waves during atrial fibrillation (AF). The aim of this study was to investigate the role of that gap in the development of sustained AF in goats.

Methods and Results: Eight female goats were instrumented with left atrium (LA) electrodes, and sustained AF (>24h) was induced by intermittent rapid atrial pacing for 9.3±4.6 days. In the process of sustained AF development, the atrial effective refractory period (AERP), refractory period during AF (RP_{AF}), mean AF cycle length (AFCL), temporal excitable gap during AF (EG_{AF}=AFCL−RP_{AF}) and degree of fractionation of fibrillation electrograms at LA were studied. When the induced AF lasted for 3–10 min, AFCL, RP_{AF} and EG_{AF} were 98.3±11.0 ms, 90.5±13.2 ms and 7.8±2.4 ms, respectively. During sustained AF, the values were 84.9±5.2 ms, 63.0±4.8 ms and 21.9±3.5 ms, respectively (P<0.05). Percentage of single potentials was 94.2±3.9% and 75.6±5.5%, respectively (P<0.05).

Conclusions: In this model progressive shortening of atrial refractoriness and widening of the temporal excitable gap induced by electrical remodeling created an electrophysiologic substrate for the perpetuation of AF. (Circ J 2010; 74: 655–663)

Key Words: Atrial fibrillation; Atrium; Electrophysiology; Remodeling

Paroxysmal atrial fibrillation (AF) often progresses to persistent AF, even in patients without underlying heart disease. In a goat model of AF, Wijffels et al demonstrated that perpetuation of AF was accompanied by a marked shortening of the atrial refractory period and loss of the normal rate adaptation (termed atrial electrical remodeling). This shortening in atrial refractoriness results in a shortening of the atrial wavelength, allowing more fibrillation waves to be simultaneously present in the available atrial tissue mass. Although this partly explains the development of persistent AF in the absence of structural heart disease, it has been found that the time required for the development of sustained AF (1–2 weeks) is longer than the time of electrical remodeling (48–72 h). Gaspo et al made similar observations. Such a time lag between electrical remodeling and the development of persistent AF has led to the suggestion that additional factors with a slower time course must be involved in the creation of the substrate of persistent AF. Most studies have followed the time course of atrial electrical remodeling by measuring the refractory period during regular pacing at relatively slow rates. However, this may not accurately reflect the time course of changes in refractoriness during AF itself.

Mapping studies have shown that during AF a short temporal excitable gap (EG_{AF}) exists between fibrillation waves and it has been suggested that the width of the EG_{AF} is a determinant of the perpetuation of AF. On the 1 hand, fibrillation waves will be more likely to die out in the presence of a short excitable gap, whereas on the other hand it may promote bifurcation of wavelets at areas of functional block. Previous studies have shown that pharmacological cardioversion of AF with class IC drugs and a pure sodium channel blocker is associated with a widening of the EG_{AF}. In the case of a longer excitable gap, wave breaks and turning of fibrillation waves may be reduced, which may lead to a reduction of the average number of fibrillation waves in the atria.

The aims of our present study were (1) to measure the time course of changes in atrial refractory period during the first week of AF, and (2) to evaluate the role of the temporal excitable gap in the transition from paroxysmal to persistent AF.
Methods

Animal handling was performed according to the guiding principles of the Declaration of Helsinki and was approved by the Animal Investigation Committee of the Chinese PLA General Hospital. Eight female goats weighing between 28 and 45 kg (mean 37±6 kg) were used for this study. They were anesthetized with 3% isoflurane and a 2:1 mixture of O2 and N2O. A left intercostal thoracotomy was performed and the pericardium was opened to expose the heart. A silicon strip measuring 10×1.2 cm and containing 5 pairs of silver electrodes (diameter, 1.5 mm; interelectrode distance 5 mm) was sutured to the free wall of the left atrium (LA) (Figure 1). After approximation of the pericardium and closure of the thorax, the electrode leads were tunneled to the neck. Ampicillin (1 g) was given prophylactically both before and after surgery.

Two weeks after surgery, while being kept in separate boxes with free access to food and water, the goats were connected to an external automatic atrial fibrillator. A cable from the ceiling of the box was plugged into the connector in the neck of the animal and the atrial electrodes were connected to a multichannel recording unit. The atria could be stimulated through any pair of the epicardial electrodes, but the pair of electrodes that connected with the fibrillation pacemaker was excluded from the measurement of the atrial effective refractory period (AERP) and atrial refractory period during AF (RPaf). The pacing electrodes for refractory period measurement and the adjacent sensing electrodes were 5 mm apart. The custom-made pacemaker generated 1 s bursts of stimuli (interval 20 ms; maximum output 6.0 V) followed by a pause of 2 s. After the goats had recovered from surgery and before they were connected to the fibrillation pacemaker, a pair of electrodes at the free wall of the LA was chosen to measure AERP during pacing at different S1:S2 intervals between 200 and 500 ms. A single premature stimulus (4× threshold) was interpolated after every 8 basic stimuli and, starting from well within the refractory period, the S1:S2 coupling interval was incrementally increased in steps of 2 ms. The shortest S1:S2 interval resulting in a propagated atrial response was taken as the AERP. After the fibrillation pacemaker had been turned on, the atrial refractory period and the duration of induced AF episodes were measured after 6, 12, and 24 h during the first day and then once daily thereafter. The mean AF cycle length (CL) was measured by averaging 200 consecutive AF intervals. Conduction velocity was not measured, because a previous study has shown that it is not affected by electrical remodeling in the goat model.3

As soon as the induced AF paroxysms lasted for longer than 3 min, a bipolar fibrillation electrogram recorded from the free wall of the LA was used to synchronize a pacemaker that delivered single stimuli through the same electrodes after every 8 sensed fibrillation cycles. Starting well within the refractory period, the coupling interval between the 8th sense stimulus and the stimulus was incrementally increased in steps of 2 ms. Each stimulus was repeated 10 times; the shortest coupling interval that captured the atrium 2 or more out of 10 times was taken as the RPaf. Criteria for capture were (1) a short latency between the stimulus and the response, (2) a short AFCL, and (3) a compensatory longer AFCL after the local capture (Figure 2). The temporal EGaf was calculated as the average AFCL minus the RPaf. The AFCL, RPaf and EGaf were measured daily until the induced AF lasted for more than 24 h spontaneously.

To evaluate the degree of fractionation of the fibrillation electrograms, all complexes were classified as single, double or multiple potentials according to criteria described previously.11 It was measured when the episode of induced AF lasted for 3–10 min, 30–60 min, then 6–8, 10–12 and >24 h.

Statistical Analysis

Statistical analysis was performed with SPSS version 10.0 (Chicago, IL, USA). Data are given as mean±standard deviation. ANOVA was used for multiple-group comparisons, followed by a Bonferroni-corrected t-test. A 2-tailed value of P<0.05 was taken as statistically significant.

Results

Stability of AF

All 8 goats successfully completed the protocol. The average duration of the first episodes of induced AF was 5.0±4.6 s.
Excitable Gap and AF

After burst pacing for 21±6h, then 2.5±1.0, 5.0±2.2, and 6.8±3.6 days, the respective duration of AF paroxysms increased to 3–10 min, 30–60 min, 6–8 h, and 10–12 h. AF became persistent in all animals after an average of 9.3±4.6 days (range 6–16 days).

**Changes in AERP and RPAF During Electrical Atrial Remodeling**

During baseline, the average AERP during regular pacing with an interval of 500, 400, 300 and 200 ms, respectively, was 140.3±15.2, 141.6±16.8, 146.0±18.8 and 136.3±13.0 ms. After 48h of AF, the AERP was shortened to 60.3±15.7, 61.3±23.1, 61.0±16.8 and 66.7±15 ms, respectively (P<0.05). In most goats, the AERP could no longer be measured after 48h because by that time premature stimulation induced long-lasting paroxysms of AF that seriously hampered the measurement. **Figure 3** shows that the AERP decreased rapidly during the first 2 days of AF, while its physiological rate adaptation became inverted. In those cases in which the AERP could be measured for longer than 48h, the shortening in atrial refractory period continued until it reached a new steady state after 4–5 days of AF. The refractory period during AF could be measured from day 1 to days 5–8. In 1 goat the RPAF of 72 ms gradually shortened to 62 ms on the 8th day, when AF became sustained (**Figure 3A**). In 3 other goats (**Figures 3B–D**), after 1 day of AF the RPAF was approximately 90 ms. It first shortened rapidly to approximately 70 ms during the 2nd day of AF and continued to decrease more gradually during the following days.

**Figure 2.** Measurement of the refractory period during atrial fibrillation. (A) Synchronized stimulus applied 88 ms after the pacing site was activated did not capture the fibrillating atrium. (B) Stimulus with a slightly longer interval of 90 ms now captures the atrium. (C, D) After 8 days of burst pacing, a stimulus with a coupling interval of 58 ms failed to capture the atrium, whereas a coupling interval of 60 ms did result in capture. The electrograms were recorded at a distance of 5 mm from the pacing site.
Figure 3. Time course of the changes in AERP$_{500}$, AERP$_{200}$ and RP$_{AF}$ during the first days of atrial fibrillation (AF) in 4 goats. The AERP decreased rapidly during the first 2 days of AF, while its physiological rate adaptation became inverted. The RP$_{AF}$ continued to shorten more gradually to approximately 60 ms after 5–8 days of AF, when AF no longer terminated spontaneously anymore. AERP, atrial effective refractory period; RP, refractory period.

Figure 4. Relationship between AFCL and RP$_{AF}$ during the first days of atrial fibrillation (AF). There was a clear, positive correlation between RP$_{AF}$ and AFCL during the process of electrical remodeling (slope: 1.32; P<0.001). CL, cycle length; RP, refractory period.
Figure 5. Measurement of the refractory period during atrial fibrillation (AF) and the AERP200 after 2 days of electrical remodeling. (A) During AF a synchronized stimulus with a coupling interval of 70 ms resulted in local capture of the fibrillating atria. (B) After spontaneous termination of AF the atria were paced regularly with an interval of 200 ms, a single premature stimulus with a coupling interval as short as 56 ms already captured the atria, leading to induction of a paroxysm of AF. AERP, atrial effective refractory period.

Figure 6. Changes in the average AFCL, RP_AF and EG_AF during the transition from paroxysmal to persistent atrial fibrillation (AF) in 8 goats. In the process of electrical remodeling, both AFCL and RP_AF decreased gradually. Because the changes of AFCL were more prominent, this resulted in a progressive widening of the excitable gap during AF. *P<0.05 vs 3–10-min episodes, †P<0.05 vs AF paroxysms of 30–60 min. CL, cycle length; EG, excitable gap; RP, refractory period.
Changes in RP_AF During 1st Week of AF
At the stage of remodeling when AF paroxysms lasted between 3 and 10 min, the average refractory period during AF was 90.5±13.2 ms. On the 1st day that AF became persistent, the RP_AF had further shortened to a mean of 63.0±4.8 ms (P<0.05). Figure 2 is an example of the measurement of the refractory period during AF. After 24 h of burst pacing, AF lasted for 5 min. A single stimulus was applied 88 ms after a fibrillation wave passed under the pacing electrodes. At this coupling interval the stimulus failed to capture the atrium, as can be seen from the long delay between the stimulus artifact and the next activation with a normal AFCL of 110 ms (Figure 2A). The coupling interval was then slightly increased to 90 ms. In this case the stimulus captured the fibrillating atrium as can be judged by (1) the short latency between the stimulus and the response, (2) the short AFCL of 90 ms, and (3) the compensatory longer AFCL after the local capture (Figure 2B). After 8 days of burst pacing, when AF had become persistent, the RP_AF was as short as 60 ms (Figures 2C, D).

The relationship between the RP_AF and the AFCL during electrical remodeling is plotted in Figure 4. There was a clear, positive correlation between the RP_AF and AFCL with a slope of 1.32 (P<0.001).

Comparison of AERP and RP_AF
At some stage of the electrical remodeling process, when the AF paroxysms were not too short and not too long, the AERP and RP_AF could both be measured. After an average of 2.7±1.6 days of burst pacing, the AERP was 60.6±10.0 ms
compared with an RP_{AF} (ACFL 89.3±7.7 ms) of 74.0±10.3 ms (P<0.01). During control conditions and after 48 h of AF, the AERP_{200} was respectively 136±13.0 and 66.7±15.0 (P<0.05). After burst pacing for 5.0±2.2 days, AF lasted for 6–8 h and the RP_{AF} was 66.5±3.4 ms (comparable to the AERP_{200} after 2 days of AF). Figure 5 is an example of the RP_{AF} and AERP_{200} after 2 days of burst pacing: the refractory period during AF was measured as 70 ms (Figures 5A, B). At the same site, during regular pacing with an interval of 200 ms, the refractory period was 56 ms (Figure 5C).

**EGG During Transition From Paroxysmal to Persistent AF**

The relationship between the changes in RP_{AF} and ACFL is plotted in Figure 6. Immediately after the onset of burst pacing, the AFCL in the LA was 152±29.0 ms. At this stage the AF episodes only lasted for 5.0±4.6 s, which was too short to measure the RP_{AF}. After 24 h of burst pacing, when the induced AF episodes lasted between 3 and 10 min, AFCL and RP_{AF} were respectively 98.2±11.0 ms and 90.5±13.2 ms. After AF had become persistent (>24 h) the AFCL and RP_{AF} were respectively 84.9±5.2 ms and 63.0±4.8 ms. Thus, during the transition from paroxysmal to persistent AF the temporal excitable gap widened from 7.8±2.4 to 21.9±3.5 ms (P<0.05).

**Electrograms Fractionation During Transition From Paroxysmal to Persistent AF**

When the induced AF lasted for 3–10 min, the percentages of single, double and multiple potentials were 94.2±3.9%, 5.5±2.6% and 0.3±0.3%, respectively. When the induced AF lasted for more than 24 h, they were 75.6±5.5%, 15.4±3.9% and 9.0±2.8%, respectively (P<0.05) (Table). Figure 7 is an example of different degrees of fractionation of fibrillation electrograms recorded from the same 2 pairs of electrodes at different stages of remodeling. When rapid atrial pacing lasted for 12 h, the induced AF lasted for 3 min, the mean AFCL was 114.1 ms, and double potentials and multiple potentials were rarely seen (Figure 7A). With rapid atrial pacing for 48 h, AF lasted for 30 min spontaneously, the mean AFCL was 90.2 ms, and fractionation of fibrillation electrograms was recorded occasionally (Figure 7B). With rapid atrial pacing for 8 days, AF lasted for 24 h, the mean AFCL was 87.2 ms, and fractionation of fibrillation electrograms occurred more frequently (Figure 7C).

**Discussion**

**Main Findings**

The main finding of the present study is that in the process of AF-induced electrical remodeling, the atrial refractory period during fibrillation shortened more than the AFCL. As a consequence, during the first days of AF the temporal excitable gap widened progressively.

**Role of Atrial Refractoriness in the Stability of AF**

The mechanisms underlying AF induction and maintenance are not fully understood. It is well accepted that the development of AF requires both a trigger and a susceptible substrate, but the intrinsic cardiac autonomic nervous system also plays an important role in the initiation and maintenance of AF. AF that results from any mechanism causes tachycardia-induced remodeling. Even if AF is initially maintained by ectopic activity or a single-circuit reentry in a given patient, electrical remodeling creates conditions favorable to multiple-circuit reentry, which may then become the mechanism that maintains AF. Thus, multiple circuit reentry may be a final common AF mechanism in many patients. The progressive shortening of atrial refractoriness and widening of the temporal excitable gap induced by electrical remodeling provide the electrophysiologic substrate of permanent AF.

Clinical findings suggest that the occurrence of AF itself may alter atrial properties, increasing the likelihood of arrhythmia maintenance and recurrence ('AF begets AF'). Electrophysiological remodeling has been well described in experimental studies. It has been demonstrated that high rates of atrial activation because of rapid atrial pacing or induced AF decreases the atrial refractory period and reduces or even inverses the physiological rate adaptation of the refractory period. There is less agreement whether atrial electrical remodeling depresses conduction velocity and increases spatial dispersion of refractoriness. It is well established that atrial electrical remodeling is associated with a maladaptation of the refractory period to changes in heart rate. This was confirmed in our present study by measuring the AERP at different pacing rates during the process of atrial remodeling. As a consequence of the inverted rate adaptation of refractoriness, the refractory period during AF was considerably longer than the AERP measured during pacing. However, the present result disagrees with that of Shinagawa et al who showed that the refractory period during AF was shorter than the effective refractory period determined by the standard extrastimulus method (basic CL 150 ms). This discrepancy might be related to the fact that they induced AF during vagal stimulation.

Atrial refractoriness shortens rapidly during AF, with most of the effect occurring within the first 48 h after onset. In contrast, the time required for AF to become sustained is significantly longer and more variable. This has led to the suggestion that additional factors with a slower time course may play a role in the development of persistent AF. In the present study, it was found that in the early stage of atrial remodeling, the RP_{AF} decreased rapidly, but from when AF paroxysms lasted for 6–8 h continued to shorten more slowly. Our data show that the RP_{AF} continued to shorten until AF became sustained. In most studies the changes in AERP has been measured during pacing at relatively slow rates, This method does not reflect the actual atrial refractoriness during fibrillation, which is affected by the abnormal rate adaptation of refractoriness induced by atrial electrical remodeling. When the normal rate adaptation is inverted, the atrial refractory period at high rates is longer than during slower rhythms. We found that the shortening of the refractory period during AF has a longer time course than the shortening in AERP measured during pacing. This may partly explain the discrepancy between the rapid time course of atrial electrical remodeling and the slower development of persistent AF.

**Role of the Excitable Gap in the Stability of AF**

The temporal excitable gap during AF has been suggested to be a critical determinant for perpetuation and/or spontaneous termination of AF. When the excitable gap is short, fibrillation waves are more likely to die out when encountering refractory tissue. However, a short excitable gap also promotes the formation of new wavelets by bifurcation and wave breaks at sites of intra-atrial conduction block. Direct measurement of the refractory period during AF can help to better understand the role of the excitable gap for perpetuation of AF. Cardioversion of persistent AF by class IC drugs...
is associated with a 2–3-fold widening of the temporal excitable gap. In a vagally-induced AF model, termination of AF by pilsicainide was associated with widening of the excitable gap from 17±3 to 42±6 mm and enlargement of the core of reentry during AF. But the role of the temporal excitable gap in the development of persistent AF has not been evaluated to date. In the present study we found that both the AFCL and the RPAF shortened during the transition from paroxysmal to persistent AF. However, because the RPAF shortened more than the AFCL, the temporal excitable gap widened when AF became progressively more sustained. The excitable gap during persistent AF in the present study was in the same range as reported by Wijffels et al (between 23±3 and 29±10 ms).

According to Moe’s multiple-wavelet hypothesis, the stability of AF is determined by the average number of wavelets. Mapping studies have shown that the number of wavelets varies considerably during AF as a result of variation in wave formation and extinction. In the early stages of atrial remodeling, the atrial refractory period is still relatively long and the chance that a fibrillation wave will encounter refractory tissue is high, which results in a short lifespan of the fibrillation waves and unless counteracted by a high rate of new wave formation, AF will self-terminate. The widening of the excitable gap during the first days of AF might prolong the lifespan and increase the average number of fibrillation waves, which will lead to greater stability of AF. This is supported by the finding that during the transition from paroxysmal to persistent AF, the fractionation of atrial electrograms increased progressively. A previous study showed that pharmacologic cardioversion of persistent AF is associated with a decrease in atrial electrograms fractionation and increased activation linking, so the underlying mechanism may be widening of the excitable gap induced by antiarrhythmic drugs. Therefore, we suggest that a proper excitable gap window is needed to maintain AF, and AF can not perpetuate when the window is too narrow or too wide.

Study Limitations
First, only electrogram criteria were used to evaluate atrial capture, which was not verified by activation maps. However, with mapping used as the gold standard, the sensitivity, specificity, and positive and negative predictive values of the electrogram criteria are high. Second, because of beat-to-beat variation in the local fibrillation interval, the intervals of the sensed atrial electrograms were variable and the refractory period would certainly be affected by this prior to the stimulation. Previous study has shown that the RPAF is not a deterministic, but rather a probabilistic variable. We arbitrarily defined the RPAF 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. Third, our results are limited to types I and AF and may not account for type II or IV AF, because of the high disassociation of activation and highly fragmented atrial electrograms. Fourth, because the RPAF was only measured in the lateral wall of the LA, it remains unknown whether the atrial refractoriness during AF develops differently in other areas such as the right atrium, Bachmann’s bundle or the area between the pulmonary veins.

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