Repetitive 4-Week Periods of Atrial Electrical Remodeling Promote Stability of Atrial Fibrillation

Time Course of a Second Factor Involved in the Self-Perpetuation of Atrial Fibrillation

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Background—Episodes of atrial fibrillation (AF) are known to cause both a rapid reduction in atrial refractoriness (atrial electrical remodeling) and a more delayed increase in AF stability thought to be due to a so-called “second factor.” The aim of this study was to quantify the effects and time course of such a factor on AF stability in the chronic goat model.

Methods and Results—AF was maintained in 6 goats by burst atrial pacing for 3 consecutive 4-week periods separated by a mean of 6.21 days of sinus rhythm. Sinus rhythm was just sufficient for refractoriness changes to reverse fully in all goats. Atrial effective refractory period, AF inducibility, and duration of individual episodes of AF were assessed at regular intervals. There was a progressive reduction from month 1 to 2 to 3 in the mean duration of burst pacing required to induce individual episodes of AF of 60 seconds (178±251, 110±102, and 21±30 hours), 1 hour (229±224, 136±104, and 68±51 hours), and 24 hours (277±218, 192±190, and 102±51 hours; P<0.03). The frequency with which AF was induced during extrastimulus pacing increased progressively from 16.7% in month 1 to 31.7% in month 2 and 46.9% in month 3 (P<0.001).

Conclusions—Sequential 4-week periods of atrial fibrillation result in a progressive increase in AF stability independent of baseline atrial refractory period. This finding suggests the presence of a second factor in the self-perpetuation of AF with a time course comparable to that of AF-induced ultrastructural changes in the atria. (Circulation. 2004;109:1434-1439.)

Key Words: fibrillation ■ atrium ■ remodeling
AF despite full reversal of refactoriness changes between successive paroxysms. No progressive increase in AF inducibility, vulnerability, or stability was demonstrable in this study, indicating that a second factor either did not exist or had a longer onset than the 5 days selected in the protocol. The aim of the present study was to retest the above hypotheses using 4-week periods of AF. This duration of AF was chosen because it has been shown to lead to structural changes in atrial myocardium,14 a putative candidate for the second factor.

Methods
Six adult female goats (mean weight, 60.5±10 kg) were used in the study. The experiments were conducted in accordance with the project license issued by the UK Home Office under the Animals (Scientific Procedures) Act 1986. The animals were allowed free access to food and water and were unrestrained in their pens throughout the experiments.

Pacemaker Implantation
Anesthesia was induced and maintained with 3% isoflurane and a 2:1 mixture of O2 and N2O. The animals were placed in the left lateral position, and 1 g flucloxacillin was given intravenously. With an aseptic surgical technique, an incision was made in the lateral aspect of the neck to expose the right internal jugular vein. The vein was mobilized and ligated, and 3 active-fixation bipolar steroid-eluting leads were introduced via a small venotomy and positioned on the septal portion of the right atrium (2 leads) and right ventricular apex (1 lead). Sensing and threshold characteristics were determined before 1 atrial lead was connected to an Intermedics pacemaker (single chamber) and the remaining leads were connected to the atrial and ventricular channels of a Medtronic Thera pacemaker (dual chamber). The leads were secured, and pacemakers were buried subcutaneously before wound closure. An ECG telemetry unit (Data Sciences Inc) was buried subcutaneously in the right flank through a further small incision.

Study Protocol
Figure 1 shows the study protocol. After pacemaker implantation, the animals were allowed to recover for ≥5 days. The animals then began repetitive induction of AF for the first 4-week period. Specifically designed software in the dual-chamber pacemaker was used to deliver burst pacing during sinus rhythm via the right atrial lead in a manner similar to that in the originally described Allessie goat model. Whenever sinus rhythm was detected, the dual-chamber pacemaker delivered a 2-second burst of atrial pacing at 64 Hz. Burst pacing was inhibited when AF was detected. During this period, measurements were made of the atrial effective refractory period (AERP), inducibility of AF by extrastimulus pacing, AF stability (mean duration of individual AF episodes), and ventricular cycle length at t=0, 4, 8, 12, and 24 hours, twice daily until 120 hours, and then every 24 hours until the end of the 4-week protocol. At the end of the 4-week period, the goats were DC cardioverted under general anesthesia. Thereafter, AERP was measured daily until it recovered to baseline. When AERP reached baseline, repetitive induction of AF was begun for the second 4-week period. At the end of the second 4-week period, the goat was again DC cardioverted, and AERP was measured daily until recovery before the third period of AF induction was begun. After the third 4-week period, the goat was DC cardioverted, and AERP was measured daily until recovery.

Measurement of Refractory Periods
The single-chamber pacemaker was used to deliver extrastimulus pacing via the external programmer to determine AERP during a basic pacing drive of 400 ms. A standard drive train of 8 extrastimuli (S1) was followed by a single S2, all at 4 times the diastolic threshold. The initial S1-S2 coupling interval was begun at a shorter interval than the expected AERP and increased incrementally in 10-ms steps until atrial capture was achieved. The AERP was defined as the longest S1-S2 that failed to capture the atrium and was taken as the mean of 3 measurements.

Inducibility of AF
At the time of AERP measurement, the presence or absence of AF induced as a response to the shortest extrastimuli resulting in atrial capture was recorded. AF was considered to be induced if the single premature response was followed by rapid irregular atrial activity lasting >1 second on at least 2 of 3 attempts.

Duration of Induced AF
The duration of induced episodes of AF was determined (1) by use of direct telemetry of the endocardial atrial signal via the pacemaker programmer and (2) from the surface ECG telemetered via the Data Sciences implant that was continuously displayed and recorded on a personal computer. In addition, the dual-chamber pacemaker software recorded a log of time and cumulative number of bursts delivered.

Data and Statistical Analyses
Statistical analysis was performed with SPSS version 8.0. Data are expressed as mean±SD. Paired data were analyzed by use of a paired t test for parametric data and Wilcoxon’s log-rank test for nonparametric data. Changes in AF duration were analyzed by converting the data to a single measure (area under the curve). Comparison of data from the three 4-week periods was performed by analysis of variance (ANOVA). The logarithm of nonnormally distributed data was used to transform data to a normal distribution for analysis by ANOVA. A 2-tailed value of P≤0.05 was taken as significant.

Results
Stability of AF
All 6 goats successfully completed the protocol. The stability of AF increased with time over each 4-week period of burst pacing in each goat. Despite a wide variation in the onset of AF stability in individual goats, there was evidence of a progressive increase in AF stability from month 1 to 2 to 3 (Figure 2). There was a progressive reduction from month 1 to 2 to 3 in the mean duration of burst pacing required to induce individual episodes of AF of 1 second (65±133, 23±48, and 1.5±2.4 hours), 10 seconds (103±145, 83±114, and 4±4.3 hours), 60 seconds (178±251, 110±102, and 21±30 hours), 1 hour (229±242, 136±104, and 68±51 hours), 6 hours (258±220, 176±187, and 87±70 hours), and 24 hours (277±218, 192±190, and 102±75 hours; P<0.03). There was considerable variability between goats, as illustrated in Figure 3.

Inducibility of AF
AF inducibility was assessed during extrastimulus pacing at the time of AERP measurement. The frequency with which AF was induced during extrastimulus pacing increased pro-
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Changes in mean duration of burst pacing required to induce AF episodes. Results are expressed as mean of values obtained in all goats. From month 1 to 3, there is progressive decrease in duration of burst pacing required to induce all pre-specified AF durations (y axis). Probability value refers to ANOVA of area under each curve.

Atrial Refractoriness

Atrial electrical remodeling (defined as a reduction in atrial refractoriness measured during a 400-ms drive) occurred in all 6 goats in the study. Measurement of atrial refractoriness was possible in all goats during the first 12 hours of maintained AF. As the duration of AF episodes became more sustained over time, the opportunities to measure atrial refractory periods became fewer. Refractory period measurements during the 3 successive 4-week periods of maintained AF are shown in Figure 4. In common with the onset of AF

Figure 2. Changes in mean duration of burst pacing required to induce AF episodes. Results are expressed as mean of values obtained in all goats. From month 1 to 3, there is progressive decrease in duration of burst pacing required to induce all pre-specified AF durations (y axis). Probability value refers to ANOVA of area under each curve.

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stability, there was considerable variation in the time course of changes in AERP among the 6 goats (Figure 5), but overall, there was a more rapid rate of remodeling during the second and third 4-week periods. To quantify the rate of AERP shortening, we calculated the duration of burst pacing required to induce half of the total reduction in AERP observed between baseline AERP and the AERP steady state when remodeling was complete (t_{1/2}). The t_{1/2} fell from 32±31 hours in month 1 to 12±6 hours in month 2 and 7.6±7 hours in month 3 (P<0.01).

After both the first and second periods of AF, atrial refractoriness had returned to baseline values (±5%) after 6 days of sinus rhythm in all 6 goats. The baseline AERP before the onset of burst pacing was 160±23, 155±21, and 155±19 ms at months 1, 2, and 3, respectively. The mean time required for AERP to return to baseline was 144±55 hours. The mean time required for AERP to return to normal did not change over the three 1-month periods (month 1, 144 hours; month 2, 156 hours; and month 3, 132 hours; P=0.78).

**Mean Ventricular Rate in AF**
The mean ventricular rate in AF remained stable from month 1 to 3 (393±65, 436±50, and 373±23 ms; P=0.1). The mean sinus cycle length measured at 24 hours after cardioversion also remained stable from month 1 to 3 (504±76, 541±60, and 551±47 ms; P=0.42).

**Discussion**

**Main Findings**
The principal finding of the present study is that repetitive 4-week periods of maintained AF have a cumulative effect on AF stability and AF inducibility that is independent of baseline atrial refractoriness. This cumulative effect has been shown previously to be absent in a similar experiment using 5-day periods of AF; ie, there is a second factor involved in the self-perpetuation of AF that takes between 5 days and 4 weeks to develop. Furthermore, the effects of this factor(s) on AF stability are progressive over a 3-month period.

**What Is the Second Factor?**
In this study, care was taken to avoid repeated cardiac instrumentation and/or tissue biopsy because such procedures would have complicated interpretation of the sequential measurements of AF stability. Consequently, the question of the precise structural and ultrastructural correlates of this second factor remains unresolved. What this study was able to show, however, was the time course and electrophysiological correlates of this factor and insight into the possible mechanism whereby it exerts its effects (see below).

**Structural Changes**
Recent work has focused attention on the structural and ultrastructural changes that occur in the atria as a response to AF (so-called structural remodeling). The first such
structural changes seen in the goat model occur after 1 week of AF and consist of redistribution of heterochromatin within the nucleus. Thereafter, progressive myolytic changes are evident until, after 16 weeks of AF, half of the atrial myocytes examined reveal evidence of myolysis.17 The fact that the effects of the second factor in the present study are progressive over a 3-month period is consistent with the possibility of such structural changes as a cause. It has been suggested that these changes may underlie the AF-induced atrial dysfunction observed in humans with AF.18 Diminished contractile function has been shown to lead to increased passive stretch and atrial dilatation, which in turn have been shown to increase the likelihood or stability of AF. More recent work suggests, however, that the principal cause of AF-induced atrial dysfunction is the downregulation of L-type calcium current and the associated depressed calcium transient; consequently, the role of myolysis in increasing stability of AF is uncertain.19,20

Changes in Connexin Distribution
Persistently AF has also been shown previously to affect atrial connexin40 (Cx 40) distribution in the goat model, resulting in an increase in heterogeneity of Cx40 distribution and an overall reduction in Cx40.21 The local absence of Cx40 in some areas does not affect overall intra-atrial conduction velocity. This is perhaps not surprising given the computer simulations suggesting that gap junction conductance would have to decrease ≥100-fold before overt slow conduction occurs.22 Nevertheless, it has been postulated that AF-induced “microheterogeneities in conduction” might increase AF stability by providing small islands of intra-atrial conduction block serving as turning points for wandering electrical wavelets.22 In time course experiments, changes in Cx distribution were shown to occur by 2 weeks of maintained AF and to be progressive until ≥16 weeks21; ie, the time course of these changes is consistent with the possibility that they are the cause of the second factor in the present study.

Effects on Conduction Velocity and Dispersion of Refractoriness
A reduction in atrial refractoriness promotes stability of AF by reducing atrial wavelength. Similarly, a reduction in conduction velocity would result in a reduction in atrial wavelength, thereby increasing AF stability. However, no changes in conduction velocity have been noted in the goat model of AF,2,23 so this cannot be considered a cause of the second factor in the present experiment. Similarly, dispersion of refractoriness3 and the formation of fibrosis14 have been shown not to occur in previous studies with the atrial-paced goat model and are unlikely to be the cause of the second factor evident in this study.

Relevance of Rate of Change of Atrial Refractoriness
The time course of the onset and offset of atrial electrical remodeling (ie, days) suggests that processes involving altered gene expression of ion channel proteins may be involved. AF-induced reduction in atrial refractoriness is associated with a reduced L-type Ca\(^{2+}\) current density of approximately 70%,24–26 with parallel changes in mRNA expression for the α1c subunit of the channel.27 It is possible that despite recovery of atrial refractoriness to control values after cessation of each period of maintained AF, mRNA and protein levels of the L-type Ca\(^{2+}\) channel remain lower than in the control situation and thus promote a more rapid onset of atrial electrical remodeling when AF is again maintained. Although this would explain the earlier fall in atrial refractoriness during the second and third periods of maintained AF in the present study, the constant time of return of atrial refractoriness to baseline values between each successive period of remodeling makes it necessary to postulate a complex situation in which the relationship between refractoriness and calcium channel density is progressively altered, possibly by a more gradual upregulation or downregulation of a different ion current. A more straightforward explanation of the progressive increase in the rate of change of refractoriness with successive months of maintained AF is that it is the consequence of the progressively increased stability of AF rather than being relevant to the nature of the second factor itself. That is, the increased duration of induced episodes of AF at the beginning of the second and third periods of maintained AF results in an increased number of myocyte stimulations per unit time and an accelerated decrease in refractoriness. This effect would be independent of the nature of the second factor.

Study Limitations
It is becoming increasingly clear that there are species differences in terms of the precise cellular mechanisms of AF-induced atrial remodeling both between the animal models studied (goat and dog) and between the animal models and patients with AF. Although it is possible that the findings in this study are limited to the goat, we believe it is the best model to study the physiological correlates of the putative structural second factors that have also been studied extensively in this species. In contrast to the findings in the goat, studies in dog models of AF have shown a measurable reduction in conduction velocity and an associated decrease in sodium current in atrial myocytes, with AF duration being related to sodium current density in each dog.28 Changes in conduction velocity have been shown to have a longer time course of onset than changes in atrial refractoriness in this species, and it is possible that conduction slowing acts as a second factor in the dog.10 Patients with AF have prolonged P-wave durations immediately after cardioversion29 that recover toward normal with a longer time course than the associated AF-induced refractoriness changes. It is likely that this represents a more complicated sequence of events than the AF-induced slowing of conduction velocity seen in the dog, however, because the sodium current is not remodeled by AF in humans.28 The mechanisms of self-perpetuation of AF are likely to be even more complex in patients with structural heart disease10; in the presence of ventricular dilation and/or failure, atrial fibrosis plays a significant role in increasing AF stability.31 The complex interactions between structural changes and electrophysiological changes were not addressed in this study.
Novel Findings and Clinical Implications

The principal novel finding in this study is that the time course of development of the second factor involved in the self-perpetuation of AF in the goat model is consistent with the AF-induced structural and ultrastructural changes (myolysis and connexins) previously described by Ausma and colleagues14 and Van-der Velden et al.21 The possibility that both of these processes may be mediated by a common mechanism (calpain-mediated proteolysis32) highlights a potential target for future therapy.

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References

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