Electrical remodeling and management of atrial fibrillation : experimental studies in the chronically instrumented goat

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Electrical Remodeling and Management of Atrial Fibrillation

Electrical Remodeling and Management of Atrial Fibrillation

Experimental Studies in the Chronically Instrumented Goat

Proefschrift

ter verkrijging van de graad van doctor

aan de Universiteit Maastricht,

op gezag van de Rector Magnificus, Prof. dr. A.C. Nieuwenhuijzen Kruseman,

volgens het besluit van het College van Dekanen,

in het openbaar te verdedigen

op vrijdag 13 december 2002 om 14.00 uur

door

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*Dankzij mijn Ouders
Voor Belleke en Charles*

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Chapter 1

Introduction

Atrial Fibrillation:

The Oldest Arrhythmia - The Newest Disease.

Mattias Duytschaever, MD

"The notion of a patient continuing to live with an auricle incapable of contraction is a somewhat novel idea...it seems necessary to devote a little study to the condition"

Thomas Lewis (1902).

Natural History of Atrial Fibrillation

THE OLDEST ARRHYTHMIA

Atrial fibrillation was a cause of speculation by physicians since early times. Initially it was referred to as rebellious palpitations, delirium cordis and pulsus irregularis perpetuus.¹ In 1902, James MacKenzie provided the first insight into the mechanism of the pulsus irregularis perpetuus.² In the "Study of the Pulse", he published a report showing that during the irregular heart rhythm the jugular "A wave" was lost. This provided the first insight into the mechanism of auricular fibrillation. Similar venous and arterial pulse recordings were made by Cushny, Edmunds and Lewis in directly observed auricular fibrillation.^{3,4} In 1909, Thomas Lewis, taking advantage of Einthoven's newly developed galvanometer, was the first to establish electrocardiographically that auricular fibrillation was the cause of pulsus irregularis perpetuus (Fig.1).⁴ As for auricular fibrillation, along the way it became "atrial fibrillation" or "atrial fib" and sometimes it is respectfully referred to as the grandfather of arrhythmias.

Figure 1. Electrocardiograms recorded by Thomas Lewis showing sinus rhythm in a normal patient (upper panel) and auricular fibrillation in another patient (lower panel). Fibrillating (f) waves are demonstrated.

THE MOST COMMON ARRHYTHMIA

With 500 million people in the European Union, and a prevalence rate approaching 2%, the number of people with AF can be estimated to be around 10 million.^{5,6} The median age of patients is 75 years with 84% are older than 65 years.⁷ The incidence and

prevalence of AF is expected to increase as the global demographic tide will result in a marked increase of the elderly population.⁸ Indeed, Braunwald, in his Shattuck lecture, referred to the growing “epidemic” of AF.⁹ The genesis of this epidemic is not only due to changes in the age-distribution of the population. The Framingham study found an increasing prevalence of AF even after adjusting for age.¹⁰ Underlying explanations remain speculative and may be multi-factorial. Since 10 to 35% of patients with CHF develop AF, the increasing number of patients with CHF may play an important role.¹¹ Indeed, atrial fibrillation and heart failure represent the intersection of two common diseases, both of which have been labeled “new epidemics”.^{9,12} Notwithstanding the increasing numbers of patients with AF, there is a strong likelihood that the incidence and prevalence of AF are even substantially underestimated. In the Stroke Prevention in Atrial Fibrillation study (SPAF-III) approximately 45% of patients had AF detected incidentally when electrocardiography was performed for an unrelated reason.¹³ In patients with symptomatic paroxysmal AF, there is a 12:1 ratio of asymptomatic versus symptomatic episodes.¹⁴ Indeed, Camm referred to silent atrial fibrillation as being another Pandora’s box.¹⁵

A TROUBLESOME ARRHYTHMIA

Atrial fibrillation has a marked effect on morbidity and mortality. Also its socio-economic consequences due to hospital admissions, chronic disease management and disabilities are substantial. Atrial fibrillation is known to be an independent risk factor for stroke.¹⁶⁻¹⁸ Overall, it is associated with a 4- to 5-fold higher risk of stroke,¹⁸ and the major cause of embolic stroke. Besides major thromboembolism, AF is also associated with intermittent ischemic attacks or even silent cerebral infarcts. According to magnetic resonance images, 40% of AF patients have one or more cerebral infarcts.^{19,20} These may cause develop apparently unaccountable neurological symptoms, ranging from forgetfulness to frank dementia. The impact of AF on mortality was less clear. However, recently the Framingham study showed that the presence of AF *independently* increased the risk for death in men (Odds ratio:1.5) and women (Odds ratio:1.9).⁶ Especially in patients with LV dysfunction, AF was found to have a detrimental role. In the Studies Of Left Ventricular Dysfunction (SOLVD) trials, the presence of AF was a powerful independent predictor of death due to pump failure and re-hospitalization for CHF.²¹

THE NEWEST DISEASE

Although the oldest arrhythmia, for some reasons atrial fibrillation seems the newest disease. The degree of interest in AF, as distinct from other forms of arrhythmias, has

increased significantly in recent years. This is illustrated in Fig.2, in which we plotted the number of full papers on AF published in *Circulation* from 1950-2000. It is clear that especially during the last decade, research was performed on atrial fibrillation. It is difficult to say what caught the attention of the cardiologist, arrhythmologist or physiologist. One explanation for this may be the fact that the successful treatment of ventricular arrhythmia and other forms of supraventricular tachycardia turned the attention of cardiologists to the remaining challenges in arrhythmia management. More likely however is the increased awareness of the considerable prevalence and morbidity of AF and that AF is just not an acceptable alternative to normal sinus rhythm.

Number of papers in
Circulation

Figure 2. Number of papers on atrial fibrillation published in *Circulation* between 1950 and 2000. Only during the last decade, the degree of interest in AF has increased significantly.

Treatment Options in Atrial Fibrillation: From Drugs to Devices

The evolution of treatment strategies in atrial fibrillation is summarized in Fig. 3. Clearly, with time there is a gradual shift towards rhythm control with non-pharmacological means to treat atrial fibrillation.

	1900-1960	1960-1990	Last Decade
Rate Control	Digoxin	Digoxin, BBL, CCBL AV ablation	Digoxin, BBL, CCBL AV ablation AV modification
Anti-Coagulation	Vit.K- antagonist	Vit.K-antagonist	Vit.K- antagonist
Rhythm Control	Class I drugs	Class I drugs Class III drugs Electrical Cardioversion	Class I drugs New Class III drugs Electrical Cardioversion Atrial Defibrillator Atrial Surgery Focal and Linear Ablation Preventive Atrial Pacing

Figure 3. Evolution of therapeutic options in atrial fibrillation. With time there is a gradual shift towards restoration of sinus rhythm (rhythm control) and non-pharmacological means to treat atrial fibrillation.

1900-1960

In the first half of the previous century the cornerstones for pharmacological treatment of AF were digoxin, anticoagulation and quinidine. In “the management of acute cardiac emergencies” De La Chapelle et al. give a point of view on the treatment of auricular fibrillation in 1951.²² Most clinicians digitalized patients “*who show signs of heart failure or who have a rapid ventricular rate as result of auricular fibrillation*”. In addition, anticoagulation was administered “*since, with this treatment the tendency for the deposition of mural thrombi and therefore of embolism can probably be diminished.*” To restore sinus rhythm “*the initial treatment should include physical and mental rest obtained by means of a sedative (preferably a barbituratic acid derivate). Quinidine is usually effective in converting paroxysmal auricular fibrillation to normal sinus rhythm and is considered by most clinicians to be the drug of choice for this purpose, regardless of the underlying type of heart disease.*”

So at the end of the first half of the previous century, treatment of AF seemed strikingly similar to actual treatment currently applied in the majority of patients with AF. Also the incremental benefit of rhythm control was acknowledged.²³ This was beautifully illustrated by Sokolow et al. who showed that cardioversion of AF resulted in marked clinical improvement, as expressed by a decrease in body weight and circulation time (Fig.4, left panel).²⁴ On the other hand, Sokolow et al already documented the pitfalls of rhythm control of AF (Fig.4, right panel). They showed that the efficacy of cardioversion by quinidine was limited by the duration of the preceding AF duration and that a high rate of recurrences of AF occurred during the first week after successful restoration of sinus rhythm.²⁴

Figure 4. Chemical cardioversion of atrial fibrillation by quinidine at the end of the first half of the previous century. Restoration of sinus rhythm resulted in marked clinical improvement, as expressed by a decrease in body weight and circulation time (left panel). Efficacy of cardioversion by quinidine depended on the preceding duration of AF (right panel).

1960-1990

A main change in the treatment of atrial fibrillation was the introduction of electrical cardioversion of cardiac arrhythmias in 1962.²⁵ This non-pharmacological approach markedly improved the efficacy of cardioversion of long lasting AF up to 90%.²⁶ However, its long-term efficacy seemed to be limited by a high rate of recurrences of AF with only 20-40% of patients maintaining sinus rhythm during the first year.²⁷⁻²⁹ Both class IA, IC and III drugs (quinidine, flecainide, propafenone, sotalol, amiodarone) were found to prevent recurrences of atrial fibrillation after cardioversion of AF.³⁰⁻³⁴ However, despite the use of antiarrhythmic drugs, the recurrence rate of AF remained as high as 25 to 50% after 1 year (Fig.5).³⁵

	Low Efficacy		Low Safety
	% of SR after 1 year	% of SR after 5 years	Mortality compared to Placebo
Quinidine	50%	18%	2.9% vs 0.8%
Flecainide	50%		7.7% vs 3.0%
Sotalol	77%		5.0% vs 3.1%
Amiodarone	53-87%		
	Hohnloser et al.	Zehender et al.	Flaker et al. (SPAAF)

Figure 5. Limited efficacy and pro-arrhythmic risks of various class I and III antiarrhythmic drug therapies for atrial fibrillation. Despite antiarrhythmic drugs, after 1 year only 50-75% of patients are still in sinus rhythm. Moreover, antiarrhythmic drugs are associated with a higher mortality compared to placebo.

Moreover, when patients were followed beyond the standard of 1 year, only 18% of patients were still in sinus rhythm 5 years after cardioversion.³⁶ More frightening was the observation that the use of antiarrhythmic drugs was associated with a higher mortality. In 1989, the CAST investigators reported an increased mortality in patients treated with class I drugs to suppress arrhythmias after myocardial infarction.³⁷⁻³⁸ Also the class III drugs (d-sotalol) were found to carry an increased risk in patients with heart failure.³⁹ In patients with congestive heart failure and atrial fibrillation, the use of antiarrhythmic drugs was associated with a higher mortality compared to placebo.^{27,40} As a result, antiarrhythmic drugs seemed to have both a low efficacy and safety for treatment of atrial fibrillation (Fig.5).

THE LAST DECADE

The limited efficacy and pro-arrhythmic risks of antiarrhythmic drug therapies for atrial fibrillation resulted in a growing interest in non-pharmacological options in the treatment of AF. Current therapeutic developments encompass new antiarrhythmic drugs, the atrial defibrillator, atrial surgery, linear and focal ablation and preventive atrial pacing (Fig.6).⁴¹ All of these interventional options are directed either at prevention or termination of atrial fibrillation. Clinical investigation of the new class III agents (dofetilide, azimilide, ibutilide, tedisamil, etc...) have shown them to offer incremental benefit over existing agents. However, the major shortfall remains the potential to cause pro-arrhythmias.⁴² The internal atrial defibrillator in theory is an excellent modality for rapid conversion of AF.⁴³ Limited data suggest that early cardioversion prolongs the interval between recurrent episodes of AF.⁴⁴ However, the drawbacks include discomfort from the shocks and immediate recurrence of AF.⁴⁵ Surgical therapy for AF (maze procedure) is highly successful in patients who require open-heart surgery for another indication.⁴⁶⁻⁴⁷ In patients with lone AF or no other indication for surgery, an intra-operative approach for the treatment of AF seems unattractive due to potential risks, discomfort and prolonged convalescence. Linear catheter ablation is still hampered by technical problems and serious complications such as cerebrovascular accidents, cardiac tamponade and loss of atrial function.⁴⁸⁻⁵⁰ Focal catheter ablation has been shown to be effective in drug-refractory patients with symptomatic paroxysmal AF.⁵¹⁻⁵⁴ However, after the initial experience, it became clear that focal ablation within pulmonary veins, pulmonary vein isolation or segmental isolation of pulmonary veins are long-lasting procedures with serious complications (pulmonary vein stenosis, thromboembolism, hemopericardium,...). Moreover, uncertainty exists about long-term results with regard to AF recurrence and PV stenosis.⁵⁵ Preventive atrial pacing was shown to decrease the likelihood of AF in patients with vagotonic AF or sinus node disease.⁵⁶⁻⁵⁷ However, atrial pacing can not be justified in patients without sinus node disease.⁵⁸

Although the wide spectrum of new approaches seems promising, they can be applied in

only a minority of patients with AF. In fact, the variety in treatment options may reflect confusion, similar to the variety of names given to AF in the beginning of the previous century (Fig.6). Despite all efforts, with a few exceptions, present-day treatment of AF can still be characterized as 'doing our best without knowing what is wrong'. To develop a more effective strategy for treatment of atrial fibrillation, one needs to further investigate the pathophysiological mechanisms underlying initiation and perpetuation of atrial fibrillation. As Thomas Lewis suggested in 1902: "The notion of a patient continuing to live with an auricle incapable of contraction is a somewhat novel idea...it seems necessary to devote a little study to the condition."

1900-1920...

- Atrial Paralysis
- Delirium Cordis
- Pulsus Irregularis Perpetuus
- Pulsus Arrhythmicus
- Auricular Fbrillation

...last decade

- New Antiarrhythmic Drugs
- Atrial Defibrillator
- Atrial Surgery
- Focal and Linear Ablation
- Preventive Atrial Pacing

Figure 6. New treatment options for AF encompass new antiarrhythmic drugs, the atrial defibrillator, atrial surgery, focal and linear ablation and preventive atrial pacing. Although the wide spectrum seems promising, the variety in treatment options probably reflects confusion, similar to the variety of names given to AF in the beginning of the previous century.

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Chapter 2

Repetitive Electrical Remodeling by Paroxysms of Atrial Fibrillation in the Goat:

**No cumulative effect on inducibility or stability
of atrial fibrillation.**

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Dorland, BS; Frans Mast, PhD; Maurits A. Allessie, MD PhD.

J Cardiovasc Electrophysiol, 1999;10:1101-1108.

Abstract

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 in the chronic goat model. The aims of this study were to examine 1) the hypothesis that an AF-induced increase in AF stability might be due to a mechanism with a longer onset and offset than that of changes in refractoriness and 2) the possibility that repeated paroxysms of maintained AF might cause a cumulative increase in AF stability independent of changes in atrial refractoriness.

Methods and Results: AF was maintained by rapid atrial pacing in 7 goats for three consecutive 5 day periods, each separated from each other by 48 hours of sinus rhythm. Assessments of atrial refractory periods, conduction velocity, AF inducibility, and duration of individual episodes of AF were attempted at intervals throughout the protocol. Forty-eight hours of sinus rhythm was just sufficient for refractoriness changes to fully reverse in all goats, with no evidence of any "residual" increase in AF inducibility. There was no significant difference between any of the 3 periods of pacing-maintained AF with regard to time to develop episodes of AF of 60 seconds duration (22.1 ± 13 hours, 23.8 ± 16 hours, 30.3 ± 29 hours), 1 hour duration (56.6 ± 28 hours, 61.3 ± 31 hours, 60.1 ± 32 hours) or 24 hours duration (84.0 ± 31 hours, 87.0 ± 33 hours, 83.5 ± 32 hours).

Conclusion: There is no evidence for a cumulative effect of AF paroxysms on AF inducibility or stability independent of changes in refractoriness. These findings highlight the importance of atrial refractoriness as a potential target for antiarrhythmic strategies aimed at inhibiting the self-perpetuation of AF.

Introduction

Atrial fibrillation (AF) has a tendency to become more persistent with time, a large percentage of patients with paroxysmal AF eventually developing chronic AF.^{1,2} Although progression of an underlying cause is one explanation for this phenomenon, in a significant proportion of cases no underlying cardiovascular disease is evident.³ Recently Wijffels and coworkers⁴ have demonstrated, in a chronically instrumented conscious goat model, that episodes of AF are self-perpetuating ("AF begets AF"). Induction of AF in this model is accompanied by a marked shortening of atrial refractory periods and loss of the normal rate-adaptation of refractoriness (termed atrial electrical remodeling). It is postulated that these effects increase AF stability by shortening atrial wavelength and increasing the potential number of reentrant wavelets in the atria.⁵ * These electrophysiological changes have a relatively rapid time course, most of the effects occurring within 48 hours of the onset of AF.⁴ In contrast, the time required for induced AF to become stable is significantly longer and more variable (from 3 to 20 days).⁴ This time delay between postulated cause and effect has led to the suggestion that additional factors with a longer time course than the shortening of atrial refractoriness may play a role in the self-perpetuation of AF. There are a number of potential candidates, including atrial dilatation and changes in atrial structure, which are likely to take more than 48 hours to develop or resolve.

The primary aim of this study was to examine the hypothesis that an AF-induced increase in AF stability might be due to a mechanism (a so-called second factor) with a longer time course than that of change in atrial refractoriness. If this were the case then it would be expected that the increase in AF vulnerability and stability that is evident following a prolonged paroxysm of AF would remain for a longer period than that required for atrial refractoriness to return to normal. A second aim of the study was to examine the possibility that repeated paroxysms of AF result in a cumulative increase in AF inducibility or stability independent of changes in atrial refractoriness. This would provide a potential means whereby recurrent paroxysms of AF might progress to persistent AF despite full reversal of refractoriness changes between successive paroxysms.

Methods

CHRONIC GOAT MODEL OF ATRIAL FIBRILLATION

Seven female goats were used for this study. Animal handling was performed according to the Dutch Law on Animal Experimentation and approved by the Animal Investigation

Committee of the University of Maastricht. The goats were anaesthetised with Thiopental (15 mg/kg) and ventilated with halothane (1-2%) and a 1:2 mixture of O₂ and N₂O. A left intercostal thoracotomy was made and the pericardium was opened to expose the heart. A silicon strip of 10 x 1.2 cm, containing 23 unipolar platinum electrodes (diameter 1.5mm, interelectrode distance 6-10mm) was guided through the anterior transverse sinus between the atria and the aortic root and sutured to the tips of both atrial appendages. In addition, two smaller silicon plaques, each containing 30 electrodes, were sutured to the lateral walls of the right and left atria (interelectrode distance 5mm). After approximation of the pericardium and closure of the thorax, the electrode leads were tunnelled subcutaneously to the neck and exteriorised by three 30-pin connectors. Three silver plates (diameter 25mm) were left subcutaneously to serve as grounding and indifferent electrodes and to record a precordial electrocardiogram. Post-operatively the animals received buprenorphine (Temgesic) for 1-3 days. Ampicillin (1000mg) was given prophylactically both before and after surgery. Approximately 2 weeks after surgery the goats were connected to an external automatic atrial fibrillator. The external automatic fibrillator consisted of a personal computer connected to a stimulator (Medtronic SP3084). The software continuously analysed one of the atrial electrograms and as soon as sinus rhythm was detected, a 1-second burst of biphasic stimuli (duration 2 ms, interval 20 ms, strength 4 times diastolic threshold) was delivered to a pair of atrial electrodes. By these automatic bursts of stimuli, atrial fibrillation could be maintained continuously 24 hours a day as has been previously described.⁴

STUDY PROTOCOL

The protocol for the study consisted of the maintenance of AF for three consecutive 5 day periods, each separated from each other by a weekend (48 hours) of sinus rhythm. Pilot studies had indicated that after 5-days of maintained AF, a period of 36-48 hours of sinus rhythm was required for refractoriness to return to control. Assessment of the atrial refractory period, conduction velocity, AF cycle length, and AF inducibility (right and left atria), was attempted during baseline and after 6, 12, 24, 48, 72, 96 and 120 hours of maintained AF (Figure 1). The duration of individual episodes of AF (the time between 2 atrial pacing bursts) was recorded automatically on a personal computer. If AF did not terminate spontaneously within 6 hours at the end of 5 days, sinus rhythm was established by external DC cardioversion. Following return to sinus rhythm, the refractory period and AF inducibility were measured 24 and 48 hours post-cardioversion.

Figure 1. The study protocol. Arrows indicate times at which measurements of atrial refractory period, conduction velocity, AF cycle length and AF inducibility are attempted.

MEASUREMENTS

The atrial effective refractory period (AERP) was measured at sites on the left and right atria (selected on the basis of good signal recording and low stimulation thresholds) during a basic pacing drive of 400ms. A single premature stimulus (4 times threshold) was interpolated at every fifth basic interval and, starting from well within the refractory period, the S1-S2 coupling interval was incremented in steps of 1ms. The shortest S1-S2 interval resulting in a propagated atrial response was taken as the AERP. Intra-atrial conduction velocity was calculated from conduction times recorded at the atrial free wall during regular pacing (400ms drive) from the left or right atrial appendage; the inter-electrode distances calculated from the known interelectrode dimensions of the silicon electrode plaques. Mean atrial fibrillation cycle length (AFCL) was determined during induced AF from a sample of 300 cycles using an automatic detection algorithm which was validated by periodic manual confirmation and continuous display of AF interval histograms.

The inducibility of AF was assessed by the response to single premature stimuli delivered during a 400ms drive at the AERP. Atrial fibrillation was considered to be induced if the single premature stimulus was followed by rapid irregular atrial activity lasting for more than one second on at least 2 of 3 attempts.

DATA ANALYSIS

Data are presented as mean $\pm$ standard deviation. Statistical analysis of the paired data was performed using the Student's t-test and in the case of multiple statistical comparisons the p value was corrected by multiplying it with the number of comparisons. A corrected p value of less than 0.05 was considered to be statistically significant.

Results

ATRIAL REFRACTORINESS

Atrial electrical remodeling (defined in this study as a reduction in atrial refractoriness measured during a 400 ms drive) occurred in all 7 goats in the study, the greatest reduction in refractoriness occurring within the first 24 hours. Measurements of refractory periods were possible in all goats for the first 24 hours of maintained AF. Because the duration of AF episodes became more sustained after this time, the opportunities to measure refractory periods became less. As a result, in only 4 of 7 goats could atrial refractory periods be measured at 48 and 72 hours, in 2 of 7 at 96 hours and in only one goat at the end of the 5 day period of AF. In Figure 2 refractory period measurements at the right and left atria (mean values all goats) are plotted during the 3 successive 5-day periods of AF. Following both the first and second periods of AF, atrial refractoriness had returned to baseline values (+5%) after 48 hours of sinus rhythm in all 7 goats. No significant differences were seen either in the degree (absolute values achieved) or the rate of remodeling between the 3 successive periods of AF. In other words no evidence could be found of any preconditioning by previous episodes of AF on the process of atrial electrical remodeling.

Figure 2. Changes in right and left atrial refractoriness. Results are expressed as means and standard deviations of values obtained in all goats.

CONDUCTION VELOCITY

Measurements of atrial conduction velocity were limited to 4 of the 7 goats (left atrial free wall only), because of lack of high quality recordings from chronic electrodes perpendicular to the activation wave during pacing. As with measurement of atrial refractory periods, conduction velocity measurements could always be made for the first 24 hours of maintained AF (each of the 3 episodes) but thereafter, because of the high inducibility of prolonged episodes of AF, measurements became more sparse. There was no evidence of a cumulative effect of episodes of pacing maintained AF on conduction velocity in the left atrium (Figure 3). Mean conduction velocity at the start of each of the 3 periods of maintained AF was 132 ± 29 cm/s, 122 ± 29 cm/s and 121 ± 50 cm/s (no significant difference). The corresponding values after 24 hours of each of the 3 periods were 134 ± 23 , 136 ± 26 and 115 ± 36 (no significant difference).

Figure 3. Example of conduction velocity measurements over the left atrium in a single goat. There is no consistent change in conduction velocity measured prior to the start of the 3 episodes of maintained AF or after 24 hours of each of these periods.

CYCLE LENGTH OF INDUCED AF

The shortening of atrial refractoriness was associated with a concomitant shortening of mean AF cycle length (Figure 4) in all goats. There was no evidence of a cumulative effect of episodes of pacing maintained AF on the cycle length of the induced episodes, either in terms of absolute changes or their rate of development.

Figure 4. Changes in right and left AF cycle length of induced episodes of AF. Results are expressed as means and standard deviations of values obtained in all goats.

INDUCIBILITY OF AF

The effects of the 3 periods of maintained AF on AF inducibility with single premature stimuli are detailed in the table. Inducibility at the beginning of the first, second and third periods was 36%, 21% and 43% respectively. The corresponding values after 12 hours of pacing were 86%, 86% and 86%, and after 24 hours of pacing were 100%, 100% and 93%. Beyond 24 hours, inducibility of AF was universally 100% whenever it could be tested. This increase in inducibility with duration of maintained AF was evident in all 3 periods of AF and there was no evidence of any "residual" increased inducibility after return of atrial refractoriness to baseline values. There was no evidence of a cumulative effect of repeated episodes of maintained AF on AF inducibility.

STABILITY OF AF

The stability of AF, as measured by the mean duration of individual episodes of induced AF, increased over each 5-day period of maintained AF in all goats, but in no goat was there evidence of a cumulative effect of repeated episodes of maintained AF. There was a wide variation in AF stability between individual goats (Figure 5). In terms of the pooled results (Figure 6) there was no significant difference between any of the 3 periods of maintained AF with regard to time to develop episodes of AF of 10 seconds duration (6.8 ± 12 hours, 10.3 ± 14 hours, 8.8 ± 11 hours), 60 seconds duration (22.1 ± 13 hours,

23.8±16 hours, 30.3±29 hours), 1 hour duration (56.6±28 hours, 61.3±31 hours, 60.1±32 hours) or 24 hours duration (84.0±31 hours, 87.0±33 hours, 83.5±32 hours). AF stability returned to baseline values within the two 48 hour periods of sinus rhythm in all goats, with mean durations of individual episodes of induced AF being 12.6±10s, 10.4±8.5 s, and 14.2±15s (not significantly different) at the beginning of each of the 3 periods of pacing-maintained AF.

Figure 5. Changes in mean duration of induced AF episodes in 2 individual goats (log scale). There is a large difference in the rate of increase in AF stability with time between the 2 goats, but there is no cumulative effect of periods of maintained AF in either individual.

Figure 6. Mean times to development of AF of 10 seconds, 1 minute, 1 hour and 24 hour durations (means and standard deviations of all goats).

Discussion

THE MAIN FINDINGS

The principal findings of this study are 1) electrical remodeling and its reversal is a rapid and reproducible process in goat atria, 2) recovery of post-AF atrial vulnerability does not have a longer time course than the time course of reversed electrical remodeling, 3) recurrent episodes of atrial remodeling do not have a cumulative effect on AF stability or inducibility independent of changes in refractoriness.

IS THERE A SECOND FACTOR?

One of the aims of this study was to examine the hypothesis that a so-called second factor with a longer time course (with respect to both onset and offset) than atrial electrical remodeling has an important role in the self-perpetuating nature of AF. If this were the case, then the increase in AF vulnerability and stability following a prolonged paroxysm of AF should endure for a longer period of time than required for reversal of atrial refractoriness. This turned out not to be the case. After a 5-day period of maintained AF, 2 days of sinus rhythm were not only sufficient for reversal of atrial refractoriness but also for full normalisation of both vulnerability and stability of AF. In other words no evidence was found for a second factor with offset kinetics slower than those of AF-induced changes in atrial refractoriness. This suggests that either 1) small progressive changes in atrial refractoriness occurring after the first 48 hours of AF are responsible for further increases in AF stability or 2) there is a second factor with slow onset but rapid offset kinetics.

The possible candidates for a second factor include atrial dilatation, increased spatial dispersion of refractoriness and changes in conduction properties of the atria. Patients with longstanding lone AF usually have dilated left atria⁷ and an increase in atrial "area" would be expected to increase AF stability according to Moe's multiple wavelet hypothesis. However patients with brief (< 2 weeks) or moderate (2-6 weeks) duration of AF do not have a measureable increase in atrial size.⁸ In contrast to atrial size, atrial mechanical systolic function is affected by short episodes of AF.⁸ Even brief (< 2 weeks) episodes of AF result in depressed peak A wave velocity and percent A-wave velocity, these changes being largely reversible within 24 hours of restoration of sinus rhythm. Longer episodes of AF result in depression of atrial systolic function that is more persistent, indicating that it is the AF rather than the DC shock that is the important factor in terms of altered systolic function. Animal studies have indicated that AF-induced atrial dysfunction has both a rapid onset and offset and is inhibited by calcium channel blockade,⁹ implicating cytosolic calcium overload as the cause. Atrial electrical remodeling is also inhibited by

calcium channel blockade, 10, 11 and it is likely that AF-induced atrial systolic dysfunction and electrical remodeling are expressions of the same phenomenon, rather than systolic dysfunction being a "second factor" with a different time course.

An increase in dispersion of atrial refractoriness might be expected to be a good candidate for a "second factor" but previous work with the goat model of AF has indicated that dispersion decreases rather than increases during pacing-maintained AF.⁴ Nevertheless refractoriness was measured in only a few sites and in theory it would only require a single site to fail to remodel (or remodel to a lesser degree) for dispersion of refractoriness to increase as AF became more prolonged. For dispersion of refractoriness to increase beyond the initial 24–48 hour period one would have to hypothesise that a) refractoriness is actually continuing to fall in some areas (i.e. not a second factor at all) or b) refractoriness increases in some cells after this period, a phenomenon that has yet to be demonstrated convincingly in the first few days of AF. Finally, one can speculate that changes in conduction properties of atrial tissue might take several days to develop and provide a means whereby AF might become increasingly stable. Studies in dog models of atrial fibrillation have shown decreasing conduction velocity in the atria¹² and prolonged surface P wave duration¹³ after prolonged periods of high rate atrial pacing. Gaspo and coworkers¹² have demonstrated that this atrial pacing-induced conduction slowing in dogs has a slower time course than that of atrial refractoriness change and suggest that this effect may constitute a "second factor". This reduction in conduction velocity is associated with a decrease in sodium current (INa) density in dog atrial myocytes, AF duration being related to INa density in each dog.¹⁴ Interestingly, no changes in atrial conduction velocity have been seen after episodes of pacing-maintained AF in goats. Wijffels and coworkers⁴ found no depressive effect of 1–4 days of AF on intra-atrial conduction velocity measured along a row of electrodes sutured onto Bachman's bundle. In the current study relatively limited measurements of left atrial conduction velocity were made but there was no evidence of a cumulative effect of AF paroxysms on this parameter. This apparent lack of effect of AF on atrial conduction characteristics may represent a real species difference in atrial electrophysiology between dogs and goats.

This study does not give information on potential contributors to AF stability for episodes of AF lasting longer than 5 days. Prolonged episodes (several weeks) of high rate atrial pacing in both dogs¹³ and goats¹⁵ have been shown to cause marked changes in atrial myocyte structure and significant increases in atrial diameter. Preliminary results of ultrastructural studies of fibrillating goat atria have indicated that it may take over one week of maintained AF before morphological changes occur.¹⁶ It would not be surprising if such changes had an additional influence on atrial electrophysiology over and above that of electrical remodeling which might contribute to the perpetuation of AF. The 5-day period was chosen because in previous experiments an increase in AF stability was clearly demonstrable after this period of maintained AF. Indeed, in 5 of the 7 goats in this study the individual episodes of induced AF were lasting longer than 24 hours by this time. So although a contribution from structural factors is likely in longer lasting AF, it is clear that such factors are not necessary for AF to become stable in the goat model.

CELLULAR MECHANISMS OF ATRIAL ELECTRICAL REMODELLING

In the original study of Wijffels et al.⁴ atrial electrical remodeling induced by maintaining AF for long periods was shown to have a rapid onset (most of the effect occurring in the first 2 days) and be completely reversible within less than one week of resumption of sinus rhythm. The current study has demonstrated that, for episodes of AF maintained for 5 days, electrical remodeling is fully reversible within 48 hours. This time course of onset and offset of electrical remodeling has implications for the underlying cellular mechanisms involved. Very short-term metabolic changes, such as changes in ionic pump activity or phosphorylation of ion channels, are unlikely to be the underlying mechanism. Similarly, long term structural changes are not likely to be implicated. In contrast, processes leading to altered gene expression of ion channel proteins would be expected to have a time course of onset and offset similar to that of electrical remodeling demonstrated in this study. This would be consistent with studies in human and dog atrial myocardium that have shown changes in ion channel expression occur in chronic AF (associated with reduced expression of Kv1.5 alpha subunit proteins and a decreased outward potassium current density)¹⁷ and after prolonged rapid atrial pacing (results in a reduction of densities of the transient outward current [Ito] and L-type calcium current [ICa]).¹⁸

RELEVANCE TO CARDIAC MEMORY

The term "cardiac memory" has been used to describe the persistence of T wave changes after termination of ventricular pacing, left bundle branch block or ventricular preexcitation¹⁹ and it has been suggested that an atrial equivalent of this phenomenon may have relevance to the mechanism of atrial electrical remodeling and its reversal following termination of atrial fibrillation. Certainly the development and persistence of altered repolarisation in response to increased rate and/or changed electrical activation sequence is a shared feature. In a canine heart model of cardiac memory such changes are abolished by pretreatment with 4-aminopyridine, a blocker of the transient outward current (Ito),²⁰ consistent with the participation of specific potassium channels in the development of the memory phenomenon. Katz²¹ discussed the modification of potassium channels as a basis for cardiac memory and summarised evidence for altered gene expression determining the structure and function of ion channels as an important mechanism for changes in cardiac repolarisation. Supporting this view are the observations that alterations of the activation sequence can lead to altered wall motion and stretch and that cell deformation can modify protein synthesis by stimulation of stretch-activated channels.²² A key aspect of the original observations of cardiac memory was the increased magnitude and slower regression of T wave changes when the heart was exposed repeatedly to the changes in ventricular activation sequence ("accumulation").¹⁹ Del Balzo and Rosen

20 demonstrated accumulation in the open-chest anaesthetised dog subjected to 3 discontinuous 20-minute periods of right ventricular pacing, each separated by a period of normal conduction sufficient for T wave changes to return to control values. They noted that the magnitude and duration of the post-pacing T wave changes increased with each successive pacing period. These findings are clearly in contrast to those of the current study, in which there was no evidence of accumulation of either the remodeling process itself (refractoriness changes) or the rate of development of stable AF. Clinical studies also indicate that cardiac memory has a much slower offset than atrial remodeling, with T wave changes remaining weeks or months after cessation of pacing. This difference in time course argues against a common mechanism.

ABSENCE OF A CUMULATIVE EFFECT OF AF PAROXYSMS

The absence of any demonstrable cumulative effect of short AF paroxysms independent of atrial electrical remodeling in the goat has significant implications. As long as there is sufficient time between paroxysms of AF to allow atrial refractoriness to return to normal, the substrate for AF is not altered and paroxysmal AF would not be expected to progress to chronic AF. In addition to the possible clinical importance of these observations (see below), this high degree of reproducibility of the remodeling process and lack of cumulative effects indicates that the model is suitable for experimental study of potential inhibitors of the process.

CLINICAL IMPLICATIONS AND LIMITATIONS

The current study has demonstrated that the general concept of "AF begets AF" is only likely to apply to recurrent paroxysmal AF if there is insufficient time between paroxysms to allow for full reversal of atrial electrical remodeling. This would imply that the development of chronic or permanent AF might be prevented by aggressive treatment of patients with very frequent paroxysms of AF with therapies aimed at either increasing the time between episodes or accelerating recovery from electrical remodeling. Of course the extent to which the remodeling process, as demonstrated in the goat, applies to the clinical situation is unknown and the results of the current study (particularly in terms of time required for recovery of remodeling) cannot be extrapolated directly to humans without further clinical studies.

This study was not designed to examine the question of why some individuals (goats or humans) are more susceptible to AF-induced electrophysiological changes than others and this aspect of the process remains to be investigated.

Table 1. Effects of Maintained Atrial Fibrillation on Inducibility of Atrial Fibrillation With Single Premature Stimuli.

		FIRST PERIOD			SECOND PERIOD			THIRD PERIOD		
Goats	Pacing Site	SR	After 12 Hours	After 24 Hours	SR	After 12 Hours	After 24 Hours	SR	After 12 Hours	After 24 Hours
1	RA	-	+	+	-	+	+	-	+	+
	LA	+	+	+	+	+	+	+	+	+
2	RA	+	+	+	-	+	+	+	+	+
	LA	+	+	+	+	+	+	+	+	+
3	RA	-	+	+	-	+	+	-	+	+
	LA	-	+	+	-	+	+	-	+	+
4	RA	-	-	+	-	-	+	-	-	-
	LA	-	+	+	-	+	+	-	+	+
5	RA	-	+	+	-	+	+	-	+	+
	LA	+	+	+	+	+	+	+	+	+
6	RA	-	+	+	-	+	+	+	+	+
	LA	-	+	+	-	+	+	+	+	+
7	RA	-	-	+	-	-	+	-	-	+
	LA	+	+	+	-	+	+	-	+	+
All Goats		5/14 (36%)	12/14 (86%)	14/14 (100%)	3/14 (21%)	12/14 (86%)	14/14 (100%)	6/14 (43%)	12/14 (86%)	13/14 (93%)

+ = inducible; - = noninducible; LA = left atrium; RA = right atrium; SR = sinus rhythm.

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Chapter 3

Widening of the Window of Inducibility of AF by Electrical Remodeling: Time course and Mechanisms.

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Abstract

Background: Electrical remodeling of the atria due to fibrillation (AF) creates a substrate for perpetuation of AF. In the present study we evaluated the effect of electrical remodeling on the window of inducibility (WI-AF) and the substrate of initiation of AF by single premature beats.

Methods: In 7 goats AF was maintained for 2 days by burst pacing. After 24 and 48h of electrical remodeling WI-AF was determined at 7 atrial sites at the right (RA) and left atrium (LA) and Bachmann's bundle (BB). During regular pacing (S1-S1:400ms), the upper and lower limit of inducibility of AF (ULI-AF, LLI-AF) by a single premature stimulus (S1-S2) were determined. Dispersion in atrial refractoriness (DISP-AERP) was defined by the difference between the shortest and longest AERP. The zone of conduction delay (CDZ) of premature beats was determined by mapping of RA, LA and BB.

Results: In all goats, 2 days of AF resulted in a marked (3 to 15-fold) widening of WI-AF. Due to AF-induced shortening of refractoriness, LLI-AF decreased at all sites whereas ULI-AF remained constant (± 150 ms). Widening of WI-AF was not due to an increase in DISP-AERP (66 ± 21 ms vs 76 ± 16 ms, $p=0.37$) or CDZ (73 ± 25 ms vs 88 ± 26 ms, $p=0.38$). Both in normal and remodeled atria, premature beats were associated with a similar zone of critical conduction at the mid-portion of BB. However only after 48h of AF, due to the short AERP, the presence of critical conduction at BB resulted in reentry and initiation of AF.

Conclusions: In the goat, electrical remodeling by two days of AF is associated with a marked widening of the window of inducibility of AF by premature beats. AF paroxysms could now be initiated by both short and late coupled premature beats. Widening of the window of inducibility was not related to an increase in dispersion of refractoriness or the zone of conduction delay/block of premature beats. Instead, enhanced vulnerability to AF could be explained by the higher likelihood of *uni-directional* block and reentry in remodeled atria.

Key-words: Fibrillation-Electrophysiology-Vulnerability.

Introduction

New approaches to treat human atrial fibrillation are evolving rapidly.¹ Part of these strategies (preventive atrial pacing, surgical maze, linear ablation, pulmonary vein ablation, antiarrhythmic drugs) is directed at the prevention of the *initiation* of atrial fibrillation (AF). However, despite a significant reduction of paroxysms of AF, in many cases recurrences still frequently occur. Gaining more insights into the pathophysiological mechanisms of the initiation of AF might help to increase the efficacy of prevention of AF. In humans paroxysms of AF are elicited by atrial premature beats or by a repetitively discharging focus.^{2,3} The actual mechanism of initiation of AF by these "triggers" is still unclear. Also little is known to what extent AF-induced electrical remodeling plays a role in the development of a substrate for initiation of AF.⁴

In the present study, we determined the effects of AF-induced electrical remodeling in the goat on the initiating mechanisms of AF by single premature beats. Both for right and left atrial premature beats, we determined the *window of inducibility* of AF before and after 1-2 days of atrial fibrillation. Programmed electrical stimulation was used to measure dispersion in atrial refractoriness and the zone of conduction delay of premature beats. The mechanism of initiation of AF was evaluated by mapping.

Methods

THE GOAT MODEL OF AF

Seven goats weighing between 42 and 58 kg (50 ± 6) were used for this study. Animal handling was carried out according to the European Directive for animal research. The study was approved by the ethical committee of the university of Maastricht. After sedation with Nesdonal (15mg/kg I.V.) the animals were anaesthetized by halothane (1 to 2%) and a mixture of O₂ and N₂O. The chest was opened through a left intercostal thoracotomy and three teflon felt plaques, each containing 23-30 silver electrodes, were sutured to the atria (Fig.1). A rectangular plaque (3 x 2.5 cm, 30 electrodes, inter-electrode distance 4 mm) was sutured on the free wall of the right (RA) and left atrium (LA). A strip of 6 x 1 cm, containing 23 electrodes (inter-electrode distance 6-10 mm) was pulled through the space between the aortic root and Bachmann's bundle (BB) and sutured to the tips of the atrial appendages. A silver plate was sutured subcutaneously to serve as indifferent electrode. The electrode leads were tunneled to the neck and exteriorized by three 30-pin connectors (Lemos®). Post-operatively the animals received

buprenorphine (Temgesic®) for 1-3 days. Ampicilline (1000mg) was given prophylactically once before and after surgery. The experiments were started 3-4 weeks after surgery. A fibrillation pacemaker was used to maintain atrial fibrillation for 48 hours.⁷ A custom made computer program automatically delivered 1-second bursts of stimuli (50-Hz) to a pair of atrial electrodes as soon as sinus rhythm was detected.

Figure 1. Localization of the epicardial electrodes. Stimulation was performed at 7 atrial sites at the right atrium (RA1 and RA2) and left atrium (LA1 and LA2) and the right (BBR), mid (BBM) and left (BBL) parts of Bachmann's bundle.

DATA ACQUISITION

After amplification (500x) and filtering (1-500Hz) all unipolar atrial electrograms were stored on tape. The spatial propagation of depolarization waves was reconstructed by custom made mapping software. Local activation times were determined automatically by an algorithm detecting the maximal negative slope of the electrograms. Isochrones were drawn manually at 5 or 10ms intervals.

EXPERIMENTAL PROTOCOL

In each animal, the protocol was performed before and after 24 and 48 hours of AF-induced electrical remodeling. Measurements were performed at least 10 minutes after spontaneous termination of a paroxysm of AF. The atria were paced by biphasic stimuli

of 2ms duration generated by a constant current stimulator (Medtronic SP3111). The minimal current intensity required for capture during slow pacing was taken as the stimulation threshold.

The window of inducibility of AF by single premature beats (WI-AF) was determined at 7 atrial sites (RA₁;RA₂;LA₁;LA₂;BB_R;BB_M;BB_L) (see Fig.1). During regular pacing with an interval of 400ms, premature stimuli (S1-S2, 4x threshold) were applied, starting with a coupling interval of 250ms. Each coupling interval was repeated 3 times and then decremented in steps of 5ms. Induction of AF by a single premature stimulus was defined as a rapid irregular atrial activity lasting for more than 2 seconds. The upper limit of the window of inducibility (ULI-AF) was determined as the longest S1-S2 coupling interval resulting in AF. The lower limit (LLI-AF) was defined by the shortest S1-S2 interval that induced AF.

The atrial effective refractory period (AERP) was determined during regular pacing with an S1-S1 interval of 400ms. After every 8th basic stimulus, premature stimuli (S1-S2, 4x threshold) were interpolated, starting with a coupling interval well within the refractory period. Incrementing the coupling interval in steps of 2ms, the shortest S1-S2 interval resulting in a propagated atrial response was taken as the AERP. Spatial dispersion in AERP (DISP-AERP) was defined as the difference between the longest and shortest AERP measured at the 7 pacing sites.

The zone of conduction delay of premature beats (CDZ) was determined during premature stimulation at RA₁ and LA₁. The longest S1-S2 interval associated with an intra-atrial conduction delay of >10ms, was taken as limit for conduction delay. Mapping of RA, LA and BB was used to partly reconstruct the activation pattern of the premature beats and initiation of AF.

STATISTICAL ANALYSIS

Data are presented as mean $\pm$ SD. Statistical analysis was performed by the paired Student's t test. A p-value of less than 0.05 was considered as statistically significant.

Results

WIDENING OF THE WINDOW OF INDUCIBILITY DURING 48 HOURS OF AF

During the first 48h of AF, we observed a marked widening of the window of inducibility of AF by single premature beats. A representative example during premature stimulation at RA₁ is shown in Fig.2. Due to the AF-induced shortening of atrial refractoriness, after 24 and 48h of AF paroxysms could be initiated by earlier premature beats (lowering of LLI-AF from 126ms to 96ms to 68ms). On the other hand the longest S1 S2 interval that could induce AF was not changed during the 2 days of electrical

Figure 2. Representative example of the widening of the window of inducibility of by single premature beats. The longest and shortest S1-S2 coupling intervals inducing a paroxysm of AF are shown. During the first 2 days of atrial fibrillation, WI-AF widened from 29 to 82ms.

remodeling (ULI-AF: from 155ms to 150ms to 150ms). As a result, the window of inducibility widened from 29ms during control to 54ms and 82ms after 1 and 2 days of AF. In Fig.3, the window of inducibility of AF of the right and left atrium is plotted for all goats. At the right atrium (left panel), WI-AF widened from 20 ± 14 ms to 45 ± 9 ms after 24h of AF ($p < 0.01$) and 73 ± 15 after 48h of AF ($p < 0.01$). At the left atrium (right panel), WI-AF widened from 8 ± 15 ms to 41 ± 18 ms ($p < 0.01$) and 62 ± 15 ($p < 0.01$). In both cases, widening of WI-AF was due to a significant shortening of LLI-AF ($p < 0.01$) while ULI-AF remained constant (p -value ranging from 0.30 to 0.87). In Table 1, the mean LLI-AF, ULI-AF and WI-AF are given for all 7 atrial sites. Two days of AF resulted in a 3 to more than 15-fold widening of WI-AF. In all cases widening of WI-AF was due to shortening of LLI-AF ($p < 0.01$) whereas ULI-AF remained constant. The LLI-AF was determined by the local AERP, whereas ULI-AF was independent of the site of premature stimulation. At the mid-portion of Bachmann's bundle (BB_M) premature stimuli never elicited a paroxysm of AF.

Figure 3. Progressive widening of the window of inducibility of AF (7 goats) by single premature stimuli applied at the right and left atrium.

SPATIAL DISPERSION OF REFRACTORINESS BEFORE AND AFTER REMODELING

We hypothesized that the upper limit of inducibility was determined by the longest refractory period present somewhere in the atria. In Fig.4 we plotted the mean AERP at 7 atrial sites before and after remodeling (7 goats). During control (left panel), AERP at BB_M (186 ± 9 ms) was significantly longer ($p < 0.01$) than the AERP at RA_1 (130 ± 14 ms), RA_2 (146 ± 18 ms), BB_R (148 ± 18 ms), BB_L (136 ± 24 ms), LA_2 (140 ± 14 ms) and LA_1 (138 ± 18 ms). There was no significant difference between the other 6 sites. The spatial dispersion in AERP was 66 ± 21 ms. After electrical remodeling by 48h of AF (right panel) the AERP was (equally) shortened at all sites. As a result, the AERP at the middle portion of BB (146 ± 16 ms) was still significantly longer ($p < 0.01$) than at each of the other 6 atrial sites (RA_1 : 74 ± 14 ms; RA_2 : 83 ± 17 ms; BB_R : 85 ± 16 ms; BB_L : 98 ± 33 ms; LA_2 : 84 ± 15 ms; LA_1 : 83 ± 11 ms). The spatial dispersion in AERP in the remodeled atria was not significantly different from control (76 ± 16 ms, $p = 0.37$).

Figure 4. Spatial distribution of atrial refractoriness before and after 48h of AF (7 goats). The spatial dispersion in AERP was not significantly altered after 48h of AF (76 ± 16 ms vs 66 ± 21 , $p = 0.37$). The exact location of atrial sites is illustrated in Fig.1.

CONDUCTION DELAY OF PREMATURE BEATS BEFORE AND AFTER REMODELING

In all goats, single premature beats elicited in the right and left atrium were associated with preferential conduction delay at the mid-portion of Bachmann's bundle. In the left panel of Fig.5 the A1-A2 intervals along BB (BB_R and BB_L) in response to S1-S2 stimulation at RA_1 are plotted. Due to AF-induced electrical remodeling, AERP had shortened from 120ms during control to 82ms after 48h of AF. Both in normal and remodeled atria, at long coupling intervals (>200ms) premature beats conducted without conduction delay along BB ($A1-A2_{BB_L} = A1-A2_{BB_R}$). However, whereas during control progressive conduction delay ($A1-A2_{BB_L} > A1-A2_{BB_R}$) became apparent at S1-S2 intervals <200ms, in remodeled atria conduction delay occurred only at S1-S2 intervals <160ms.

In the right panel of Fig.5, the conduction time along BB in response to S1-S2 stimulation at RA is plotted for 7 goats. The relative prematurity of the S1-S2 interval is defined as the degree of prematurity relative to the AERP (S1-S2 interval - AERP). Both during control and after 48h of AF, premature beats were associated with similar intra-atrial conduction delay. In both cases, with increasing degree of prematurity a progressive increase in conduction time from 30ms to 85ms became apparent. In normal atria, the zone of conduction delay of RA premature beats was 73 ± 25 ms compared to 88 ± 26 ms after 48h of AF ($p=0.38$). Also for LA premature beats CDZ had not changed after 48h of AF (62 ± 14 ms vs 74 ± 18 ms, $p=0.36$).

Figure 5. Left panel: representative A1-A2 curves along BB during S1-S2 pacing at RA. See text for explanation. Right panel: mean conduction time along BB for 7 goats. The prematurity of the S1-S2 interval is presented as the degree of prematurity relative to the AERP (S1-S2 interval - AERP). Both during control and after 48h of AF, premature beats were associated with similar intra-atrial conduction delay.

CRITICAL CONDUCTION AND REENTRY BEFORE AND AFTER REMODELING

In all goats we observed that due to AF-induced shortening of atrial refractoriness, in remodeled atria critical conduction at BB_M resulted more readily in reentry of the premature beat and initiation of AF. A representative example is shown in Fig.6. The unipolar electrograms were recorded at RA, BB_R , BB_M , BB_L and LA. In normal atria (left panel), a premature beat with a relative coupling interval of 50ms was associated with preferential conduction delay at BB_M , as evidenced by an increase in conduction time

Figure 6. Unipolar electrograms recorded at RA, BB_R , BB_M , BB_L , and LA. In normal atria, critical conduction at BB_M does not result in initiation of AF, whereas in remodeled atria fragmentation at BB_M is associated with proximal reentry and initiation of AF.

along BB and fragmented, low amplitude electrograms recorded at BB_M . However, despite the presence of critical conduction and fragmentation at the mid-portion of BB, this premature beat did not evoke AF. After 48h of AF (right panel), a premature beat (relative $S1-S2$: 50ms) was associated with similar conduction delay and fragmentation at BB_M . However, now critical conduction at BB_M resulted in reentry and initiation of AF proximal to the area of fragmentation at BB_M . In Fig.7, the corresponding activation maps of RA, BB and LA are shown. During regular pacing ($S1-S1$:400ms) the wavefront propagated uniformly from RA along BB to LA. The premature beats, both in normal and remodeled atria, were associated with preferential conduction delay at the mid-portion of BB_M (grey area). In both cases, the left side of BB was activated after a considerable conduction delay of >40ms. However, only in remodeled atria, critical conduction

at BB_M was associated with initiation of AF. From the activation map it can be seen that during the first beat of AF, the earliest activation occurred at BB_R proximal to area of fragmented potentials. The combination of low-voltage potentials at BB_M and early re-excitation of BB_R strongly suggests that AF was initiated by block and reentry in Bachmann's bundle. However, the activation maps can not distinguish between micro-reentry within BB and macro-reentry involving the inter-atrial septum.

The higher likelihood of reentry of premature beats in remodeled atria was observed in all goats, both during RA and LA premature stimulation. Reentry of the premature beat could be explained by the short AERP present in remodeled atria ($<100\text{ms}$). In the presence of critical conduction, a short AERP will allow more readily reentry of the premature beat proximal to the zone of critical conduction (*uni-directional block*). On the other hand, in non-remodeled atria, despite the presence of critical conduction, a long AERP ($>130\text{ms}$) will prevent reentry of the premature beat and initiation of AF (*bi-directional block*).

Figure 7. Representative activation maps of RA premature beats before and after remodeling. Both in normal and remodeled atria, premature beats were associated with critical conduction at the mid-portion of BB (grey area). In remodeled atria, during the first beat of AF, the earliest activation occurred at BB_R proximal to area of fragmented potentials.

Discussion

MAIN FINDINGS

In the present study we determined in the caprine model, the effect of 2 days of maintained atrial fibrillation on the substrate of initiation of AF by single premature beats. The main results were as follows. (1) In the goat, electrical remodeling by 2 days of AF is associated with a marked (3 to 15-fold) widening of the window of inducibility of AF by premature beats. Due to AF-induced shortening of atrial refractoriness, AF paroxysms could be initiated by shorter coupled premature beats (shortening of the lower limit of inducibility). However, electrical remodeling did not shorten the upper limit of inducibility which remained constant (± 150 ms). (2) In the goat, the mid-portion of Bachmann's bundle is an area of preferential conduction delay of premature beats. Critical conduction at the center of BB was associated with fragmented low-voltage potentials at Bachmann's bundle. The combination of low-voltage potentials together with early re-excitation proximal to this area strongly suggest that initiation of AF was due to conduction block and reentry at Bachmann's bundle. Critical conduction along Bachmann's bundle could be explained by a considerable longer refractory period at its mid-portion. (3) In the goat, electrical remodeling by 2 days of AF had no effect on the spatial dispersion of atrial refractoriness or the zone of conduction delay of premature beats. As a result, both in normal and remodeled atria, premature beats were associated with a similar zone of critical conduction at the mid-portion of BB. (4) Enhanced vulnerability to initiation of AF by "late" premature beats was due to AF-induced shortening of atrial refractoriness itself. Due to the short AERP, in remodeled atria critical conduction of premature beats resulted more readily in reentry and initiation of AF (*uni-directional block*).

WIDENING OF THE WINDOW OF INDUCIBILITY OF AF

Previous studies on the window of inducibility of AF by single premature beats were performed only in non-remodeled atria. In canine hearts, the window of inducibility of AF was 22 ± 9 ms.⁸ In patients, the "zone of induction of repetitive atrial firing" by applying single premature stimuli was 23 ± 5 ms.⁹ Widening of the window of inducibility due to atrial fibrillation implies a highly vulnerable substrate for (re-)initiation of AF in electrically remodeled atria. After cardioversion of human AF, atrial premature beats both *early and late* in diastole are expected to re-initiate paroxysms of AF. This might explain the high incidence of recurrences of atrial fibrillation during the first days after restoration of sinus rhythm.^{10,11}

Although our caprine model of AF can not emulate the more complex substrate in human atrial fibrillation, the present findings may help to guide and optimise treatment of atrial fibrillation in patients. The window of inducibility of AF seems a useful tool to quantify atrial vulnerability for AF by single ectopic beats. The lower limit of inducibility is determined by the local AERP, whereas the upper limit of inducibility was independent of the site of premature stimulation. Treatment strategies aimed at shortening of WI-AF are expected to reduce the statistical likelihood of recurrence of AF. However, it remains to be seen whether antiarrhythmic drugs, linear ablation or preventive pacing can effectively reduce the window of inducibility of human AF.¹² On the other, in patients with a large window of inducibility, the beneficial effect of a treatment shortening WI-AF might be negligible and useless. Evaluation of the window of inducibility in patients would imply Holter monitoring, body surface mapping or programmed electrical stimulation.

BACHMANN'S BUNDLE: THE "INITIATOR" OF AF

In the present study we observed preferential conduction delay at the center of Bachmann's bundle. The fragmented low-voltage potentials recorded at Bachmann's bundle could be the result of non-uniform anisotropy, slow discontinuous conduction, longitudinal dissociation or conduction block.¹³⁻¹⁵ Critical conduction along Bachmann's bundle could be explained by a considerable longer refractory period at its mid-portion. These findings imply a critical role of the mid-portion of Bachmann's bundle (roof of the inter-atrial septum) in the genesis of human atrial fibrillation. Also in human atria, the inter-atrial septum seems to play a critical role in the genesis of AF. The inter-atrial septum was found to have the longest atrial refractory period and also to be the area of preferential conduction delay of premature beats.¹⁶⁻¹⁷ More recently, it was shown that in patients with rheumatic heart disease, earliest activity during induced AF was located near the inter-atrial septum (ostium of the coronary sinus).¹⁸ Treatment strategies aimed at de-activation of this initiator are expected to prevent recurrences of AF. In a recent study in the goat, we showed that pacing at the mid-portion of Bachmann's bundle effectively reduced the propensity of right or left atrial premature beats to induce AF.¹² Also in patients preventive pacing at BB seems to have incremental benefit over pacing at RA.¹⁹⁻²⁰ Likewise, ablation of the mid-part of the inter-atrial septum was shown to reduce the likelihood of initiation of AF.²¹ However we want to emphasize that due to age, dilatation, fibrosis, etc... in patients with AF other areas than the inter-atrial septum may become the "initiator" of AF.²²⁻²³ Identification of the "initiator" in patients would imply an electrophysiological study to localize the areas of critical conduction and fragmentation and the site(s) of earliest re-activation during AF.

EFFECT OF ELECTRICAL REMODELING ON THE SUBSTRATE OF INITIATION OF AF

In the present study we observed no change in spatial dispersion of refractoriness or zone of conduction delay of premature beats after 2 days of maintained atrial fibrillation. Investigators have observed varying alterations in dispersion of AERP after maintained episodes of high atrial rates.^{6-7, 24-28} Our data are in agreement with previous studies in the goat reporting that the difference between RA and LA refractory period was not altered during remodeling.⁶⁻⁷ On the other hand, Nattel et al. showed that enhanced vulnerability due to rapid atrial pacing was due to an increase in refractoriness heterogeneity.²⁴ Also in patients with atrial fibrillation, the increase in atrial vulnerability is often ascribed to an increase in dispersion in atrial refractoriness or a widening of the zone of conduction delay of premature beats.²⁴⁻²⁸

In the present study we showed that enhanced atrial vulnerability might be due to shortening of AERP alone. Due to AF-induced shortening of AERP, critical conduction resulted more readily in reentry (uni-directional block). These findings imply that already in normal human atria (no ageing, no remodeling, ...), due to functional properties of the atrial tissue, a marked substrate for initiation of AF may already exist. However, this substrate of initiation may remain *concealed* until either shortening of atrial refractoriness (metabolic changes, dilatation, ...) or additional changes in the substrate (cardiac disease, fibrosis, ...) will result in reentry and initiation of AF.

LIMITATIONS

In the present study, atrial vulnerability and the substrate for reentry were measured by applying external extrastimuli during sinus rhythm. Since in the goat, spontaneous atrial premature beats are virtually absent, we could not study the effect of remodeling on the initiation of AF by spontaneous ectopic beats. Also we do not know whether the substrate for initiation of AF would be the same after AF of >48 hours of duration. During chronic AF other changes (like a decrease of connexin 40, an increase in size of the atrial myocytes, loss of sarcomeres, dedifferentiation, atrial dilatation etc.) might alter the substrate for initiation of AF.

The limited epicardial mapping, does not allow complete reconstruction of all the complex interactions between ectopic premature beats and the different pacing modes to prevent initiation of AF. Since no endocardial recordings were made, the three-dimensional structure of the atrial wall, which might play a role in the activation during AF, was not taken into account.

Table 1. Window of Inducibility of AF by Single Premature Stimuli.

	CONTROL (NO REMODELING)			AFTER 24H OF AF			AFTER 48H OF AF		
Pacing Sites	LLI-AF (ms)	ULI-AF (ms)	WI-AF (ms)	LLI-AF (ms)	ULI-AF (ms)	WI-AF (ms)	LLI-AF (ms)	ULI-AF (ms)	WI-AF (ms)
RA1	130±14	151±4	20±14	97±11†	145±14	45±9†	74±14†	151±7	73±15†
RA2	146±18	152±11	10±13	107±18†	144±16	39±16†	83±17†	158±20	76±22†
BBR	148±18	147±16	4±7	112±19†	145±15	31±20†	85±16†	151±11	66±13†
BBM	No AF	No AF	-	No AF	No AF	-	No AF	No AF	-
BBL	136±24	141±15	10±21	111±23†	144±19	32±25†	98±33†	139±16	43±24†
LA2	140±14	140±6	4±10	108±12†	147±13	52±40†	84±15†	143±18	59±16†
LA1	138±18	143±18	8±15	104±12†	146±20	41±18†	83±11†	145±19	62±15†

Mean values (±SD) for 6 goats; LLI-AF: lower limit of inducibility of AF; ULI-AF: upper limit of inducibility of AF; WI-AF: window of inducibility of AF; † : p<0.01 compared to control.

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Chapter 4

The Supervulnerable Phase Immediately after Termination of Atrial Fibrillation.

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Abstract

Background: Recent studies with the implantable atrial cardioverter have shown that in about 27% of the cases AF recurs almost immediately after successful cardioversion. In the present study we determined the electrophysiological properties of the caprine atrium immediately after spontaneous termination of AF both before and after 48h of AF-induced electrical remodeling.

Methods: In 8 goats, atrial effective refractory period (AERP), intra-atrial conduction velocity (CV) and atrial wavelength (WL) were measured during sinus rhythm, both before ($t=0$) and after 48 hours ($t=48$) of electrically maintained AF (baseline). After baseline, a 5-min paroxysm of AF was induced during which refractory period (RP_{AF}) was determined. AERP, CV and WL were also measured immediately after spontaneous restoration of sinus rhythm (post-AF values).

Results: Both in normal and remodeled atria, immediately after AF, AERP and CV were markedly decreased compared to baseline ($p<0.01$). In normal atria, post-AF AERP (107 ± 14 ms) gradually prolonged from its AF-value (114 ± 17 ms) to its baseline value (138 ± 13 ms). CV decreased from 130 ± 9 cm/s to 117 ± 9 cm/s. After 48 hours of AF, AERP had shortened to 74 ± 8 ms. RP_{AF} was 89 ± 9 ms. Surprisingly, immediately after termination of AF, AERP was further shortened to 58 ± 6 ms ($p<0.01$). Post-AF CV decreased from 136 ± 11 cm/s to 122 ± 10 cm/s ($p<0.01$). As a result, the post-AF WL became as short as 7.1 ± 1 cm. These changes were transient and all parameters gradually returned to baseline within 1-2 minutes after conversion of AF.

Conclusions: Due to a combined decrease in AERP and CV, a marked shortening of the atrial wavelength occurs during the first minutes after conversion of AF. In electrically remodeled atria, this results in a transient ultra-short value of AERP (<60 ms) and atrial wavelength (WL: 7.1 cm). These observations imply a highly vulnerable substrate for reentry immediately after termination of atrial fibrillation. During this supervulnerable phase, both early and later premature beats re-initiated immediate recurrences of AF.

Key Words: Electrophysiology-Atrium-Atrial fibrillation.

Introduction

Clinical studies with the implantable atrial defibrillator have reported a high incidence of immediate recurrences of AF (IRAF) within 1 minute after successful electrical cardioversion (27%).^{1,3} Mapping of immediate recurrence demonstrated that all IRAF's were triggered by premature ectopic beats, mostly originating from atrial foci in the pulmonary veins or crista terminalis.^{4,5} Also after spontaneous restoration of sinus rhythm, a high incidence of immediate AF recurrences has been reported.⁶ Immediate recurrences of AF after electrical or spontaneous cardioversion of AF may be due to increased atrial ectopy during the first minutes of sinus rhythm and/or to a higher vulnerability for the *initiation* of reentry.

It is not clear to what extent AF-induced electrical remodeling plays a role in the occurrence of IRAF. A progressive shortening of the atrial action potential and refractory period during the first 1-2 days of AF has been shown to increase both inducibility and persistence of AF.⁷ An additional post-cardioversion shortening of atrial refractoriness and slowing of conduction may further enhance the vulnerability of the atria. The aim of the present study was to evaluate the electrophysiological substrate for reentry during the first 5 minutes after spontaneous termination of AF, both in remodeled and non-remodeled atria.

Methods

Eight goats weighing between 40 and 58 kg (50 ± 6) were used for this study. The study was approved by the ethical committee of the university of Maastricht and animal handling was carried out according to the European Directive for animal research. After sedation with Nesdonal (15mg/kg I.V.) the animals were anaesthetized by halothane (1-2%) and a mixture of O₂ and N₂O. The chest was opened through a left intercostal thoracotomy and three teflon-felt plaque electrodes were sutured to the atria (Fig1). Two oval-shaped plaques (3.5 x 2.5 cm, 30 electrodes, inter-electrode distance 4 mm) were sutured to the free walls of the right (RA) and left atrium (LA). A strip of 6 x 1 cm, containing 23 electrodes (inter-electrode distance 6-10 mm) was pulled through the space between the aortic root and Bachmann's bundle and sutured to the tips of the atrial appendages. A small plaque containing 4 electrodes was sutured to the free wall of the left ventricle (LV). A silver plate was implanted under the skin of the thorax to serve as indifferent electrode. The electrode leads were tunneled subcutaneously to the neck and exteriorized by three 30-pin connectors (Lemos®). Post-operatively the animals received buprenorfine (Temgesic®) for 1-3 days. Ampicilline (1000mg) was given prophylactically both before and after surgery.

Figure 1. Localization of the chronically implanted epicardial electrodes (upper panel). Two oval plaques, each containing 30 electrodes, were sutured to the free wall of the RA and LA. One strip with 23 electrodes was positioned on Bachmann's bundle. In the lower panel the function of the fibrillation pacemaker is illustrated. As soon as sinus rhythm is detected, a 1 second burst of stimuli (50Hz) was delivered to induce AF. BB: Bachmann's bundle; RA: right atrium; LA: left atrium; PV: pulmonary veins; SCV: superior caval vein; ICV: inferior caval vein; PAF: paroxysmal atrial fibrillation.

DATA ACQUISITION

After the goats recovered from surgery (2-3 weeks) they were connected to a data acquisition system recording all unipolar atrial electrograms simultaneously (gain 300; bandwidth 1-500 Hz). Atrial conduction velocity was measured during sinus rhythm from the conduction time between two electrodes on Bachmann's bundle (inter-electrode distance 4-6.6cm). Local activation times were determined by a custom made algorithm for automatic detection of the intrinsic negative deflection of the electrogram. The maximal negative slope of the unipolar electrogram was taken as the local activation time. Uniform propagation along Bachmann's bundle was verified by mapping. It was defined as homogeneous propagation with synchronous activation of the two rows of electrodes. Atrial fibrillation cycle length (AFCL) was determined from a unipolar atrial electrogram from the right or left atrium by an algorithm detecting the maximal negative slope of the fibrillation electrogram. The median and 5th percentile of the AFCL were calculated from 100 consecutive AF cycles.

ELECTRICAL STIMULATION

A pair of electrodes in the center of the electrode-array at the free wall of the right ($n=5$) or left atrium ($n=3$) was used for stimulation. Biphasic stimuli of 2ms duration were applied by a constant current stimulator (Medtronic SP3111), equipped with an amplifier recording a bipolar electrogram from the pacing electrodes. The stimulation threshold (mA) was determined during slow pacing. Atrial fibrillation was maintained by a fibrillation pacemaker delivering a 1-second burst of stimuli (50 Hz, 4 $\times$ diastolic threshold) as soon as sinus rhythm recurred.^{7,8} The atrial effective refractory period (AERP) was determined during sinus rhythm, during incremental pacing and during regional entrainment of AF. During sinus rhythm, the premature stimuli were synchronized to the bipolar electrogram recorded from the pacing electrodes. After 8 sinus beats, a single stimulus (4 $\times$ threshold) was delivered and the coupling interval was incremented in steps of 2ms. The shortest coupling interval resulting in a propagated early response 4mm from the pacing site was taken as the AERP. During incremental pacing (S1-S1: 400-160ms) the AERP was determined by interpolated premature stimuli (4 $\times$ threshold). Starting well within the refractory period, after every 8th basic stimulus, the coupling interval was incremented in steps of 2ms. The shortest S1-S2 interval resulting in a propagated response was taken as the AERP. The refractory period during AF (RP_{AF}) was measured during regional entrainment of AF, as recently described.⁹ The right or left atrium was paced with an interval equal to the median AFCL (4 $\times$ diastolic threshold). Capture during AF could be differentiated from fibrillatory activity by the latency and morphology of an electrogram recorded 4mm from the pacing site. In case of capture, the electrograms show a constant morphology (low amplitude S-wave) together with a short and constant interval between stimulus and response. In addition, mapping was used to verify a radial spread of activation from the pacing site. After 8 entrained beats, a single premature stimulus was applied with a coupling interval starting well within the refractory period. The S1-S2 interval was changed in steps of 2ms. In case of capture, the premature electrogram showed a similar morphology and latency as during entrainment. The longest S1-S2 interval that failed to capture the atria was taken as the RP during entrainment of AF.⁹ Atrial vulnerability was measured by the incidence of AF during measurement of the AERP. In electrically remodeled atria (100% induction of AF), the *window* of inducibility (WI-AF) was used to quantify the vulnerability of the atria. The lower limit of the WI-AF was given by the AERP at the site of pacing, whereas the upper limit of inducibility was determined by the longest S1-S2 interval still inducing AF.

EXPERIMENTAL PROTOCOL

In each animal, the same protocol was executed twice, once before and once after electrical remodeling (48 hours of AF). Both in non-remodeled and remodeled atria, first a

baseline study was performed to determine the AERP and conduction velocity during steady-state sinus rhythm and the rate adaptation of the AERP during incremental pacing. In case of electrical remodeling, these measurements were performed at least 10 minutes after a 48 hours period of AF maintained by a fibrillation pacemaker. After the baseline study, a paroxysm of 5 minutes of AF was induced during which the AFCL and RP_{AF} were determined. In normal atria, switching off the fibrillation pacemaker resulted in prompt restoration of sinus rhythm (13 ± 14 seconds, range: 4–46). In electrically remodeled atria, AF persisted some what longer (119 ± 127 seconds, range: 54–320) before sinus rhythm resumed.

Immediately after spontaneous termination of AF, we determined the time course of changes in CV and AERP during the first 5 minutes of sinus rhythm (post-AF values). After restoration of sinus rhythm, CV was measured continuously from the conduction times along Bachmann's bundle. The change in AERP was measured 10 times during the first 5 minutes of sinus rhythm. Single extrastimuli were introduced starting with a coupling interval slightly shorter than the expected refractory period and incremented after every 4 sinus beats in steps of 2ms. For statistical analysis the exact moments of measurement of the AERP were divided in 5 classes: 10sec (range 0–20); 30sec (range 20–40); 1min (range 40–1:20); 2min (range 1:20–2:40); 5min (4–6min). If during measurement of the post-AF AERP AF of >8 sec was induced, the measurements were interrupted and the heart was kept in sinus rhythm for 10 minutes. During this period the AERP returned to its baseline sinus rhythm value. Then, a new paroxysm of AF (5 minutes) was induced and measurement of the post-AF AERP was continued. For each experiment, about 3 to 5 paroxysms were needed to collect at least 10 data points during the first 5 minutes after a paroxysm of AF. No cumulative effect of repetitive AF inductions on AERP was observed.

STATISTICAL ANALYSIS

Data are presented as mean $\pm$ SD. Statistical analysis was performed by the paired Student's t-test. A p-value of less than 0.05 was considered as statistically significant.

Results

In Fig 2 the rate adaptation of the atrial refractory period before and after 48 hours of AF is plotted for all goats. In normal atria, the AERP during baseline sinus rhythm was 138 ± 13 ms. Due to the physiological rate adaptation the refractory period shortened to

128±8ms during rapid pacing ($p<0.05$, S1-S1:180ms). During entrainment of AF (S1-S1:145±12ms) the AERP was 114±17ms (-17%, $p<0.01$, Table 1). After 48 hours of AF-induced electrical remodeling, the AERP during sinus rhythm was shortened to 74±8ms and the physiological rate adaptation was lost. Since in some goats an inversed rate adaptation was present, the average AERP slightly prolonged from 74±12ms during slow pacing (400ms) to 84±12ms during rapid pacing ($p<0.05$, S1-S1:170ms). During entrainment of AF (S1-S1:108±10ms) the AERP was 89±9ms (+21%, $p<0.01$, Table 1). There were no differences between RA and LA.

Figure 2. Rate adaptation of atrial refractoriness in normal atria (open symbols) and after 48 hours of AF (filled symbols). The leftmost data points (diamonds) were obtained during regional entrainment of AF. Due to AF-induced electrical remodeling, the AERP was markedly shortened and its normal physiological rate adaptation was lost or became even slightly inversed. As a result, the AERP during entrainment of AF was longer than during sinus rhythm. AERP: atrial effective refractory period; SR: sinus rate; AF: entrainment of atrial fibrillation.

ATRIAL WAVELENGTH IMMEDIATELY AFTER CONVERSION OF ATRIAL FIBRILLATION

Both in normal and remodeled atria, atrial refractoriness was transiently shortened immediately after termination of AF. A representative example is given in Fig 3. In the absence of electrical remodeling, immediately after termination of AF, AERP during sinus rhythm gradually prolonged from its AF value (124ms) to baseline (155ms). In electrically remodeled atria, the AERP during baseline sinus rhythm (at least 10 minutes

Figure 3. After restoration of sinus rhythm, in normal atria the AERP gradually prolongs from its AF value to the baseline value during sinus rhythm. In contrast, after 48 hours of electrical remodeling, immediately after cardioversion of AF the AERP shortened markedly from 86ms during AF to 56ms after 10 seconds of sinus rhythm. As a result, both in normal and remodeled atria, after termination of a 5-min paroxysm of AF, the AERP was approximately 20% shorter than during baseline sinus rhythm.

after spontaneous cardioversion of a 48-hours period of AF) was as short as 76ms. During entrainment of AF, the refractory period was 86ms. Immediately after termination of a new paroxysm of AF (5min), the AERP was extremely shortened to a value of 56ms. Also intra-atrial conduction was temporarily depressed immediately after termination of AF. A representative example is shown in Fig 4. Both in normal and remodeled atria, the conduction velocity of the first sinus beats after termination of a 5min paroxysm of AF was significantly decreased (110 and 116cm/s respectively). Normal atrial conduction velocity was restored rapidly within one minute of sinus rhythm (not shown).

Figure 4. Slowing of atrial conduction velocity along Bachmann's bundle immediately after termination of a 5min paroxysm of AF. Conduction velocity during sinus rhythm was calculated from the relative activation times of two electrodes on the right and left part of Bachmann's bundle (interelectrode distance 6.6 cm). Both in normal atria (CV:129cm/s) and after 48 hours of AF (CV:135cm/s), atrial conduction velocity during the first sinus beats after spontaneous conversion of AF was depressed by about 14%.

Figure 5. Mean and standard deviation (8 goats) of AERP, CV and WL during sinus rhythm both before (open symbols) and after 48h of AF (filled symbols). After 48h of AF the baseline measurements were made 20 minutes after spontaneous termination of AF. Both in normal atria and after a 48h episode of electrical remodeling, a 5min paroxysm of AF caused a transient shortening of AERP and lowering of CV. As a result, immediately after conversion to SR, the WL was temporarily shortened by approximately 30%. Due to the cumulative effects of electrical remodeling and immediate post-AF changes in AERP and CV, after 48h of AF the postcardioversion atrial wavelength shortened by >60% to as short as 7.1cm (supervulnerable phase). (* $p < 0.05$; ** $p < 0.01$).

In Fig 5 and Table 1 the AERP, CV and WL are given for all goats ($n=8$). Whereas in normal atria the AERP shortened from 138 ± 13 ms during sinus rhythm to 114 ± 17 ms during AF, in remodeled atria (48h of AF) the AERP actually slightly prolonged from 74 ± 8 ms to 89 ± 9 ms. After restoration of sinus rhythm, in normal atria the AERP gradually prolonged from its AF value to the baseline value during sinus rhythm. In contrast, after 48 hours of electrical remodeling, immediately after cardioversion of AF the AERP shortened markedly from 89 ± 9 ms during AF to 58 ± 6 ms after 10 seconds of sinus rhythm. As a result, after termination of a 5-min paroxysm of AF, the AERP was respectively 22 and 21% shorter than during baseline sinus rhythm ($p<0.01$). Both in remodeled and non-remodeled atria, this post-AF shortening of the AERP lasted only for a few minutes and after 5 minutes of sinus rhythm the AERP had already returned to its baseline value. Atrial conduction velocity directly after termination of AF was slightly decreased (-10% , $p<0.01$) both in normal and remodeled atria. Normal atrial conduction velocity was restored within 2 minutes of sinus rhythm ($p=0.32$ and $p=0.13$). Due to the post-AF changes in AERP and CV, immediately after cardioversion the atrial wavelength was shortened by respectively 30% and 29% ($p<0.01$). Due to the combination of electrical remodeling and the immediate post-AF changes in AERP and CV, after conversion of 48 hours of AF the atrial wavelength became temporarily as short as 7.1 ± 1 cm. Compared to a wavelength of 17.7cm in normal atria, immediately after cardioversion of AF the WL in remodeled atria was shortened by more than 60%. These immediate post-AF changes were not associated with alterations in sinus rate. Three to five sinus beats after cardioversion, sinus rate was equal to the rate before the paroxysm of AF.

VULNERABILITY TO ATRIAL FIBRILLATION IMMEDIATELY AFTER TERMINATION OF AF

Both in normal and remodeled atria, the vulnerability for initiation of AF was higher immediately after termination of AF. In normal atria a single early premature beat evoked a short paroxysm of AF in only 2 of 8 goats. Immediately after a 5-min paroxysm of AF, an early premature beat reinduced AF in 7 of 8 goats. In electrically remodeled atria, already during baseline, AF was induced in 8/8 goats (Table 1). To assess the difference in post-cardioversion vulnerability, the window of inducibility by single premature beats was determined within 30 seconds after termination of AF. An example is shown in Fig 6. Immediately after termination of AF the window of inducibility was widened from 74 to 94ms. In Table 2 the data are given for all 8 goats. In remodeled atria (48h of AF) the window of inducibility was 90 ± 15 ms (lower limit: 74 ± 8 ms; upper limit: 167 ± 11 ms). Immediately after spontaneous conversion of 5min of AF, the window of inducibility was increased by 20% to 109 ± 22 ms ($p<0.05$). This was due to shortening of the refractory period from 74 ± 8 ms to 57 ± 10 ms ($p<0.01$) without a concomitant change in the latest premature beat that induced AF (168 ± 19 versus 167 ± 11 , $p=0.8$).

Electrical Remodeling

Window of Inducibility: 74ms

Immediate Post-AF

Window of Inducibility: 94ms

Figure 6. Widening of the window of inducibility immediately after termination of AF. The earliest and latest premature beat (S_1 - S_2) that induced AF were taken as the lower and upper limit of the window of inducibility. After 48 hours of AF the WI-AF was 74ms (left panel). Immediately after termination of a new 5min paroxysm of AF this window of inducibility was widened to 94ms (right panel). Now, shorter coupled premature beats also induced AF (LLI-AF:66ms) whereas the upper limit of inducibility remained unchanged (160ms).

CHARACTERISTICS OF IMMEDIATE RECURRENCES OF ATRIAL FIBRILLATION (IRAF)

To study the effects of the transient shortening of the atrial refractory period after termination of AF in remodeled atria, we analyzed the AFCL during the first phase of immediate recurrences of AF. In Fig 7 an example is given of an immediate reinitiation of AF, 4 seconds after spontaneous termination of a 5min episode of AF. A single premature stimulus was applied with a coupling interval as short as 46ms. This very early premature stimulus restarted AF. Interestingly, at the beginning of IRAF, the AF cycle length was markedly shortened (65ms) to prolong again during the first 5-6 seconds of the recurrence of AF.

In the 8 remodeled atria (48h of AF) the median AFCL during the 5-min paroxysms of AF was 107 ± 9 ms (5th percentile: 90 ± 12 ms). The AERP measured during entrainment of AF was 89 ± 9 ms. Immediately after termination of AF (5 ± 3 sec), the AERP during sinus rhythm was as short as 57 ± 10 ms (-36%, $p < 0.01$). Also, the AFCL during the first second of IRAF was significantly shorter (66 ± 6 ms, $p < 0.01$). However, already after 6 seconds, AFCL had returned to its baseline value (104 ± 23 ms, $p = 0.46$). This observation strongly supports the transient shortening of the atrial refractory period immediately after termination of AF.

Paroxysmal AF

Spontaneous Cardioversion

Immediate Recurrence

Figure 7. Four seconds after spontaneous termination of a 5min paroxysm of AF, an immediate recurrence of AF was induced by a single early premature stimulus. The median AFCL during the preceding AF paroxysm was 99ms, with a 5th percentile of 80ms. Spontaneous termination of AF was preceded by a spontaneous progressive prolongation of AFCL to 180ms. Immediately after reinitiation of AF (IRAF) the AFCL was very short (65ms) indicating a shortened atrial refractory period. During the first 5-6 seconds of IRAF the AFCL progressively prolonged to its baseline value.

Discussion

IMMEDIATE RECURRENCE OF AF

Immediate recurrences of AF can occur both after internal (27%) and external (26%) electrical defibrillation.^{1,5,11-15} The time between successful cardioversion and immediate recurrence varies between 8 ± 5 seconds (range 2-20) and 16 ± 17 seconds (range 2-55).^{2,3} All IRAF's are triggered by premature ectopic beats, the coupling interval ranging between 310 and 580ms (418 ± 79). Ectopic beats with a coupling interval longer than 600ms never lead to recurrence of AF. Immediate recurrences of AF may be caused by an increase in *triggers* (enhanced atrial ectopy) or a vulnerable substrate for *initiation* of reentry during the first minutes of sinus rhythm after cardioversion.

A SUPERVULNERABLE SUBSTRATE FOR REENTRY IMMEDIATELY AFTER TERMINATION OF AF

In the present study we evaluated the electrophysiological substrate immediately after termination of AF in the caprine model. The main findings were the following: (1) During the first minutes after spontaneous termination of AF, both in normal and remodeled atria, a transient shortening of atrial refractoriness occurred. (2) These changes were associated with a transient depression of atrial conduction velocity, resulting in a marked temporal shortening of the atrial wavelength directly after termination of AF. (3) In remodeled atria, due to the cumulative effects of AF-induced electrical remodeling and immediate post-cardioversion changes, the AERP and wavelength could become as short as 58ms and 7.1cm respectively. (4) In remodeled atria, during the first minute of sinus rhythm, the window of inducibility of AF by single premature beats was markedly widened.

These observations imply that directly after termination of atrial fibrillation a highly vulnerable substrate for initiation of reentry exists.¹⁶⁻¹⁷ Especially after cardioversion of AF lasting longer than 24 hours, the cumulative effects of electrical remodeling and the immediate post-AF changes will result in a "supervulnerable phase" during the first minutes of sinus rhythm. During this supervulnerable phase, both early and later premature beats can be expected to initiate immediate recurrences of AF.

However for immediate re-initiation of AF, the spontaneous occurrence of atrial triggers is at least as important as a vulnerable substrate for reentry. This is illustrated by the fact that due to the absence of atrial ectopy, in our goat model not a single case of spontaneous IRAF was observed.

ATRIAL REFRACTORINESS

It is already known for a long time that the refractory period adapts to acute changes in cycle length.¹⁸⁻²¹ Both experimental and clinical studies have shown that at increasing heart rates the atrial refractory period becomes progressively shortened (physiological rate adaptation).^{16-17, 19-21} Although the duration of the refractory period is predominantly determined by the preceding cycle length,¹⁹ after a marked change in heart rate, it may take up to several hundred beats before a new steady-state has been reached.²²⁻²⁴ As in the studies by Daoud et al.¹⁸ and Yu et al.,²⁵ we observed that in normal atria, after cardioversion of AF it takes about 5 minutes for the refractory period to adapt to the slower sinus rhythm. The duration of the atrial refractory period is largely determined by the duration of the plateau phase of the action potential, carried predominantly by the calcium current. Calcium overload, resulting from the high rate during AF, causes inactivation of the L-type calcium channels (calcium-induced inactivation) which, in combination with the decreased gradient of Ca^{++} , will reduce the magnitude and duration of calcium inflow and shorten the plateau phase of the action potential.²⁶⁻²⁷ After conversion to sinus rhythm, it may take several minutes for the Na/Ca exchanger and the ATP-driven active Ca^{++} transport to lower the cytoplasmic Ca^{++} concentration. The gradual reduction of the calcium-induced inactivation of calcium-current will result in a progressive prolongation of the action potential.

The observed acute post-cardioversion shortening of atrial refractoriness in electrically remodeled atria was quite unexpected. As shown by several studies,^{7, 28-30} the physiological rate adaptation of the atrial refractory period is lost after long-lasting AF due to a reduction of the L-type calcium current.³¹⁻³² In the presence of a flat rate adaptation curve we expected no effect anymore of changes in heart rate on the atrial refractory period. At this moment one can only speculate about the mechanisms underlying the transient shortening of the action potential after cardioversion of remodeled atria. Changes in neurohumoral balance do not seem to play a major role since sinus rate after cardioversion was equal to the rate before the paroxysm of AF. During a sudden change in heart rate various metabolic alterations occur like changes in intra- and extracellular ion concentrations, ion pump activity, phosphorylation of ion channels, intracellular acidosis, etc.^{22, 34} It is yet unclear whether the increase in *metabolic* demand during AF may lead to atrial ischemia.³⁵

ATRIAL CONDUCTION VELOCITY

Although it is well known that at high heart rates atrial conduction velocity is decreased,^{16-17, 36} the present study is the first to measure atrial conduction velocity after transition from AF to sinus rhythm. Immediately after termination of AF conduction velocity was 10% slower than during normal sinus rhythm. After cardioversion atrial

conduction velocity was restored quickly within 1-2 minutes. The slowing of atrial conduction after AF is probably due to an increase in intracellular sodium and extracellular potassium concentration due to the high atrial rate during AF.^{37,38} The associated decrease in membrane resting potential and driving force for sodium ions will lower the upstroke velocity of the atrial action potential and consequently lower the conduction velocity of the first sinus beats. In addition, a high intracellular Ca^{2+} concentration after conversion of AF may lead to a decrease in electrical coupling of the atrial myocardium and a slowing of conduction of the atrial impulse.³⁹ It may take 1-2 minutes before the normal intracellular sodium and calcium concentrations are restored and a new metabolic steady-state is reached.

POSSIBLE CLINICAL RELEVANCE

In previous studies in the goat we measured *steady-state* changes in atrial refractoriness induced by prolonged episodes of maintained AF.⁷ It was found that atrial fibrillation itself induced a progressive shortening of the atrial refractory period from about 140 to less than 80ms (electrical remodeling).⁷ This shortening of the action potential has been shown to be due to a down regulation of the L-type calcium current (altered gene expression).^{31,33} In the present study the *transient* changes were studied occurring immediately after termination of AF. During the first minutes of sinus rhythm an additional shortening of atrial refractoriness occurred, resulting in a refractory period transiently shorter than 50ms. The transient post cardioversion shortening of refractoriness should not be confused with AF-induced electrical remodeling.¹⁸ This is clearly illustrated by the markedly different time course of recovery of the immediate post-AF shortening (minutes) compared to electrical remodeling (days).⁸

Recurrence of atrial fibrillation during the first days after electrical cardioversion has been ascribed to fibrillation-induced electrical remodeling of the atria.⁴⁰ The high incidence of *immediate* recurrences of AF (during the first minutes) can be explained by the additional transient shortening of RP described in the present study (supervulnerable phase). On the other hand, together with electrical remodeling, the supervulnerable phase may also play an important role in the persistence of AF. In patients with chronic AF, it has been shown that AF still terminates spontaneously but that AF recurs within seconds (immediate re-initiation).⁶

It still has to be shown whether prevention of post-AF shortening of refractoriness by antiarrhythmic drugs might reduce the incidence of immediate and early recurrences.⁴¹ On the other hand, the postcardioversion shortening of atrial refractoriness might also be prevented by preventing the sudden change in atrial rate after cardioversion of AF. Decremental rapid post-AF pacing might prevent the incidence of IRAF, not only by overdrive suppression of atrial ectopy, but also by preventing the immediate post-AF shortening of AERP.

LIMITATIONS

In the present study, the supervulnerable phase was evaluated after *spontaneous* termination of AF. In case of direct-current cardioversion the electrical shock may cause transient electrophysiological changes. Thus, after electrical cardioversion, the substrate for reentry and the vulnerable phase may be different than after spontaneous cardioversion of AF. Also we do not know whether the supervulnerable phase would be the same after AF of >48hours of duration. During chronic AF other changes (like a decrease of connexin 40, an increase in size of the atrial myocytes, loss of sarcomeres, dedifferentiation, atrial dilatation etc.) might alter the immediate post-AF vulnerability. Another limitation is that we could not study the incidence of spontaneous IRAF since the goat did not show any atrial ectopy. Because of this our study does not provide information about the important question whether immediately after cardioversion atrial triggers might occur more frequently or whether atrial ectopic beats are more premature.

Table 1. Atrial Electrophysiology Immediately After Termination of AF.

	AERP (ms)				CV (cm/s)				WL (cm)				INDUCIBILITY (%)	
Time	Normal	$\Delta\%$	48h AF	$\Delta\%$	Normal	$\Delta\%$	48h AF	$\Delta\%$	Normal	$\Delta\%$	48h AF	$\Delta\%$	Normal	48h AF
Baseline	138 $\pm$ 13		74 $\pm$ 8		130 $\pm$ 9		136 $\pm$ 11		17.7 $\pm$ 1		10.0 $\pm$ 1		2/8	8/8
Rapid Pacing	128 $\pm$ 8	-7% $\pm$	84 $\pm$ 12	+14% $\pm$										
AF	114 $\pm$ 17	-17% $\dagger$	89 $\pm$ 9	+21% $\dagger$	-	-	-	-	-	-	-	-	-	-
Post-AF														
10sec	107 $\pm$ 14	-22% $\dagger$	58 $\pm$ 6	-21% $\dagger$	117 $\pm$ 9	-10% $\dagger$	122 $\pm$ 10	-10% $\dagger$	12.4 $\pm$ 1	-30% $\dagger$	7.1 $\pm$ 1	-29% $\dagger$	7/8	8/8
30sec	119 $\pm$ 17	-14% $\dagger$	62 $\pm$ 6	-16% $\dagger$	123 $\pm$ 10	-6% $\dagger$	126 $\pm$ 10	-7% $\dagger$	14.2 $\pm$ 2	-19% $\dagger$	7.8 $\pm$ 1	-22% $\dagger$	5/8	8/8
1min	125 $\pm$ 16	-10% $\dagger$	65 $\pm$ 7	-12% $\dagger$	126 $\pm$ 9	-4% $\pm$	129 $\pm$ 9	-5% $\pm$	15.5 $\pm$ 2	-12% $\dagger$	8.4 $\pm$ 1	-16% $\dagger$	3/8	8/8
2min	131 $\pm$ 15	-5% $\pm$	69 $\pm$ 7	-6% $\pm$	129 $\pm$ 9	-1%	134 $\pm$ 10	-1%	16.6 $\pm$ 2	-6% $\pm$	9.2 $\pm$ 1	-8% $\pm$	2/8	8/8
5min	135 $\pm$ 14	-2%	72 $\pm$ 8	-3%	130 $\pm$ 9	0%	136 $\pm$ 11	0%	17.0 $\pm$ 2	-2%	9.7 $\pm$ 1	-3%	2/8	8/8

Mean values ($\pm$ SD) for 8 goats; AERP, atrial effective refractory period; CV, conduction velocity; WL, wavelength; $\pm$ $p < 0.05$, $\dagger$ $p < 0.01$

Table 2. Atrial Vulnerability immediately after Conversion of AF.

	AFTER 48 HOURS OF AF (REMODELING)		
	WI-AF (ms)	LLI-AF (ms)	ULI-AF (ms)
Baseline	90±15	74±12	167±11
Post-AF	109±22	57±10	168±19
Δ %	+20% (p<0.05)	-23% (p<0.01)	1% (n.s.)

WI-AF: window of inducibility of AF; LLI-AF: lower limit of inducibility of AF; ULI-AF: upper limit of inducibility of AF

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Chapter 5

Is there an Optimal Pacing Site to Prevent Atrial Fibrillation?

**An experimental study
in the chronically instrumented goat.**

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Abstract

Background: Atrial pacing is able to reduce the number of recurrences of atrial fibrillation (AF). It is unclear to what extent this effect is determined by the site(s) of pacing.

Methods: In 6 electrically remodeled goats (24h AF), the window of inducibility of AF (WI-AF) was determined by applying premature stimuli (S1-S2) at the right (RA) or left (LA) atrium (baseline values). Determination of WI-AF at RA and LA was repeated during preventive pacing at 4 sites: RA, LA, Bachmann's Bundle (BB) and RA+LA (Bi-atrial). Mapping was used to elucidate the mechanisms of prevention of AF.

Results: At baseline, WI-AF was 49 ± 9 ms at RA and 45 ± 17 ms at LA. Initiation of AF was associated with conduction block of the premature beat at BB. Preventive pacing at BB markedly shortened WI-AF at RA (25 ± 11 ms) and LA (17 ± 8 ms, $p < 0.01$). Pacing at RA only shortened WI-AF at LA (23 ± 9 ms, $p < 0.01$), whereas pacing at LA only shortened WI-AF at RA (23 ± 11 ms, $p < 0.01$). Bi-atrial pacing failed to shorten WI-AF both at RA and LA. Prevention of AF by pacing was due to prolongation of the premature interval at BB.

Conclusions: In the caprine model of AF, Bachmann's Bundle is a critical area of conduction of premature beats and initiation of AF. Pacing at BB can prevent but not completely abolish the initiation of AF by single premature beats (*shortening of the window of inducibility*). Prevention of AF by pacing is based on prolongation of the premature interval at BB thus preventing conduction block and reentry. The optimal site for preventive pacing is close to the area of block and remote from the origin of premature beats.

Key-words: Fibrillation-Electrophysiology-Pacing.

Condensed abstract

In the goat model of atrial fibrillation (AF), we compared the effect of different sites of preventive atrial pacing on the window of inducibility of AF by right and left atrial premature beats. Initiation of AF was due to critical conduction of the premature impulse at Bachmann's bundle. The optimal site of preventive pacing was close to the area of block and remote from the origin of premature beats. Preventive pacing was based on pre-excitation of the critical area of conduction.

Introduction

Atrial pacing has been shown to reduce the number of paroxysms of AF in patients with sinus node dysfunction.^{1,5} Daubert et al. demonstrated that bi-atrial pacing reduced the likelihood of atrial flutter and fibrillation in patients with advanced inter-atrial conduction delay.³ Saksena et al. showed that both single- and dual-site pacing of the right atrium markedly prolonged the arrhythmia-free interval.^{4,5} Recently, several studies have shown that after coronary artery surgery the incidence of AF is markedly reduced by atrial pacing.^{6,7} The mechanisms of prevention of AF by atrial pacing are not clear.⁸⁻¹⁰ Especially it is still uncertain to what extent the location and number of pacing sites can improve the efficacy of prevention.^{4,7,9,10}

Atrial pacing might prevent AF by inhibiting the *triggers* of AF due to prevention of bradycardia or suppression of atrial premature beats.^{2,9} In the present study we investigated the mechanisms by which atrial pacing can attenuate the *substrate* of initiation of AF by atrial premature beats. It was found that atrial pacing can reduce the window of inducibility of AF. The degree of this anti-arrhythmic effect was largely dependent on the pacing site relative to the site of origin of the premature beat and the area of intra-atrial conduction block.

Methods

GOAT MODEL OF ATRIAL FIBRILLATION

Six goats weighing between 40 and 58 kg (48 ± 7) were used for this study. Animal handling was carried out according to the European Directive for animal research. After sedation with Nesdonal, the animals were anaesthetized by halothane and a mixture of O_2 and N_2O . Two rectangular plaques (3×2.5 cm, 30 electrodes, inter-electrode distance 4 mm) were sutured on the free wall of the RA and LA. A strip of 6×1 cm, containing 23 electrodes (inter-electrode distance 6-10 mm) was pulled through the space between the aortic root and Bachmann's bundle (BB) and sutured to the tips of the atrial appendages (Fig.1). The electrode leads were tunneled to the neck and exteriorized by three 30-pin connectors. After the goats had recovered from surgery (2-3 weeks) they were connected to a data acquisition system recording all unipolar atrial electrograms simultaneously (gain 300; bandwidth 1-500 Hz). Local activation times were determined automatically by an algorithm detecting the maximal negative slope of the electrograms. Isochrones were drawn manually at 10ms intervals. Atrial pacing was performed using a stimulator generating biphasic stimuli of 2ms. In the present study, AF was maintained for 24 hours by a fibrillation pacemaker.^{11,12}

Figure 1. Localization of epicardial electrodes. Preventive pacing was performed at the right and left atrium and the middle portion of Bachmann's bundle. Premature stimuli were elicited at RA or LA.

ELECTROPHYSIOLOGICAL MEASUREMENTS

After 24h of AF, programmed electrical stimulation (S1-S2) was performed both at RA and LA to determine the window of inducibility of AF (WI-AF) at baseline (Fig.1). During regular pacing (S1-S1:400ms), premature stimuli (S1-S2, 4x threshold) were interpolated, starting with a coupling interval of 250ms. Each coupling interval was applied 3 times before it was decremented in steps of 5ms. The upper limit of inducibility (ULI-AF) was determined as the longest coupling interval (S1-S2) inducing AF (>1sec), whereas the lower limit (LLI-AF) was taken as the shortest S1-S2 interval that still induced AF. WI-AF was defined as the time window between the longest and shortest S1-S2 coupling interval inducing AF. The atrial effective refractory period (AERP) was determined at 5 sites: RA, BB_{right} , BB_{mid} , BB_{left} and LA. During regular pacing (S1-S1:400ms), premature stimuli (S1-S2, 4 x threshold) were interpolated, starting well within the expected refractory period. After every 8th basic interval, the coupling interval was incremented in steps of 2ms. The longest S1-S2 interval that failed to capture the atrium was taken as AERP.

PREVENTIVE ATRIAL PACING PROTOCOL

In each goat, the effects of different sites of atrial pacing on WI-AF were determined in random order. Pacing (S1-S1:400ms) was performed at 4 sites: RA, LA, BB_{mid} and RA+LA (Fig.1). During preventive pacing, single premature stimuli were applied at RA and LA. A stimulator was used which also recorded a bipolar atrial electrogram at the site of stimulation (Medtronic SP3111). The degree of prematurity was varied by changing the coupling interval relative to the preceding bipolar electrogram (A1-S2 interval). Starting with a coupling interval of 250ms, the A1-S2 interval was decremented in steps of 5ms. Each interval was repeated 3 times. WI-AF was defined as the time window between the longest and shortest A1-S2 coupling interval inducing AF.

STATISTICAL ANALYSIS

Data are presented as mean $\pm$ SD. Statistical analysis was performed by the paired Student's t test. WI-AF during preventive pacing (A1-S2) was compared to WI-AF at baseline (S1-S2). A p-value of less than 0.05 was considered as statistically significant.

Results

INITIATION OF AF BY SINGLE PREMATURE BEATS

A representative example of WI-AF after 24h of AF is shown in Fig.2. Due to AF-induced electrical remodeling, AERP was 94ms. Single premature stimuli with coupling intervals between 96 and 150ms all induced AF (WI-AF:54ms). In Table 1, WI-AF values at baseline are given for 6 goats. LLI-AF, determined by the AERP, was 101 ± 6 ms at RA and 108 ± 10 ms at LA. ULI-AF was similar in both atria (150 ± 6 ms and 152 ± 9 ms). Thus, at baseline WI-AF at RA (S1-S2) was 49 ± 9 ms and 45 ± 17 ms at LA. Measurement of WI-AF was highly reproducible and varied less than <5 ms during a time period of 4 hours. All premature coupling intervals in this window invariably induced AF (no gap). Initiation of AF by single premature stimuli was found to be associated with critical conduction at Bachmann's bundle. In Fig.3, a representative example is given (S1-S2 at RA:150ms). Unipolar electrograms recorded at RA, BB_{right}, BB_{mid}, BB_{left} and LA are shown together with activation maps of BB, RA and LA. During regular pacing (S1) the wavefront propagated uniformly from RA along BB to LA. During the premature beat (S2), the unipolar electrogram at BB_{mid} became fragmented and of low amplitude.

Figure 2. After 24h of electrical remodeling, AF was induced by single premature stimuli with coupling intervals between 96 and 150ms (WI-AF at baseline: 54ms).

*Initiation of AF by a Single Premature Stimulus
(S1-S2 at RA)*

Figure 3. Initiation of AF by a single premature stimulus (S_1 - S_2 : 150ms). Unipolar electrograms at RA, the right, middle and left part of BB and LA are shown together with the activation maps during initiation of AF.

From the activation map it can be seen that BB_{left} was activated after a considerable delay (A1-A2 interval at BB_{left} :197ms). During the first beat of AF, the earliest activation occurred at BB_{right} proximal to area of fragmented potentials. The combination of low-voltage potentials at BB_{mid} and early re-excitation of BB_{right} strongly suggests that AF was initiated by block and reentry in Bachmann's bundle. However, the activation maps can not distinguish between micro-reentry within BB and macro-reentry involving the inter-atrial septum.

ELECTROPHYSIOLOGICAL PROPERTIES OF BACHMANN'S BUNDLE

In all goats, complete conduction curves along BB were determined during S1-S2 stimulation at RA and LA. In Fig.4, the average A1-A2 intervals along BB are given for 6 goats during S1-S2 pacing at RA. At long coupling intervals (S1-S2:250-180ms), premature beats conducted normally along BB. However, at S1-S2 intervals <180ms, a progressive conduction delay along BB became apparent ($A1-A2_{BBleft} > A1-A2_{BBright}$, $p < 0.05$). At coupling intervals shorter than 158 ± 16 ms, the electrogram at BB_{mid} became fragmented and of low amplitude and all premature stimuli were associated with markedly prolonged A1-A2 intervals at BB_{left} ($p < 0.01$ compared to BB_{right}). Conduction delay at RA (prolongation of $A1-A2_{BBright}$) only became apparent at S1-S2 intervals shorter than 120ms. The events during premature stimulation at LA were almost an exact mirror image of pacing at RA (not shown).

Critical conduction at BB seemed to be due to a longer AERP at BB_{mid} . In Fig.4 (right panel) we plotted representative tracings of the shortest S1-S2 intervals at RA, BB_{right} , BB_{mid} , BB_{left} and LA resulting in a propagated atrial response. Clearly, at BB_{mid} AERP was markedly longer than AERP at other sites. For all goats, the average AERP at BB_{mid} was 163 ± 15 ms, significantly longer ($p < 0.05$) than in the right and left part of BB (117 ± 14 and 115 ± 22 ms) or RA (101 ± 6 ms) and LA (108 ± 10 ms).

Conduction Curves along Bachmann's bundle

Intra-atrial Dispersion in AERP

Figure 4. Left panel: conduction curves along BB during S1-S2 pacing at RA ($n=6$). At S1-S2 intervals shorter than 158 ± 16 ms, the electrogram at BBmid became fragmented and of low-voltage. In the right panel the marked spatial dispersion in AERP was illustrated.

EFFECT OF PREVENTIVE ATRIAL PACING ON THE WINDOW OF INDUCIBILITY OF AF

In Fig.5, representative tracings are shown of the effects of preventive pacing on the inducibility of AF by right and left premature stimuli (A_1 - S_2 :150ms). Without preventive pacing a premature beat with an S_1 - S_2 interval of 150ms invariably had induced AF.

Figure 5. Representative tracings of the effects of preventive pacing at different sites in case of right and left atrial premature stimuli (A_1 - S_2 :150ms).

During preventive pacing, AF was prevented in 4 specific situations. In case of premature stimuli at RA, preventive pacing at LA and BB were both effective. During premature stimulation at LA, in contrast preventive pacing at RA and BB were effective. In Fig.6, the effects of preventive pacing on WI-AF are given for all goats ($n=6$). Without preventive pacing WI-AF was 49 ± 9 ms at RA, and 45 ± 17 ms at LA (Table 1). Preventive pacing at RA was not effective against premature stimuli at RA (WI-AF: 55 ± 11 ms, $p=0.17$). On the other hand it significantly shortened WI-AF by premature stimuli at LA (23 ± 9 ms, $p<0.01$). This was due to a marked lowering of ULI-AF from 152 ± 9 ms to 122 ± 14 ms ($p<0.01$). In neither case preventive pacing affected the lower limit of inducibility (AERP) and the same degree of prematurity was achieved during all modalities of preventive pacing. Preventive pacing at LA showed the opposite effect. Now, initiation of AF by premature stimuli originating in the RA was reduced (WI-AF: 23 ± 11 ms, $p<0.01$), whereas it had no effect on induction of AF by premature stimuli at LA (WI-AF: 52 ± 10 ms, $p=0.35$). Again the shortening of WI-AF was due to a lowering of ULI-AF

(from 150 ± 6 ms to 125 ± 9 ms, $p < 0.01$). Preventive bi-atrial pacing failed to shorten WI-AF both in case of premature stimuli delivered at RA (56 ± 12 ms, $p = 0.69$) and LA (54 ± 8 ms, $p = 0.13$). Pacing at BB exerted the largest preventive effect and shortened the window of inducibility to 25 ± 11 ms (RA) and 17 ± 8 ms (LA, $p < 0.01$). Again this protective effect was due to lowering of LLI-AF to 123 ± 15 ms and 119 ± 8 ms ($p < 0.01$).

Figure 6. Effects of preventive pacing on the window of inducibility of AF during premature stimuli at RA and LA ($n=6$). Pacing at BB reduced WI-AF both in case of RA and LA premature stimuli. Bi-atrial pacing did not shorten WI-AF in either case.

MECHANISMS OF PREVENTIVE PACING

In all cases, the preventive effect of pacing was due to prevention of critical conduction of premature beats at BB. This is clearly illustrated in Fig.5. In cases in which pacing failed to prevent AF, the premature stimulus was blocked at BB_{mid} (fragmented, low-voltage potentials associated with early proximal re-excitation). When pacing prevented induction of AF, the premature impulse conducted normally along BB_{mid} (high-amplitude negative deflections). The prevention of conduction block of premature beats could be explained by marked prolongation of the premature interval at BB_{mid}. When AF was prevented A1-A2 at BB_{mid} was longer than 191 ms compared to < 156 ms when pacing failed to prevent AF.

Figure 7. Diagrammatic explanation of the prevention of initiation of AF by atrial pacing. Left: the spatial relationship between the site of preventive pacing, the site of ectopy and the area of critical conduction. j Conduction time (CT) between site of pacing and area of block, k CT between site of pacing and site of ectopy, l CT between site of ectopy and area of block. Right: the premature interval at the area of block ($A1-A2_{Block}$) is determined by the sum of the prematurity at the site of ectopy ($A1-A2_{Ectopy}$) + $CT_k - CT_j + CT_l$.

In Fig.7 the mechanisms of preventive atrial pacing are illustrated schematically. Prolongation of the premature interval at the area of block by preventive pacing is due to pre-excitation of the area of block relative to the site of ectopy (left panel). As illustrated in the right panel, the prematurity at the area of block ($A1-A2_{Block}$) is determined by the sum of the prematurity at the site of ectopy + the conduction time from the site of preventive pacing to the site of ectopy ② - the conduction time from the site of pacing to the area of block ① + the conduction time of the premature beat to the area of block ③. In Table 2, these conduction times and the resulting prolongation of the premature interval at the area of block are given for all goats. Preventive pacing at RA only prolonged the premature interval of *left* ectopic beats (by 48 ± 14 ms), whereas preventive pacing at LA only prolonged the prematurity at BB during *right* ectopy (by 39 ± 6 ms). Preventive bi-atrial pacing did not prolong the premature intervals of either right or left ectopic beats. Preventive pacing at BB, prolonged the premature interval of both right and left premature stimuli (by 38 ± 8 ms and 50 ± 15 ms, respectively) because in this case the area of block was always pre-excited relative to the site of ectopy.

Discussion

SHORTENING OF THE WINDOW OF INDUCIBILITY BY PREVENTIVE ATRIAL PACING

The main findings of the present study in the caprine model of AF show that: (1) Bachmann's bundle is an area of critical conduction of premature beats. The fragmented low-voltage potentials recorded at BB can be the result of non-uniform anisotropy, slow discontinuous conduction, longitudinal dissociation or conduction block. The combination of low-voltage potentials together with early re-excitation proximal to this area strongly suggest that initiation of AF was due to conduction block and reentry at BB. Critical conduction of premature impulses along BB can be explained by a considerable longer refractory period at the mid-portion of BB. (2) Atrial pacing can prevent but not completely abolish the initiation of AF by single premature beats. The shortening of the window of inducibility was due to prevention of AF by *late* premature beats. Atrial pacing could not prevent the induction of AF by very early premature beats. (3) In neither case pacing affected the atrial effective refractory period and the same degree of prematurity was achieved during all modalities of preventive pacing. (4) The mechanism of prevention of AF is a prolongation of the premature interval at the area of critical conduction (Bachmann's bundle). During atrial pacing, the premature interval at the area of block can be calculated by the following formula: $A1-A2_{Block} = A1-A2_{Ectopy} + (CT_{Pace\ to\ Ectopy} - CT_{Pace\ to\ Block}) + CT_{Ectopy\ to\ Block}$. (5) The optimal site for preventive pacing is close to the critical area of block and remote from the origin of premature beats. In this case the area of block is maximally pre-excited relative to the site of ectopy. Other pacing sites remote from the area of block may be as effective as long as the area of block is equally pre-excited. Bi-atrial pacing can be less effective than single-site pacing when one of the pacing sites is close to the origin of premature beats.

IS THERE AN OPTIMAL PACING SITE TO PREVENT ATRIAL FIBRILLATION?

The present findings suggest that Bachmann's bundle (area of critical conduction) may be an effective pacing site for preventive atrial pacing. This finding is in agreement with a recent clinical study by Bailin et al. showing that in patients with paroxysmal AF pacing at Bachmann's bundle had an incremental benefit (compared to right atrial pacing) in the prevention of chronic AF.¹³ However, it should be emphasized that our caprine model of AF does not emulate the more complex substrate of different types of human AF. Several differences have to be taken into consideration comparing our experimental

findings to preventive pacing in human AF. First of all, the *substrate* for initiation of AF might be very different in patients compared to normal, electrically remodeled caprine atria. Although also in patients the atrial septum, including the ostium of the coronary sinus and Bachmann's bundle, seems to be a preferential site for intra-atrial conduction delays,¹⁴⁻¹⁸ abundant patho-anatomical changes may occur in the atrial myocardium as a result of ageing, atrial dilatation and coronary artery disease.^{19,20} In such diseased atria, other areas may act as critical sites of conduction block and reentry. In addition, the *triggers* of human AF may be different from the single premature stimuli applied in our experimental study. Although also in humans single premature beats have been shown to initiate AF,^{21,22} other onset-mechanisms like multiple premature beats, focal atrial tachycardia and atrial flutter may trigger AF as well. In these cases, the efficacy and mechanism of preventive pacing may be different from the mechanisms shown in our experimental study. Finally, the beneficial effect of the observed shortening of the window of inducibility by atrial pacing may be rather limited. Since in patients with AF the window of inducibility may be wide,²² a reduction by 30 to 40ms as found in our study may exert only a negligible effect. In such case choosing an optimal site for preventive pacing might have only a minor effect on recurrences of AF.

Nevertheless, although our observations can not provide guidelines for preventive pacing in humans they may help to gain insights into the basic mechanism(s) of prevention of AF. Our findings imply that the optimal site for preventive pacing depends on the site of origin of atrial premature beats and the location of areas of critical conduction. The best pacing site may differ from case to case and therefore should be determined on an individual basis. Characterization of the nature of triggers of AF and localization of areas of critical conduction delay, may help to define the optimal site for preventive pacing.

SINGLE-SITE VERSUS MULTI-SITE PACING

Single-site pacing at the right atrium was found to reduce recurrences of AF in patients with sinus node dysfunction.^{1,2,4,5} According to our data, this might not only be due to prevention of bradycardia-dependent triggers but also by lowering the chance of initiation of AF by atrial premature beats. In our study, pacing at the right atrium was effective in preventing AF induced by *left* atrial premature beats. Since in the majority of patients with paroxysmal AF the atrial ectopic beats originate from the pulmonary veins,^{14,23} the efficacy of right atrial preventive pacing in these cases might be explained by the mechanisms as proposed in our experimental study. Clinical studies on the additional benefit of bi-atrial pacing are controversial.^{3,6,7,24} Daubert et al. showed a marked effect of bi-atrial pacing on recurrent atrial arrhythmias in patients with advanced inter-atrial conduction delay.³ After coronary artery surgery, bi-atrial pacing was found to be more as well as less effective than right atrial pacing.^{6,7} Like in our experimental study, in one clinical study, bi-atrial pacing was found to be less effective than single-site pacing in

preventing AF by premature stimuli.²⁴ Saksena et al. reported a beneficial effect of dual-site pacing in the right atrium. Pacing both at the ostium of the coronary sinus and the high right lateral atrium, prolonged the arrhythmia-free interval more than pacing at the right atrium or coronary sinus alone (195 ± 96 days vs 143 ± 110 days).⁵ These clinical studies are not necessarily conflicting with our experimental findings. In case of an optimal single pacing site multi-site pacing could be of limited value, whereas in case of a more complex atrial substrate with multiple areas of conduction delay, multisite pacing might be better than single site pacing. On the other hand, multi-site (or dual-site) pacing may also have favorable hemodynamic effects explaining their incremental benefit in the prevention of AF.²⁵

Limitations

The limited epicardial mapping, does not allow complete reconstruction of all the complex interactions between ectopic premature beats and the different pacing modes to prevent initiation of AF. Because of the absence of endocardial recordings the 3-D structure of the atria was not taken into account.

Another limitation our study is that we did not study the effects of preventive atrial pacing on initiation of AF by *spontaneous* atrial premature beats (which are virtually absent in the goat) and *multiple* atrial premature beats.

Table 1. Window of Inducibility of AF by Single Premature Beats (Baseline Values).

	LOWER LIMIT OF INDUCIBILITY (ms)		UPPER LIMIT OF INDUCIBILITY (ms)		WINDOW OF INDUCIBILITY (ms)	
Goats	S1-S2 at RA	S1-S2 at LA	S1-S2 at RA	S1-S2 at LA	S1-S2 at RA	S1-S2 at LA
1	100	108	145	150	45	42
2	104	122	150	145	46	23
3	92	96	144	164	52	68
4	96	111	150	140	54	29
5	106	112	160	160	54	48
6	105	97	150	155	45	58
Mean	101ms	108ms	150ms	152ms	49ms	45ms
SD	±6	±10	±6	±9	±9	±17

Table 2. Prolongation of the Premature Interval at the Area of Block (Bachmann's bundle) during Preventive Pacing (6 goats).

	PREVENTIVE PACING AT RA		PREVENTIVE PACING AT LA		PREVENTIVE BI-ATRIAL PACING		PREVENTIVE PACING AT BB	
	Premature Stim. at RA	Premature Stim. at LA	Premature Stim. at RA	Premature Stim. at LA	Premature Stim. at RA	Premature Stim. at LA	Premature Stim. at RA	Premature Stim. at LA
○ 1 CT _{Pace to Block}	25±4	66±10	57±9	33±13	25±4	33±13	17±3	20±6
○ 2 CT _{Pace to Ectopy}	2±1	83±7	75±4	6±3	2±1	5±4	36±10	41±10
○ 3 CT _{Ectopy to Block}	24±4	31±12	21±1	29±13	25±4	30±14	21±1	30±12
Prolongation of the Premature Interval at BB _{mid}	1ms ±1	48ms ±14	39ms ±6	2ms ±3	3ms ±2	2ms ±3	40ms ±9	50ms ±16

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Chapter 6

Pro-fibrillatory Effects of Verapamil but not of Digoxin in the Goat Model of Atrial Fibrillation.

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Abstract

Background: Verapamil (V) and digoxin (D) have been shown to modulate tachycardia-induced atrial electrical remodeling. The goal of the present study was to determine the direct effects of V and D on AF, before and after electrical remodeling.

Methods and Results: In 6 goats we measured the cycle length (AFCL) and duration of AF (DurAF) of 50 consecutive induced paroxysms, before ($t=0$) and after 24h ($t=24$) of electrical remodeling. During AF conduction velocity (CV_{AF}), refractory period (RP_{AF}) and the type of AF (I-II-III) were determined. V was administered by a loading dose of 0.1 mg/kg, followed by a continuous (2-hour) infusion of 5 μ g/kg/min. D was given intravenously as a single bolus of 0.02 mg/kg. At $t=0$ and $t=24$, D and V caused a significant slowing of the ventricular rate of $>40\%$. D had no effect on DurAF, AFCL, CV_{AF} or RP_{AF} . Infusion of V had a direct pro-arrhythmic effect. Both at $t=0$ and $t=24$, AFCL and RP_{AF} were shortened by about 15%. During acute AF, V prolonged the average duration of AF-paroxysms from 7 to 16 seconds. After 24 hours of AF, the proarrhythmic effect was much stronger. Shortly after start of the infusion (6 ± 2 min) V converted paroxysmal into sustained AF. As long as V infusion was maintained, in all goats AF no longer terminated. This effect was associated with an increase in AF-fragmentation from type I to type II-III.

Conclusions: Verapamil shortens AFCL and RP_{AF} in the presence and absence of electrical remodeling. After 24 hours it exerted a marked proarrhythmic effect and converted paroxysmal (type-I) into sustained (type-III) AF. In contrast, digoxin had no effect on rate or stability of AF.

Key Words: Atrial fibrillation- Electrical Remodeling- Verapamil- Digoxin- Pro-arrhythmia.

List of Abbreviations

AF	: Atrial Fibrillation
V	: Verapamil
D	: Digoxin
AFCL	: Atrial Fibrillation Cycle Length
RR _{AF}	: Ventricular Rate during AF
DurAF	: Duration of induced AF episodes
TypeAF	: Complexity of AF (type I-II-III)
CV ₄₀₀	: Atrial Conduction Velocity during Slow Pacing (S1-S1:400ms)
CV _{AF}	: Atrial Conduction Velocity during AF
AERP ₄₀₀	: Atrial Effective Refractory Period during Slow Pacing (S1-S1:400ms)
RP _{AF}	: Atrial Refractory Period during AF

Introduction

Digoxin and verapamil are commonly used to control the ventricular rate during atrial fibrillation (AF).¹ Recent experimental and clinical studies suggest that digoxin and verapamil might influence the natural course of AF.²⁻⁴ Tieleman et al. demonstrated that verapamil attenuated the tachycardia-induced shortening of atrial refractoriness (electrical remodeling).² In contrast, digoxin had no detectable effect on remodeling, but slightly delayed its recovery (reverse electrical remodeling).¹ Clinically, there are indications that patients treated with calcium lowering drugs have fewer relapses of AF after electrical cardioversion, whereas digoxin may increase the likelihood of recurrences.⁴

However, little is known about the direct effects of digoxin and verapamil on the electrophysiological properties of the atria and on the characteristics of atrial fibrillation. The goal of the present study was to compare the effects of both drugs on the refractory period and conduction velocity during AF and to determine the pro- and antiarrhythmic effects on rate and duration of AF. To investigate whether AF-induced electrical remodeling affected the action of digoxin and verapamil, they were administered both during acute AF (no remodeling) and after 24 hours of AF (remodeling).

Methods

THE GOAT MODEL OF ATRIAL FIBRILLATION

Six goats weighing between 42 and 74kg (56 ± 12) were used for this study. Animal handling was carried out according to the Dutch Law and the European Directive for protection of vertebral animals. Anesthesia was induced by intravenous administration of Nesdonal (15mg/kg) and maintained by ventilation with halothane (1-2%) and a mixture of O_2 and N_2O . The chest was opened through a left intercostal thoracotomy. Three teflon felt plaques, each containing 23-30 silver electrodes, were sutured to the atrial epicardium (Fig1). Two oval-shaped plaques (3.5x2.5cm, 30 electrodes, inter-electrode distance 4mm) were sutured on the free wall of the right (RA) and left atrium (LA). One strip of 6x1cm, containing 23 electrodes (inter-electrode distance 6-10mm), was pulled through the sinus between the atria and the aortic root and sutured to the atrial appendages. A plaque containing 4 electrodes was sutured on the free wall of the left ventricle (LV). A silver plate was left under the skin of the thorax to serve as indifferent electrode. The electrode leads were tunneled subcutaneously to the neck and exteriorized by three 30-pin connectors (Lemos@). Post-operatively the animals received buprenorfine (Temgesic®) for 2-4 days. Experiments were started 3-4 weeks after surgery.

Figure 1. Localization of chronically implanted electrodes. Two plaques, containing 30 electrodes were sutured on the free wall of the atria. One strip with 23 electrodes was positioned along Bachmann's bundle. In the lower panel the fibrillation pace-maker is illustrated. As soon as sinus rhythm is detected, a 50Hz burst was delivered to induce AF. In this example (no remodeling) only short episodes of AF were induced.

EXPERIMENTAL PROTOCOL

The effects of verapamil and digoxin were measured both before and after electrical remodeling by 24 hours of AF. AF was maintained by a fibrillation pacemaker. This custom-made computer program automatically delivered a 1-second burst of stimuli (50Hz, 4xdiastolic threshold) to a pair of atrial electrodes as soon as sinus rhythm was detected (Fig1). By these automatic bursts of stimuli, AF could be maintained continuously during 24 hours as has been previously described.⁵

First the effects of verapamil were studied both during normal sinus rhythm (no remodeling) and after 24 hours of AF. Then digoxin was administered both before and after AF-induced electrical remodeling. Between all 4 experiments the goats were left in sinus rhythm for at least one week. During this period the atria recovered completely from earlier remodeling and drug administration.⁶ Before each drug a baseline study was performed. First, during regular pacing (S_1S_1 :400ms) the left atrial effective refractory period ($AERP_{400}$) and conduction velocity along Bachmann's bundle (CV_{400}) were measured. The double row of electrodes on Bachmann's bundle was used to verify propagation parallel to the electrodes. Cycle length and duration of AF were measured during 50 consecutively induced episodes of AF. The mean AFCL and RR-interval were determined from 100 consecutive AF and RR cycles from unipolar left atrial and ventricular electrograms. Verapamil was administered by a loading dose of 0.1 mg/kg, followed by a continuous (2-hour) infusion of 5 μ g/kg/min. Digoxin was given intravenously as a single bolus of 0.02 mg/kg. These dosages were chosen, based on previous studies in the goat^{2,3} and because they prolonged the average RR-interval during AF to a similar extent ($\pm 45\%$). Immediately after drug administration, repeated induction of AF was continued for a period of 2 hours. The mean AFCL and RR interval were measured every 15 minutes after start of the infusion, whereas the duration of the paroxysms of AF was monitored continuously for a period of 2 hours. After 1 and 2 hours of drug administration, the atrial refractory period and conduction velocity were measured during regular pacing with an interval of 400 ms.

ELECTROPHYSIOLOGY OF AF

The type of atrial fibrillation (type_{AF}), the conduction velocity and the refractory period during AF were measured after 1 and 2 hours of drug infusion. To determine the type of AF (I-II-III)⁷ fibrillation maps were recorded from the free wall of the left and right atrium. Time windows of 4 seconds were analyzed excluding the first and last seconds of AF. The software used for mapping (custom-made) included an algorithm for automatic detection of the intrinsic negative deflections of unipolar electrograms and interactive editing of local activation times. Isochrones were drawn manually at 5-ms intervals. Type I AF was defined by broad single wavefronts propagating without apparent

conduction delay. Type II AF was characterized either by the presence of two wavelets or by lines of intra-atrial conduction block. During type III, multiple wavelets were present under the mapping array which continuously changed their size and position. During a 4 second-sample of AF (25 to 40 AF cycles) beat to beat variations in AF pattern were observed. The degree of disorganization during AF was determined by calculating the percentage of beats with a type II or III pattern.

To measure the atrial conduction velocity during AF, the fibrillation maps were triangulated and the local conduction vectors calculated from the activation times at the corners (Fig 2). When neighboring electrodes were activated almost simultaneously (either by collision or by epicardial breakthrough of fibrillation waves) high virtual conduction velocities were measured. Values of more than 140 cm/sec were discarded from analysis. The CV_{AF} histogram, obtained in this way, contained about 700-900 data points. The median value was taken as an index of conduction velocity during AF.

Figure 2. Measurement of intra-atrial conduction velocity during AF. After triangulation of the fibrillation map (left) local conduction vectors were calculated from the activation times at the corners (middle). All local conduction velocities <140 cm/s were collected in a velocity histogram (right). The median conduction velocity was taken as the conduction velocity during AF.

The refractory period during AF was measured during regional entrainment of the free wall of the left atrium with a pacing interval equal to the median AFCL (Fig 3). A pair of electrodes in the center of the mapping array was selected for stimulation whereas local capture was verified at a neighboring electrode 4 mm from the pacing site. Capture was defined by the absence of R-waves and a short and constant latency between stimuli and response. After 8 entrained beats, single premature stimuli were applied starting well within the refractory period. In the upper panel of Fig 3, a premature stimulus with a coupling interval of 112ms did not capture the atrium. The post-stimulus electrogram appeared after a long latency and exhibited a clear R-wave. Failure of capture was confirmed by the activation map, showing a fibrillation wave propagating from left to right under the pacing electrode. A coupling interval of 116ms (lower panel, Fig 3) resulted in

local capture. The activation map showed radial spread from the pacing site at the center of the mapping array and the neighboring electrogram exhibited a similar response as during the previous entrainment of AF. The shortest coupling interval that captured the atrium during AF was not constant. When stimuli with the same coupling interval were applied repeatedly, capture sometimes occurred and sometimes not. This shows that, instead of deterministic, the RP_{AF} is a probabilistic measurement. Therefore each coupling interval was repeated 10 times before it was incremented by 2 ms. Because we were interested in the largest possible excitable gap during AF, the refractory period during AF was taken as the shortest coupling interval that resulted in 20 % of capture (2 of 10 attempts).

Figure 3. Measurement of the atrial refractory period during entrainment of AF. The atrium was paced at the median AFCL at the center of the mapping area. The electrogram recorded 4 mm from the pacing site, showed local entrainment of AF. A premature stimulus with a coupling interval of 112ms failed to capture the atrium (upper panel). The lower panel shows that a coupling interval of 116ms resulted in radial spread of activation from the pacing site (capture).

STATISTICAL ANALYSIS

Data are presented as mean $\pm$ SD. In case the data were not normally distributed the median value and its range are given. Statistical analysis was performed by the paired Student's *t* test or the Wilcoxon matched pairs signed ranks test. A *p*-value of less than 0.05 was considered as statistically significant.

Results

EFFECTS OF DIGOXIN AND VERAPAMIL ON ATRIAL RATE AND STABILITY DURING AF

A representative example of the effects of digoxin and verapamil on RR interval and AFCL is given in Fig 4. During acute AF (no remodeling) the average RR interval was 573 ± 104 ms and the mean AFCL 164 ± 11 ms. Digoxin progressively slowed the ventricular rate during the first 15 minutes after administration of the single bolus. Then, during 2 hours, the average RR interval remained constant (908 ± 315 ms). AFCL remained unchanged (164 ± 13 ms). Verapamil equally prolonged the RR interval to 849 ± 365 ms. However, in contrast to digoxin, the AFCL was shortened to 138 ± 12 ms. After 24 hours of AF, the RR interval was unchanged (630 ± 84 ms), while the AFCL had shortened to 132 ± 12 ms (atrial electrical remodeling). Again digoxin prolonged the RR interval (927 ± 319 ms), but left the AFCL unchanged (130 ± 9 ms). Infusion of verapamil prolonged the RR interval to 908 ± 355 ms and again shortened AFCL. As a result of the combination of electrical remodeling and verapamil, the AFCL became as short as 109 ± 15 ms (lower right panel).

Figure 4. Representative ventricular and atrial tracings during acute AF and after 24 hours. Both digoxin and verapamil caused a significant slowing of the ventricular rate. In contrast to digoxin, verapamil caused a shortening of the fibrillation interval, both in acute AF and after 24 hours.

The effect on the duration of AF is illustrated in Fig 5. In the non-remodeled state (acute AF) the AF paroxysms were short lasting (median 6 seconds, range 2-24). Digoxin had no effect on the duration of the AF episodes, whereas verapamil (lower panel) slightly prolonged the AF paroxysms (median 15 seconds, range 2-72 sec). After 24 hours of electrical remodeling, the AF paroxysms lasted longer with a median duration of 54 seconds (range 8-230 sec). Again digoxin did not alter the duration of AF. Verapamil now had a dramatic effect on the stability of AF. During the first 5 minutes after start of the infusion, AF was still self-terminating (7 episodes). However, the 8th paroxysm of AF, induced after 5 minutes, no longer terminated and persisted as long as verapamil was given (2 hours).

Figure 5. Effects of digoxin and verapamil on the stability of AF. Acute AF was short-lived and verapamil caused only a slight increase in its duration. After 24 hours, the atrial substrate was changed and AF paroxysms now lasted approximately one minute. Although digoxin did not affect AF stability, verapamil made AF sustained.

Analysis of fibrillation maps showed no effect of digoxin on the degree of fragmentation, either during acute AF or after 24 hours of electrical remodeling. However, administration of verapamil increased the fragmentation of the fibrillation waves in the remodeled atria (24h AF). In Fig 6 an example is given. Before verapamil was given (upper panel), the AFCL was 119 ± 11 ms. The majority of the electrograms consisted of single high-amplitude complexes, separated by isoelectrical segments. The fibrillation maps revealed single broad wavefronts propagating without much conduction delay (type I). Only incidentally dissociation by a line of functional conduction block was observed (type II). Administration of verapamil accelerated AF (AFCL 99ms) and the fibrillation electrograms became more fragmented and lower in amplitude. The fibrillation maps

now showed a more complex activation pattern. Often multiple wavelets were seen separated by lines of functional conduction block (type II and III).

Control (after 24 h of AF)

Verapamil

Figure 6. Increased fragmentation of AF by verapamil. Compared to control the AFCL was shortened and the fibrillation electrograms were more fragmented and lower in amplitude. The fibrillation maps showed a more complex type of activation (type II-III).

In Fig 7 and Table 1 the effects of digoxin and verapamil on the characteristics of AF are given for all six goats. Both during acute AF and after 24 hours, digoxin and verapamil caused a similar prolongation of the RR interval by > 40 %. In all goats, administration of digoxin progressively slowed the ventricular rate during the first 15 minutes, after which the average RR interval remained constant for 2 hours. Digoxin had no effect on AFCL, neither during acute AF (151 ± 17 versus 151 ± 15 ms) nor after 24 hours of AF (115 ± 12 versus 116 ± 13 ms). In contrast, verapamil clearly accelerated the rate of AF. In non-remodeled atria, AFCL shortened from 152 ± 15 to 129 ± 9 ms (-15%) and in remodeled atria from 121 ± 10 to 102 ± 8 ms (-16%). The duration of AF was not influenced by digoxin, neither before nor after remodeling. Verapamil prolonged the duration of AF. The median duration of paroxysms of acute AF was prolonged from 7 to 16 seconds ($p=0.06$). After AF of 24h duration, the effect of verapamil on the stability of AF was more pronounced. Only during the first 6 ± 2 minutes of verapamil infusion, AF was still self-terminating. Then, verapamil converted paroxysmal into sustained atrial fibrillation

in all 6 goats and as long as verapamil infusion was maintained (2 hours), AF no longer terminated.

Whereas the degree of fragmentation of AF was not affected by digoxin, the effects of verapamil were largely determined by the degree of electrical remodeling. During acute AF verapamil had no effect on the complexity of AF. However, after 24 hours of AF-induced electrical remodeling, verapamil shifted AF from type I to type II-III. The percentage of type II+III beats after administration of verapamil was $96 \pm 6\%$ compared to $25 \pm 11\%$ at baseline ($p < 0.01$).

Figure 7. Effects of verapamil and digoxin on acute and 24-h AF in 6 goats. Verapamil had a similar effect on RR-interval as digoxin, but preferentially shortened AFCL and increased the stability of AF. The degree of fragmentation, plotted as the percentage of type II+III beats, also increased by verapamil. (* $p < 0.05$, † $p < 0.01$)

EFFECTS OF DIGOXIN AND VERAPAMIL ON ATRIAL REFRACTORY PERIOD AND CONDUCTION VELOCITY

Both digoxin and verapamil had no effect on atrial conduction during slow pacing either before or after AF-induced electrical remodeling. Conduction during AF was also not changed by digoxin (acute AF: 75 ± 15 versus 76 ± 12 cm/s; 24h of AF: 74 ± 8 versus 74 ± 10 cm/s) (Table 1). The effects of verapamil on conduction during AF are illustrated in the CV_{AF} histograms given Fig 8. During acute AF, verapamil had no effect on the CV_{AF} histogram. However, after 24 hours of AF, the histogram was clearly skewed to the

left, indicating an increase in the incidence of intra-atrial conduction block. The contribution of local conduction velocities of $<20\text{cm/s}$ increased from 0 to 12% and the percentage of CV_{AF} between 20 and 40 cm/s increased from 4 to 18%. As a result the median CV_{AF} decreased from 80 to 60cm/s. In Table 1 the effects of digoxin and verapamil on CV_{AF} both during acute AF and 24h of AF are given for all six goats.

Figure 8. Conduction velocity histograms during AF both before and after verapamil. In acute AF, verapamil had no effect on atrial conduction during AF. After 24 hours, verapamil skewed the histogram to the left, indicating a higher incidence of slow conduction and conduction block.

The atrial refractory period, both during slow pacing and atrial fibrillation was not affected by digoxin (Table 1). During slow pacing in non-remodeled atria also verapamil had no significant effect on atrial refractoriness (157 ± 19 versus $152\pm 19\text{ms}$). Because in remodeled atria verapamil resulted in persistent AF, the AERP_{400} could not no longer be determined. The shortening of the AFCL by verapamil was paralleled by a shortening of the atrial refractory period during AF. In Fig 9, an example of the shortening of RP_{AF} by verapamil is shown. During control, the shortest coupling interval that captured the atrium during entrainment of AF was 116ms (upper panel). Verapamil accelerated AF to a median AFCL of $108\pm 15\text{ms}$. During regional entrainment of AF with a pacing interval of 108ms, the shortest coupling interval which captured the atria was now 94 ms (lower panel). In Table 1 the average results were listed. During acute AF, RP_{AF} shortened from 130 ± 14 to $113\pm 11\text{ms}$ (-13% , $p<0.01$). After 24 hours of AF, verapamil shortened the RP_{AF} from 106 ± 7 to $90\pm 6\text{ms}$ (-16% , $p<0.01$).

Figure 9. Verapamil-induced shortening of the atrial refractory period during AF. The unipolar electrogram was recorded 4 mm from the pacing site. Verapamil shortened the RPAF from 116 ms to 94 ms.

Discussion

Both experimental and clinical studies have suggested that digoxin and verapamil might alter the natural course of atrial fibrillation by modulating tachycardia-induced electrical remodeling.^{2,4,11} AF-induced electrical remodeling was first described by Wijffels et al. as a progressive shortening of the atrial refractory period during the first days of AF.⁵ Two years later Yue et al. showed that the shortening of the action potential was due to downregulation of the L-type calcium current.⁶ It is believed that this process of electrical remodeling is mediated by intracellular calcium overload resulting from the extremely high atrial rate.^{9, 10}

Initially, experimental and clinical studies demonstrated that verapamil could attenuate tachycardia-induced electrical remodeling.^{2,11} Patients treated with intracellular calcium lowering drugs experience fewer relapses of AF after electrical cardioversion.⁴ However, more recently it has been shown that long-term tachycardia-induced shortening of atrial refractoriness can not be prevented by verapamil.¹²

Digoxin, which increases intracellular calcium content, had no significant effect on the

time course of electrical remodeling, but delayed its recovery (reverse electrical remodeling).³ Clinically, there are indications that digoxin might increase the likelihood of recurrence of AF.⁴

DIGOXIN

Clinical studies on the effects of digoxin on the stability of AF are controversial. Some authors suggested that digoxin might cardiovert recent onset AF,¹³ whereas others reported that digoxin prolonged the duration of paroxysmal AF.¹⁴ In isolated human atrial tissue it has been shown that ouabaine shortens the atrial action potential due to its parasympathetic effect.¹⁵ Rawles et al. hypothesized that this might increase the atrial rate of AF.¹⁴ In the present study, we showed that (1) intravenous administration of digoxin (0.02 mg/kg) had no effect on atrial fibrillation cycle length, degree of dissociation of AF waves or atrial electrophysiology during AF, neither in the non-remodeled and remodeled atria; (2) digoxin had no effect on the duration of electrically induced AF episodes. Even in the remodeled atria AF paroxysms still terminated spontaneously without intervention during digoxin administration. These data are in agreement with recent randomized studies demonstrating that digitalization of patients with recent onset AF does not increase the likelihood of restoration of sinus rhythm.^{16,17} Obviously, intravenous administration of digoxin does not exert much parasympathetic effect on the atrial myocardium. The lack of effects of digoxin on AF could not be due to inadequate plasma levels or insufficient time to reach its maximal effect because, in all goats, after 15 minutes of digoxin administration a maximal prolongation of the RR-interval (>40%) was observed. The early onset of prolongation of the RR-interval is in agreement with the clinical study by Powell et al. in which a maximal effect on atrio-ventricular conduction was already reached 10–20 minutes after intravenous administration of digoxin (1 mg).¹⁸

PRO-FIBRILLATORY EFFECT OF VERAPAMIL

Both experimental¹⁹ and clinical²⁰ studies have shown that verapamil enhances the maintenance of electrically induced AF episodes. However, in non-remodeled atria this pro-fibrillatory effect was only moderate. In patients verapamil prolonged the duration of electrically induced AF from 5 to 39 min, but all paroxysms were still self-terminating.²⁰ Friedman et al. suggested that the verapamil-induced increase in catecholamines^{19,21} promoted the maintenance of AF but they could not identify the electrophysiological mechanism by which this occurred. In vitro, calcium antagonists cause a decrease in the action potential duration due to shortening of the calcium dependent plateau of the action potential.²² However, clinical studies showed no effects of verapamil on the atrial refractory peri-

od during slow pacing (S_1S_1 : 500-700ms).²³⁻²⁴ Only limited data are available on the effects of verapamil at high atrial rates.²⁵

In the present study we showed that (1) the direct pro-arrhythmic effect of verapamil was based upon an increase in the atrial rate during AF due to a shortening of the refractory period at AF rates, both in the presence and absence of electrical remodeling but that (2) the pro-fibrillatory effect of verapamil on stability of AF was markedly enhanced by electrical remodeling. In non-remodeled atria, the pro-fibrillatory effect of verapamil was negligible and resulted in a non-significant prolongation of the average duration of AF from 7 to 16 seconds. In contrast, in electrically remodeled atria, verapamil converted paroxysmal into sustained atrial fibrillation, shortly after start of the infusion. As long as verapamil infusion was maintained (2 hours), AF no longer terminated.

Also in the present study there was no effect of verapamil on atrial refractoriness during slow pacing. The preferential shortening of the refractory period during high atrial rates might be due to a rate-dependent block of the L-type calcium channels. Due to their low lipid solubility, phenylalkylamines (verapamil) access their intracellular binding site predominantly via the open channel and because these compounds are charged they unbind slowly from their receptor.²⁶ Therefore verapamil exhibits a marked use-dependent block. Since during AF the excitable gap comprises not more than 20-25% of the cycle length, the L-type calcium channels are in the open state more than 75% of the time. This might explain the preferential shortening of the atrial refractory period by verapamil during AF. An alternative explanation is that shortening of the atrial refractory period at high rates is mediated by the neurohumoral effects of verapamil. In a canine model of AF, sympathetic stimulation shortened the atrial refractory period especially at high atrial rates, resulting in a shortening of the AFCL by 12 %.²⁷ This resulted in a slight prolongation of AF duration similar to the pro-fibrillatory effect of verapamil in the non-remodeled atria of the goat.

In the present study we demonstrated that the effect of verapamil on AF stability strongly depended on the degree of electrical remodeling. It is very likely that the profibrillatory effects of electrical remodeling and of verapamil both are based upon a shortening of the atrial refractory period. This will shorten the pathlength and increase the number of atrial fibrillation waves. The resulting increase in the average number of intra-atrial circuits could explain the increased stability of AF.²⁸ However, since the atrial refractory period (and circuit size) have to be shortened below a critical value,⁵ only after the additive effect of electrical remodeling and verapamil, fibrillation became sustained. This is supported by the observation that only in the remodeled atria, verapamil resulted in a marked increase in the fragmentation of the atrial activation during AF.

It is unclear whether verapamil would also shorten the RP_{AF} in chronic AF, since Yue et al.⁸ reported a significant reduction in the L-type calcium current after 6 weeks of rapid atrial pacing. In addition, after long-standing AF, structural changes may occur which could alter the effects of verapamil on atrial electrophysiology. A recent study by Ramanna et al. reported that also in patients with chronic AF verapamil increases the atrial rate during AF.²⁹ This implies that also after long-term electrical remodeling (>24h AF), verapamil could shorten the refractory period during AF.

CLINICAL IMPLICATIONS

The choice of drugs for rate control of AF might not only be determined by the efficacy of regulating the ventricular rate but also by their possible effects on atrial fibrillation itself. This is emphasized by our present study in which digoxin and verapamil exerted a similar effect on the RR interval but different effects on rate and stability of AF. In patients with paroxysmal AF, the proarrhythmic effect of verapamil might prolong the duration of AF paroxysms. When an episode of AF has proceeded long enough for electrical remodeling to occur, rate control by verapamil might even reduce the likelihood of spontaneous termination of AF. Furthermore, the acceleration of atrial rate might also reduce the efficacy of chemical cardioversion.

LIMITATIONS OF THE STUDY

The question whether the observed pro-fibrillatory effect of verapamil is mediated through an increase in sympathetic tone remains unanswered. The occurrence of severe bradycardia in the goat by the combination of verapamil and beta-blockade precluded experimental evaluation of this hypothesis. Furthermore, the direct effect of verapamil as described in this paper is clinically relevant, since also in patients verapamil is preferably administered in the absence of beta-blockade.

The different experiments were not performed in a random order. However, sufficient time was allowed between experiments for complete washout of the previously administered drug or complete recovery from electrical remodeling (reversed remodeling).⁶ In addition, serum levels of digoxin and verapamil were not measured. However, the significant slowing of the ventricular rate during AF (>40%) by verapamil and digoxin implied adequate serum levels of both drugs.

Since, even after prolonged periods of AF, the goat does not get spontaneous paroxysms of AF, it is unknown whether digoxin or verapamil would affect the triggers for initiation of AF. Also it remains to be seen how these drugs affect AF of longer than 24h duration. However, preliminary reports suggest that also in patients with chronic AF, verapamil increases the atrial rate during AF.²⁹

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Table 1. Effects of digoxin and verapamil on acute and 24-hours AF.

	RR _{AF} (ms)	AFCL (ms)	DurAF (s)	Type II+III (%)	CV ₄₀₀ (cm/s)	CV _{AF} (cm/s)	AERP ₄₀₀ (ms)	RP _{AF} (ms)
Acute AF								
Control	424±72	151±15	10 (4-34)	29±12	136±17	75±15	151±31	129±24
Digoxin	624±77	151±17	11 (4-175)	32±10	133±14	76±12	147±32	129±27
Δ %	+47% (p<0.01)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Control	469±112	152±15	7 (4-86)	29±8	134±12	77±14	157±19	130±14
Verapamil	671±109	129±9	16 (4-132)	26±19	138±15	80±12	152±19	113±11
Δ %	+43% (p<0.01)	-15% (p<0.01)	(p=0.06)	n.s.	n.s.	n.s.	n.s.	-13% (p<0.01)
24h of AF								
Control	447±143	116±13	76 (30-284)	30±19	138±15	74±8	116±23	100±9
Digoxin	630±153	115±12	72 (34-587)	28±13	134±16	74±10	114±25	100±10
Δ %	+41% (p<0.02)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Control	460±120	121±10	74 (24-140)	25±11	136±8	77±15	120±12	106±7
Verapamil	666±120	102±8	Sustained	96±6	-	42±19	-	90±6
Δ %	+45% (p<0.01)	-16% (p<0.01)	-	+291% (p<0.01)	-	46% (p<0.05)	-	-16% (p<0.01)

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Chapter 7

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

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Abstract

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 (RP_{AF}) and 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-AFCL 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: 100x) 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 ($n: 250-500$) the paced AFCL was plotted against its coupling interval and capture was determined by statistical shortening of the AFCL ($RP_{AF}: 71 \pm 17$ 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 the most attractive technique because of the ease of acquiring the data and the clear graphic result.

Key Words: Atrial fibrillation; Refractory Period; Excitable Gap.

Condensed Abstract

Methods to assess the refractory or excitable period during AF so far have been limited to monophasic action potential recordings or extensive pace-mapping protocols. In the present study we evaluated 5 different methods to measure the RP_{AF} in the chronically instrumented goat. 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. The results of the other methods all correlated well with the gold standard (activation maps).

Introduction

Recently it has been suggested that the temporal excitable gap during 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. On the other hand, a short excitable gap may also promote the formation of new wavelets due to an increase in the likelihood of intra-atrial conduction block. So far, determination of the excitable gap during AF has been limited to the recording of monophasic action potentials.^{3,4} Recently, RP_{AF} has been measured directly by programmed electrical stimulation.^{2,5} Cardioversion by class I drugs was found to be associated with a 2-3 fold widening of the temporal excitable gap.²

The aim of the present study was to develop clinical tools for the measurement of the refractory period 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 eleven goats (51±8kg) two plaques (3.5x2.5cm, 30 electrodes, distance 4mm) were sutured to the free wall of the right (RA) and left atrium (LA). One strip (6x1cm, 23 electrodes, distance 6-10 mm) was pulled along Bachmann's bundle and sutured to both atrial appendages. The leads were tunnelled subcutaneously to the neck and exteriorized by three 30-pin connectors. A subcutaneous silver plate served as indifferent electrode. After recovery from surgery, persistent AF was produced by a fibrillation pacemaker.⁶ After amplification (300x) and filtering (1-500Hz) 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 AFCL of 98±14ms. A central pair of electrodes on RA (n=7) or LA (n=4) was used for stimulation. Biphasic stimuli of 2ms were generated by a constant current stimulator equipped with an amplifier recording a bipolar electrogram from the pacing electrodes (Medtronic SP3111). The minimal current required for regional entrainment of AF was taken as the threshold for stimulation.⁷

METHODS TO MEASURE THE REFRACTORY PERIOD DURING AF

In Fig.1 the five methods to measure RP_{AF} are illustrated. Mapping was used as the gold standard. Single premature stimuli of 4xthreshold were synchronized to the fibrillation waves at the pacing site. Capture was verified by activation maps around the pacing site. In case of capture the atrium was activated in a radial fashion from the site of pacing.

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 2ms. Capture was determined by the latency and morphology of an electrogram recorded 4mm from the pacing site. In case of capture, the premature electrogram showed a similar morphology and latency as 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 resulting in a statistically significant difference in AFCL-histogram was taken as the RP_{AF} (Wilcoxon two-sample test).

A fourth method to determine RP_{AF} is to pace the fibrillating atria at a slow fixed-rate (1-Hz). Because of the irregularity of AF, slow pacing actually results in a series of randomly applied premature stimuli. A computer algorithm was used to determine the coupling interval at the pacing site (AF-S) and the associated AFCL at a neighboring electrogram. At least 250 consecutive stimuli were analyzed. The shortest coupling interval (classes of 5ms) resulting in a significant shortening of AFCL (Kolmogorov-Smirnov one-sample test) was defined as the RP_{AF} . Since 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 estimate RP_{AF} is to determine the 5th percentile of the AFCL-histogram, constructed from at least 100 consecutive AF cycles from a unipolar RA or LA electrogram.

THE EXCITABLE GAP DURING AF

The term "excitable gap" refers both to the excitable *tissue* between fibrillation waves (spatial EG) and the *time* window of excitability during the AF interval (temporal EG). We used the term *excitable period* (EP_{AF}) to indicate the temporal excitable gap. With all 5 methods, EP_{AF} can be calculated as the difference between the median-AFCL and RP_{AF} . During fixed-rate pacing, EP_{AF} could be directly measured from the histogram of the interval between the stimulus and the first response (S-AF). Whereas during sub-threshold pacing the S-AF intervals were equally distributed, pacing at 4xthreshold caused a gap in the S-AF histogram. The longest S-AF interval occurring less frequently than expected (95%-confidence interval) was taken as the EP_{AF} .

Mapping

Entrainment

Synchronized Stimuli

Fixed Rate Pacing

p5 AFCL

Figure 1. Five methods to determine RPAF: (1) mapping of premature stimuli during AF; (2) premature stimuli (S1-S2) during entrainment of AF; (3) statistical analysis of the effect of synchronized premature stimuli (n:100); (4) fixed-rate pacing (1Hz) during AF resulting in randomly applied premature stimuli; (5) the 5th percentile of the AFCL.

Results

MAPPING OF THE REFRACTORY PERIOD DURING AF

In Fig.2 an example is given of the RP_{AF} determined by mapping. In the upper panel, a stimulus was applied 70ms 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 75ms (lower panel) resulted in capture as evidenced by radial spread of activation from the pacing site. However, when stimuli with the same coupling interval were repeated 20x, sometimes capture occurred and sometimes not. In Fig.3 we plotted the percentage of capture at different coupling intervals. At short coupling intervals (<65ms) the atrium was never captured. Prolonging the interval from 65 to 90ms resulted in a progressive increase in the probability of capture. A coupling interval longer than 90ms 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 at least 20% of the times. 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.

Figure 2. A representative example of capture by a single synchronized stimulus. The electrograms were recorded at the pacing site (bipolar) and a neighboring electrode (unipolar).

Figure 3. Probability of capture of AF by premature stimuli. The S-shaped curve shows the temporal variation in RPAF.

REFRACTORY PERIOD DURING ENTRAINMENT OF AF

An example of the RP during entrainment of AF is shown in Fig.4. Entrainment was performed at the LA with an S1-S1 interval of 110ms (median-AFCL:113ms). 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 90ms did not capture the atrium, whereas an S2 stimulus with a coupling interval of 92ms did capture the atrium (radial spread). For determination of the RP_{AF} , 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 as during entrainment of AF (no R-wave). The RP during entrainment, defined as the longest S1-S2 interval that did not capture the atrium, was 77 ± 17 ms (6 goats).

Figure 4. Measurement of RP during entrainment of AF. The electrogram was recorded 4 mm from the pacing site. A premature stimulus with a coupling interval of 90ms did not capture the atrium, whereas an S₁-S₂ interval of 92ms captured the atria (no R-wave; short latency).

MEASUREMENT OF THE RP_{AF} 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 Fig.5 an example is given of a premature stimulus with a coupling interval of 50ms. The control-AFCL histogram (sampled one second before each premature stimulus) shows a normal distribution with a median-AFCL of 80ms. In contrast, the paced-AFCL histogram showed a bimodal distribution. Due to capture, 39% of the AF-cycles were now shorter than 60ms. The Wilcoxon two-sample test revealed a significant difference between the two 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 RP_{AF} 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.

Figure 5. Determination of RPAF by statistical analysis of the paced-AFCL histogram close to the pacing site. During administration of premature stimuli with a coupling interval of 50ms (n:100) the histogram showed a shift to the left due to capture of part of the stimuli.

MEASUREMENT OF THE RP_{AF} BY FIXED-RATE PACING

An alternative way to determine RP_{AF} is to stimulate the fibrillating atria at a slow fixed-rate of 1Hz, resulting in a series of randomly applied premature stimuli. In Fig.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:88ms). At longer coupling intervals the normal variation in AFCL was lost and, due to capture, the paced-AFCL was now determined by the coupling interval of the stimulus. At intermediate coupling intervals (48-69ms) only part of the stimuli captured the atrium, illustrating the temporal variation in RP_{AF} (grey column). In all goats the range of temporal variation of the RP_{AF} was 24 ± 7 ms. The shortest coupling interval producing a significant shortening of AFCL, as determined by the Kolmogorov-Smirnov test, was 71 ± 17 ms. This method was applied only in the last 6 of 11 goats since it was developed during evaluation of the other techniques in the first 5 goats.

AFCL
(ms)

Figure 6. Determination of the RPAF by slow fixed-rate pacing (1Hz). During 250 consecutive stimuli the paced-AFCL is plotted against its coupling interval. The grey column indicates the range of refractory periods during AF.

Using fixed-rate pacing, EP_{AF} can be directly visualised (Fig.7). During 16-17 minutes of 1Hz pacing all post-stimulus intervals ($n:1000$) were plotted in a histogram. During sub-threshold pacing the S-AF intervals were equally distributed with a mean incidence of 10.7 ± 2.9 (95%-confidence interval between 4.9 and 16.5). When stimulus strength was set at $4 \times \text{threshold}$, 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 30ms markedly decreased. These intervals disappeared because they were shortened by capture of the premature stimuli. The distribution of S-AF intervals longer than 35ms did not change during pacing. The upper limit of the excitable period was determined by the longest S-AF interval occurring less frequently than the 95%-confidence interval. 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. Due to variation in AFCL and RP_{AF} , during many cycles EP_{AF} will actually be shorter. This is illustrated in Fig.7 by the atrial responses still occurring during the measured excitable period.

Figure 7. Determination of the excitable period during AF by fixed-rate pacing. The horizontal dotted line represents the 95%-confidence limit of the incidence of the S-AF intervals during sub-threshold pacing.

THE P₅-AFCL VALUE AS AN INDEX OF THE RP_{AF}

In 11 goats the median AFCL measured at RA or LA was 98 ± 14 ms. The 95th and 5th percentile of the AFCL were 120 ± 16 and 77 ± 12 ms. The 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 RP_{AF} as obtained by the 5 methods are listed for all goats. The RP_{AF} measured by mapping is considered as gold standard. All methods yielded an RP_{AF} between 70 and 80 ms. During entrainment the RP was 4 ± 5 ms longer than the RP_{AF} determined by mapping (77 ± 17 vs 73 ± 15 ms, $p=0.12$). The correlation between the values obtained by entrainment and mapping was 0.88 ($p<0.01$). The RP_{AF} determined by synchronized stimuli yielded the same values as obtained by mapping (70 ± 13 vs 70 ± 12 ;

$R^2:0.94$; $p<0.01$). The RP_{AF} measured by fixed-rate pacing also gave a similar result (71 ± 17 vs 73 ± 15 ms; $R^2:0.84$; $p<0.01$). The 5th percentile of the AFCL-histogram was 7 ± 4 ms longer than RP_{AF} measured by mapping (77 ± 12 versus 70 ± 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 RP_{AF} were consistently longer and showed more variation in the RA ($p:0.33-0.47$).

Discussion

THE REFRACTORY PERIOD DURING ATRIAL FIBRILLATION

In the present study it was shown that the refractory period during AF exhibits a considerable temporal variation. Measurement of RP_{AF} by mapping or fixed-rate pacing revealed a time window of 20-25ms during which premature stimuli sometimes captured the atrium and sometimes not (Fig.3 and 6). This probabilistic nature of RP_{AF} is probably due to 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 RP_{AF} .^{8,9} The RP_{AF} measured by mapping was defined as the coupling interval resulting in >20% capture. Also the other methods determined the "shortest" refractory period during AF. The values obtained by the 5 methods ranged between 70 ± 12 and 77 ± 17 ms (70-80% 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 RP_{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 RP_{AF} in the right atrium may be associated with a more complex geometry, allowing 3-D propagation during AF.¹⁰

COMPARISON OF DIFFERENT TECHNIQUES TO MEASURE RP_{AF}

The different methods to measure RP_{AF} all have certain advantages and disadvantages (Table 2). Mapping of premature stimuli is the most reliable technique and can be regarded as gold standard. However, due to its complexity and invasive nature, it can only be used under exceptional circumstances. Measurement of the refractory period during entrainment of AF has the advantage that it uses the conventional extrastimulus tech-

nique (S1-S2) and that it can also be applied during paroxysmal AF. The somewhat longer values obtained during entrainment are probably due to electrotonic prolongation of the action potential at the pacing site.⁹ On the other hand, 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 RP_{AF} . However, it is a rather complex technique 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 about 15 minutes during which no intervention of the electrophysiologist is needed. This method produces a clear graphic output from which both RP_{AF} and EP_{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 RP_{AF} became considerably shorter than the p5-AFCL.²

THE EXCITABLE PERIOD DURING ATRIAL FIBRILLATION

In the present study EP_{AF} , calculated as the difference between AFCL (98 ± 14 ms) and RP_{AF} (70 ± 12 ms), was 28 ± 8 ms. The EP_{AF} measured directly during fixed-rate pacing was 27 ± 4 ms (Fig. 7). However, also EP_{AF} must show a considerable temporal variation. In our experiments the p5 and p95-AFCL were 77 ± 12 and 120 ± 16 ms. This variation in AFCL (43 ± 8 ms) can not completely be explained by the variability in RP_{AF} (19 ± 4 ms). This implies that at short AF cycles the excitable period may become as short as 7ms, whereas during long cycles the excitable period might be as long as 50ms. This beat-to-beat variability in EP_{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, EP_{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, EP_{AF} is caused by the curvature of the circulating wavefront at pivot points. Due to the high curvature, the excitatory current generated by the turning wavefront may not be enough to make a rapid 180° turn¹⁵⁻¹⁷ 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 due to the antero-grade and retrograde conduction time to the site of reentry.¹ On the other hand, the excitable period will be shortened by epicardial breakthrough of wavefronts 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 3-D nature

of the reentrant process but also by narrowing its excitable gap.

Recently we 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 wavefront whose circulating pathway is ultimately interrupted. However, the possibility that under other circumstances cardioversion of AF might be due to closure of the excitable period can not be excluded. Measurement of RP_{AF} and EP_{AF} during pharmacological cardioversion of AF using 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} . Since no endocardial recordings were made, the three-dimensional 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. Since in the present study RP_{AF} was measured by chronically implanted epicardial electrodes, clinical application of these methods must await validation using multipolar endocardial catheters.

Table 1. RP_{AF} values obtained by different Methods.

Goat	Site	AFCL (median)	Refractory Period during AF (ms)				
			Mapping	Entrainment	Synchronized Stimuli	Fixed-Rate Pacing	p5AFCL
1	RA	105	75	-	80	-	82
2	RA	107	70	-	70	-	79
3	LA	88	55	-	55	-	64
4	RA	88	65	-	65	-	70
5	LA	93	70	-	70	-	74
6	LA	86	65	72	65	68	69
7	RA	90	70	66	70	58	72
8	RA	127	95	100	100	98	104
9	LA	103	75	74	70	68	88
10	RA	113	80	90	75	83	85
11	RA	80	50	58	50	53	64
Mean		98	70 (73)	(77)	70	(71)	77
SD		14	12 (15)	(17)	13	(17)	12
R2				0.88*	0.94*	0.84*	0.90*

Numbers between brackets indicate mean and standard deviation of goats 6-11; * $p < 0.01$.

R2, correlation of the RPAF measured by the different techniques compared to mapping.

Table 1. Comparison of Different Techniques to measure the RP_{AF}

TECHNIQUES	EQUIPMENT	TIME	ADVANTAGES	DISADVANTAGES
Mapping	<ul style="list-style-type: none"> - High-density mapping - Programmed electrical stimulation 	1 hour	Gold standard	Only in exceptional circumstances
Entrainment	<ul style="list-style-type: none"> - Programmed electrical stimulation - Multipolar catheter 	15 min	<ul style="list-style-type: none"> - Conventional S1-S2 pacing - Also in Paroxysmal AF 	Not always possible
Synchronized Stimuli	<ul style="list-style-type: none"> - Stimuli synchronized to pacing site - Multipolar catheter 	30 min	Statistical determination of RP_{AF}	Complex
Fixed-Rate Pacing	<ul style="list-style-type: none"> - Fixed-rate pacemaker - Multipolar catheter 	15 min	<ul style="list-style-type: none"> - Easy to use - Direct measurement of EP_{AF} 	Takes 15 minutes
p5-AFCL	<ul style="list-style-type: none"> - Recording of atrial electrogram 	3 min	No pacing required	Reliability questionable

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Chapter 8

General Discussion

New Perspectives for Atrial Fibrillation.

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Introduction

In recent years the interest in AF has increased explosively. One explanation might be that the successful treatment of other arrhythmias has shifted the attention of cardiologists to the remaining challenges in arrhythmia management. Also an increased awareness of the considerable morbidity and mortality associated with AF has caused a more aggressive attitude towards attempts to restore normal sinus rhythm (rhythm versus rate control).^{1,5} Finally, the discovery that 'AF begets AF' has emphasized that accepting atrial fibrillation to persist, may lead to irreversible atrial remodeling and a progressive inability to treat the arrhythmia.^{6,7} These considerations have boosted the development of both pharmacological and non-pharmacological therapies for treatment of atrial fibrillation.^{8,10} Current non-pharmacological treatments of AF encompass atrial surgery, linear and focal RF ablation, single and dual site preventive pacing, and the implantable atrial defibrillator. However, despite all these efforts, present-day treatment of AF can still be characterized as 'doing our best without knowing what is wrong'. To develop a more effective strategy for treatment of AF, one needs to know the underlying pathophysiological mechanisms in a given individual patient.²⁰ In this respect, one has to realize that the diagnosis of atrial fibrillation is merely a description of a certain electrocardiographic pattern which can be generated by different pathophysiological mechanisms.

Triggers, Initiators and Perpetuators

Three different 'players' are involved in the electropathology of atrial fibrillation: triggers, initiators and perpetrators (Fig. 1). Atrial fibrillation can be *triggered* by single premature stimuli originating from the crista terminalis, ostium of the coronary sinus, interatrial septum and atrial free wall.^{21,23} Also other onset-mechanisms like multiple premature beats, focal atrial tachycardia, atrial flutter and AV nodal tachycardia can start AF.

Figure 1. The pathophysiological triad of AF. The "players" involved in the electropathology of AF include triggers that elicit the arrhythmia and a substrate for initiation and perpetuation of intra-atrial reentry.

Recent pioneering work by Haissaguerre et al. showed that in patients with frequent paroxysms of AF the vast majority of AF episodes (95%) were due to ectopic foci in the wall of the pulmonary veins.¹¹ This was confirmed and strengthened by other investigators and led to the introduction of radiofrequency catheter ablation of these pulmonary vein foci.^{13-18, 24-25} However, if one believes that atrial fibrillation is due to a reentrant mechanism, apart from the trigger also a substrate for initiation of atrial reentry is required (*initiator*). Initiators are thought to consist of areas of prolonged atrial refractoriness, depressed or delayed conduction and/or a short atrial wavelength.²⁶⁻²⁸ Also factors such as local wavefront curvature and sink-source mismatch may be relevant for initiation of AF.²⁹⁻³⁰ The area where atrial reentry occurs and AF is actually initiated may be located remote from the trigger site. In normal atria AF is short-lasting and is expected to terminate spontaneously within minutes. A variety of different abnormalities in the atria may act as *perpetuators*.³¹ Persistence of AF can result from atrial dilatation, electrical contractile and structural remodeling, pro-fibrillatory effects of drugs and pathoanatomical changes like apoptosis, fibrosis and fatty degeneration.^{6, 7, 32-34} Knowledge about how AF is initiated and perpetuated in a single patient could help in choosing between the different treatment options. It is quite feasible that different underlying mechanisms of AF require different therapies (Fig. 2). Such a *differential diagnosis* of the underlying pathophysiological mechanisms (trigger-initiator-perpetuator) would imply a clinical history, echocardiography, Holter monitoring, autonomic and invasive electrophysiological testing. Although in many cases the underlying pathophysiology will be multifactorial, in some patients a dominant pathophysiological mechanism might be involved. Presently available catheters and sophisticated mapping systems allow detailed analysis of the atrial electrophysiological substrate of AF, which might be useful to choose the best treatment in individual cases.³⁵

	Trigger	Initiator	Perpetuator
AAR Drugs	+	+	+
Atrial Surgery			++
Focal Ablation	+++		
Linear Ablation		++	++
Preventive Pacing	++	++	

Figure 2. Different underlying mechanisms of AF require different treatment strategies. In patients with "triggered" AF, focal ablation of premature beats or discharging foci may eradicate atrial arrhythmia. Suppression of atrial arrhythmia may also be accomplished by atrial-based preventive pacing or antiarrhythmic drugs. In patients with a manifest "initiator", ablation or pacing at the culprit area of critical conduction might prevent recurrence of AF. In patients with a marked substrate for self-perpetuation of AF, reducing atrial size by atrial surgery or linear ablation may help to terminate and prevent AF.

The Initiation as Target for Non Pharmacological Treatment of AF

Most cases of AF are self-perpetuating and after a short time become independent of the trigger site. It is widely accepted that atrial fibrillation is maintained by single or multiple reentrant wavefronts. In a caprine model of AF we recently evaluated the substrate for *initiation* of atrial reentry by single premature beats.³⁶ Premature beats and initiation of AF were found to be associated with critical conduction delay at Bachmann's bundle. Also during rapid left atrial pacing, AF initiated remote from the rapidly firing focus.³⁷ The preferential conduction delay and fragmented low-voltage potentials recorded at the mid-portion of Bachmann's bundle can result from non-uniform anisotropy, slow discontinuous conduction, longitudinal dissociation or conduction block.^{26, 38-39} In the goat, critical conduction along Bachmann's bundle might be explained by the considerably longer refractory period measured at the mid-portion of BB. There is evidence that also in human atria the inter-atrial septum plays a role in the genesis of AF. In several studies it was found to have a longer atrial refractory period and be an area of preferential conduction delay of premature beats.^{23, 28, 40-41} In patients with rheumatic heart disease the earliest activity during induced AF was located near the inter-atrial septum (ostium of the coronary sinus).⁴² It seems quite likely that due to ageing, atrial dilatation, fibrosis, etc., also other areas than the inter-atrial septum may become an 'initiator' of AF.⁴³⁻⁴⁴ Localisation of areas of critical conduction delay and fragmentation and site(s) of earliest activation during onset of AF might provide a new target for modification of the substrate of AF by RF ablation in patients. Because of their anisotropic properties and strategic position, Bachmann's bundle and the inter-atrial septum are an attractive target for radiofrequency ablation.⁴⁵⁻⁴⁶ In a canine model of sterile pericarditis, Kumagai et al. showed that ablation of BB successfully terminated and prevented AF.⁴⁵ In another canine study Tondo et al. showed that ablation of the mid-part of the inter-atrial septum significantly reduced the likelihood of initiation of AF.⁴⁶ In the caprine model of AF we showed that the site of initiation of AF might also be an optimal site for preventive pacing. Pacing at the mid-portion of Bachmann's bundle significantly shortened the window of inducibility of AF by single premature beats, originating either from the right or the left atrium.⁴⁷ These experimental findings suggest that Bachmann's bundle (area of critical conduction) may be an effective pacing site for preventive pacing. In patients with recurrent AF, Padeletti et al. showed that atrial septal pacing was successful in reducing the recurrences of AF (65% in sinus rhythm after 17 months).¹⁵ Similarly, Bailin et al. showed that in patients with paroxysmal AF, pacing at Bachmann's bundle had an incremental benefit compared to right atrial pacing.¹⁶

Need for New Antiarrhythmic Drugs

Despite the success of non-pharmacological approaches, drug therapy will remain the mainstay in the management of atrial fibrillation in the general population.⁴⁸⁻⁴⁹ Non-pharmacological approaches, although eagerly welcomed by cardiac arrhythmologists and industry, are expensive, time-consuming and limited to a small subset of the general population.¹⁹ In Fig.3 we plotted the estimated number of patients with atrial fibrillation in Belgium, according to age-related prevalence of AF.⁵⁰ The total number of people with AF is estimated to be 121,000. Of this population, 85% or actually 102,000 patients are older than 65 years. In these elderly, the underlying pathophysiology of AF is expected to be *multifactorial* and AF is likely to recur or persist even after treatment of the initial "culprit" mechanism. Most likely in these patients antiarrhythmic drugs will be needed in an effort to attain the goal of arrhythmia management (reduction of AF burden and prevention of symptoms, cerebral embolism and aggravation of heart failure).

Figure 3. Age-related distribution of patients with AF in Belgium. The vast majority of people with AF (85%) are older than 65 years. In these patients pharmacological treatment will be needed in an effort to attain the goal of arrhythmia management. Beyond finding effective treatment in patients with AF (secondary prevention) it should be possible to prevent AF in high-risk patients (primary prevention).

Until now, antiarrhythmic drugs have been limited and far from ideal.⁹ In the search for the ideal drug the aim should be for superior efficacy, low risk of proarrhythmia, few adverse events, no serious interactions and appropriate pharmacokinetics. New drugs like ibutilide, dofetilide and azimilide might offer additional benefit in cardioverting AF.⁴⁸ However, better ion channel-targeted drugs aimed at suppression of atrial foci or prolongation of atrial refractoriness at slow heart rates may become equally important. Also it must be better understood how antiarrhythmic drugs terminate AF (widening of the excitable gap) and why they ultimately lose their efficacy for cardioversion of AF.⁵¹⁻⁵³ The progressive inability of drugs to terminate AF might be due to an increase in stability of the arrhythmia,⁵² and/or a progressive loss of efficacy of antiarrhythmic drugs by electrical remodeling.⁵³ Also it remains to be seen whether antiarrhythmic drugs restoring the shortened action potential in remodeled atria (such as $I_{Ca,L}$ agonists) are able to promote restoration and maintenance of sinus rhythm in patients with atrial fibrillation.⁵⁴⁻⁵⁵

Primary Prevention of AF

Since atrial fibrillation is widespread and associated with significant morbidity and mortality, prevention seems essential in managing AF (Fig.3). Beyond finding effective pharmacological and non-pharmacological treatments for patients with atrial fibrillation (secondary prevention), it should be possible to prevent the arrhythmia in high-risk patients (primary prevention). To focus on means for prevention necessitates considering both clinical and pathophysiological factors. The Cardiovascular Health Study and the Framingham study have shown that some of the *clinical* risk factors for AF (e.g. systolic blood pressure, blood glucose and valvular heart disease) are subject to control.^{1,2} The most common clinical settings for AF are hypertension and congestive heart failure.⁵⁶⁻⁵⁷ Identification and aggressive management of these clinical risk factors may play an important part in the primary prevention of AF. For instance, administration of angiotensin-converting enzyme inhibitors is expected to reduce the incidence of atrial fibrillation in patients with congestive heart failure.⁵⁸⁻⁶⁰ Primary prevention strategies should also focus on the prevention of the *electropathological* factors of AF (trigger, initiator and perpetuator).²⁰ The inter-atrial septum (initiator) seems an attractive target for radiofrequency ablation in high-risk patients. Also the development of a substrate for self-perpetuation of AF, the point of no return, must be forestalled. The latter may be accomplished by prevention of electrical remodeling, by either early cardioversion of AF (implantable atrial defibrillator) or administration of antiarrhythmic drugs (calcium blockade).⁶¹⁻⁶³ On the other hand, therapies aimed at the prevention of fibrosis, atrial distension or age-related structural and electrical changes may offer incremental benefit.

Interest in atrial fibrillation is growing at an impressive rate and tools are becoming available to study the electrophysiologic mechanisms responsible for human atrial fibrillation. These tools could be the basis for the development of better pharmacologic and nonpharmacologic ways for primary and secondary prevention of atrial fibrillation. Patients already benefit substantially from these efforts. However, treatment of AF will remain a challenge and it seems likely that a few years more will be required before the "vicious circle" of AF is cut short.^{64,65}

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Summary

In the present thesis, we studied in the caprine model of AF, the role of AF-induced electrical remodeling in the management of atrial fibrillation.

In **Chapter 1** the background to the present study is given. In recent years the interest in AF has increased explosively. Especially the increased awareness of the considerable prevalence, morbidity and mortality associated with AF has shifted the attention of cardiologists to the oldest arrhythmia (atrial fibrillation: the oldest arrhythmia-the newest disease). The renewed interest in AF has boosted the development of both pharmacological and non-pharmacological therapies for treatment of atrial fibrillation. However, despite all these efforts, present-day treatment of AF can still be characterized as 'doing our best without knowing what is wrong'. To develop a more effective strategy for treatment of AF, one needs to study the underlying pathophysiological mechanisms of AF. Or, as Thomas Lewis laconically suggested in 1902, *"The notion of a patient continuing to live with an auricle incapable of contraction is a somewhat novel idea....it seems necessary to devote a little study to the condition."*

In **Chapter 2** we described the cumulative effect of repeated paroxysms of AF on inducibility and stability of AF in the goat. Two days of sinus rhythm were found to be enough to recover completely from AF-induced electrical remodeling by 5 days of atrial fibrillation. Also we observed no cumulative effect of repeated 5 day paroxysms of AF on inducibility and stability. These findings highlight the importance of atrial refractoriness as a potential target for antiarrhythmic strategies aimed at inhibiting the self-perpetuation of AF.

In **Chapter 3** we studied the effect of AF-induced electrical remodeling on atrial vulnerability by single atrial premature beats. In the goat, 2 days of atrial fibrillation was associated with a marked widening of the window of inducibility of AF by single premature beats. Enhanced vulnerability in remodeled atria was due to a higher likelihood of reentry in the presence of critical conduction at Bachmann's bundle. These findings suggest that, during the first days of after cardioversion of human AF, both early and late premature beats can initiate early recurrences of AF. In patients, measurement of the window of inducibility might be useful to predict likelihood of recurrence of AF and to evaluate the efficacy of new treatment strategies.

In **Chapter 4** we described the transient changes in atrial electrophysiology immedi-

ately after spontaneous termination of atrial fibrillation. In the goat, during the first 2 minutes of sinus rhythm after spontaneous termination of AF there was a transient decrease in both atrial refractory period and conduction velocity. In remodeled atria, the cumulative effect of electrical remodeling and this short-term changes created an ultra-short atrial wavelength after termination of AF. In patients, immediate re-initiation of AF (IRAF) can be explained by this transient supervulnerable phase. However for immediate re-initiation of AF, the spontaneous occurrence of atrial triggers is at least as important as a vulnerable substrate for reentry.

In **Chapter 5** we determined the effects of different atrial pacing sites on the inducibility of AF by both right and left single atrial premature beats. It was found that, in remodeled atria, preventive atrial pacing markedly could reduce the window of inducibility of AF by single premature beats. The optimal site for preventive pacing was found to be at Bachmann's bundle, close to the area of critical conduction delay. Also in patients, the inter-atrial septum (Bachmann's bundle) might play a critical role in the genesis of AF. Pacing at Bachmann's bundle might be optimal pacing to prevent AF by both right and left atrial premature beats. Multi-site pacing seems of limited additional value.

In **Chapter 6** the effects of both digoxin and verapamil were evaluated on the duration and electrophysiological properties of paroxysmal atrial fibrillation. In the goat, verapamil but not digoxin exerted a direct pro-fibrillatory effect on paroxysmal AF. Especially in remodeled atria, intravenous administration of verapamil converted paroxysmal into persistent AF. This pro-fibrillatory effect of verapamil was due to shortening of atrial refractoriness at high AF rates (rate-dependent shortening of AERP). These findings suggest that in patients with paroxysmal AF, administration of verapamil might reduce the likelihood of spontaneous termination of the atrial arrhythmia. In contrast, digoxin is expected to reduce ventricular rate during AF without any effect on atrial electrophysiology during AF.

In **Chapter 7** we described five methods to determine the refractory and excitable period during chronic AF. 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 seemed the most attractive technique because of the ease of acquiring the data and the clear graphic result.

Finally, in the last chapter of this thesis (**Chapter 8**) the possible implications were discussed. To develop a more effective strategy for treatment of AF one needs to know the underlying pathophysiological mechanisms of AF. Three different “players” are involved in the electropathology of AF: triggers, initiators and perpetuators. Although in many cases the underlying pathophysiology will be multifactorial (e.g. elderly), in some patients a dominant pathophysiological mechanism might prevail. It is quite feasible that different underlying mechanisms of AF require different therapies (differential treatment of AF).

Nawoord

Het werd me al duidelijk tijdens mijn eerste jaar opleiding inwendige geneeskunde. Ik zou "wetenschappelijk onderzoek" gaan doen. Het waarom is moeilijk uit te leggen, maar ik denk dat vooral het gevoel primeerde dat ik iets moest bewijzen, meer nog tegenover mezelf dan tegenover anderen. Sommige mensen noemen het ambitie.

Dus zei ik op een dag tegen **Isabelle**: "Belleke, ik zou voor een jaartje naar Maastricht kunnen, voor onderzoek in de electrofysiologie, ...het is een buitenkans". We woonde toen nog in de Patiëntjestraat te Gent en waren pas een jaar getrouwd. Liefste Belle, je wist toen al beter en je vermoeden werd bevestigd toen ik uiteindelijk er nog drie jaar langer bleef voor het vervolledigen van een doctoraat. Steeds heb je me gesteund in mijn keuze, ook al was ik de godganse weken in Maastricht en liep ik ook in de weekends hoofdzakelijk te piekeren. Het was niet altijd even gemakkelijk voor jou, maar dankzij je zin voor realiteit en je enorme relativeringsvermogen, is het ons gelukt deze ommeweg te "overleven". Belleke, je mag er van op aan, ik blijf nu thuis en alle liefde en aandacht die je nog tegoed hebt, krijg je dubbel en dik terug.

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Gedurende een werkweek, reed ik twee keer per week op en af tussen Gent en Maastricht. In de vroege ochtend, sliep ik op verlaten parkings langsheen de autostra-

de. In den beginne was het, als "kleine Belg" tussen de Nederlanders, niet altijd even eenvoudig. Maar vooral de laatste jaren heerste er op het werk een gezellige sfeer en drukte. **Peter, Sabine, Frits, Jan, Frans, Jannie, Ullie, Cliff, Marcus, Shan, Paolo, Claire, Theo, Ruuth, Leo, Natascha, Olivier, Bodo, Maurits** aan jullie allen bewaar ik de beste herinneringen. Peter en Sabine waren ervan bij het beginne bij. Met Peter besprak ik de toekomst in de cardiologie die steeds zover afleek omdat de grote hindernis van het proefschrift ons het zicht belemmerde. Bientje was onze troost, ze is verstanding en realistisch en ze zorgde voor onze gemoedsrust door gewoon zich zelf te blijven. Jan, speciaal voor jou nog een dankwoord, want zonder jou was dit gewoonweg niet gelukt en was er geen proefschrift.

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Curriculum Vitae

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1982-1988	Latijn-Griekse Humaniora, St.-Barbara college Gent, Grote Onderscheiding.
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