'Early' and 'late' class III drugs for the treatment of atrial fibrillation : a pre-clinical study in the goat

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'Early' and 'Late' Class III Drugs for the Treatment of Atrial Fibrillation

A Pre-clinical Study in the Goat

Proefschrift

ter verkrijging van de graad van doctor
aan de Universiteit Maastricht
op gezag van de Rector Magnificus, Prof. mr. GPMF Mols
volgens het besluit van het College van Decanen
in het openbaar te verdedigen
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Voor Thea en Koen

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Prologue

Atrial fibrillation (AF) is a common arrhythmia that affects about 0.4% of the general population and its incidence increases with age to ~6% in patients over the age of 80 years.¹ Atrial fibrillation is associated with a significant morbidity and mortality.²⁻⁴ Strategies for the treatment of AF aim at reducing symptoms and improving long-term prognosis. Basically, this can be achieved by slowing down the fast ventricular heart rate during AF ('rate' control) or by restoring the normal sinus rhythm and preventing recurrences of AF ('rhythm' control).

The hallmark of the treatment of AF has been the use of anti-arrhythmic drugs. Already back in 1918 Walter Frey described the anti-fibrillatory action of quinidine.⁵ At present, more than 80 years later, several anti-arrhythmic drugs are available for the treatment and prevention of AF. Unfortunately, so far the clinical use of anti-arrhythmic drugs for AF has been far from successful. Most drugs are only moderately effective in restoring sinus rhythm. For example, the commonly used class IC drug flecainide and the class III drug ibutilide can terminate AF in ~60 to 80% of the patients if they are treated within the first 1-3 days after the onset of the arrhythmia.⁶⁻¹⁰ In contrast, if the arrhythmia is present for a longer period of time, pharmacological cardioversion is only successful in 20 to 30% of the patients.^{6-9,11} Also, anti-arrhythmic drugs have not been very effective in preventing AF. In a large Canadian trial (CTAF) it was demonstrated that despite 1 year treatment with amiodarone, dl-sotalol or propafenone still respectively 31, 61 and 61% of the patients experienced a recurrence of the arrhythmia.¹² Besides being only moderately effective, anti-arrhythmic drugs are also poorly tolerated. In the large AFFIRM-trial (Atrial Fibrillation Follow-Investigation of Rhythm Management) it was shown that respectively 12.3, 11.1 and 28.1% of patients receiving amiodarone, sotalol and class I drugs discontinued their medication because of side effects.¹³ Anti-arrhythmic drugs may cause serious ventricular pro-arrhythmia. Large clinical trials even demonstrated an increased mortality of flecainide or encainide in patients with a previous myocardial infarction.¹⁴ For these reasons, alternative (non-pharmacological) treatment strategies have been developed, including catheter ablation,¹⁵ surgery¹⁶ and pacemakers.^{17,18} However, because of the large number of people with AF, the only way to manage AF in the general population is to use safe anti-arrhythmic drugs.

In the last decade important progress has been made in the understanding of the electrophysiological mechanism of AF. In 1995 Wijffels et al. published a paper in which they showed that 'AF begets AF' because of AF-induced atrial remodeling.¹⁹ In this experimental study it was demonstrated that repetitive induction of AF by burst pacing, progressively prolonged the duration of AF-episodes until after 1-2 weeks the arrhythmia no longer terminated spontaneously anymore. The underlying

electrophysiological mechanism proposed was a progressive shortening of the atrial action potential that produced a substrate for AF by decreasing the wavelength of the fibrillation waves.^{20,21} Since then, others have demonstrated electrical atrial remodeling in different species, including man.^{22,23} At a cellular level, several changes have been described and downregulation of the L-type Ca^{2+} current has been shown to play a central role in the shortening of the atrial action potential.²³

OBJECTIVES OF THE STUDY

In my thesis I evaluated the electrophysiological effects of experimentally and clinically available anti-arrhythmic drugs in a goat model of AF. An important focus was to investigate the consequences of AF-induced electrical remodeling for the action of anti-arrhythmic drugs. It was hypothesized that due to AF-induced shortening of the action potential, the sensitivity for specific channel blockers may be altered. This hypothesis is supported by the clinical observation that the efficacy of anti-arrhythmic drugs declines when AF persists for a longer period of time.

In Chapter 1 several animal models of AF are compared and their clinical relevance is discussed. In Chapter 2 we evaluated the anti-arrhythmic properties of two Na^+/H^+ exchanger blockers EMD125021 and EMD87580. The rationale behind this study was the hypothesis that prevention of atrial acidosis during AF by blocking the Na^+/H^+ exchanger could prevent or revert AF-induced electrical remodeling. In chapter 3 we describe the effects of AF-induced electrical remodeling on the action of class IC and class III drugs (flecainide, *d*-sotalol and ibutilide). In chapter 4 we evaluated the anti-arrhythmic properties of a novel drug AVE0118 that blocks I_{Kur} , I_{to} and I_{KACh} currents. This drug is believed to have a selective class III action on the atria. In chapter 5 we evaluated the effects of combined administration of AVE0118 with the currently available I_{Kr} blockers. We demonstrate in goats with persistent AF that this drug combination could restore sinus rhythm in 100% of the cases. In Chapter 6 we evaluated whether the spatial distribution of the atrial refractory period and the vulnerability to AF are altered by long-term changes in the sequence of atrial activation. In chapter 7 we explored whether the class III drug dofetilide may facilitate the initiation of AF by increasing atrial refractoriness on Bachmann's bundle.

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CHAPTER 1

Pre-Clinical Models of AF: What Lessons Have We Learned ?

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INTRODUCTION

Atrial fibrillation (AF) is a common arrhythmia responsible for a substantial morbidity¹ and mortality² in the general population. It affects mainly the elderly, with a prevalence of 0.5% in patients aged 50 to 59 years, incrementing to almost 9% in men aged over 80 years.³ Atrial fibrillation sometimes occurs in individuals without cardiac abnormalities (lone atrial fibrillation).⁴ More often it is associated with an underlying heart disease such as hypertension, valvular disorders, coronary artery disease, and/or congestive heart failure.³

In the last century, several animal models of atrial fibrillation were developed. This has led to a considerable improvement in our understanding of the mechanisms of the arrhythmia. The aim of the current chapter is to review the various animal models of atrial fibrillation and assess what they have taught us about the treatment of AF.

AF is due to Multiple Mechanisms

Atrial fibrillation can easily be diagnosed on the basis of the characteristic features of the surface electrocardiogram. However, this does not provide much information on the many possible causes and mechanisms of the arrhythmia in individual patients. Experimental and clinical studies have revealed that AF has several different etiologies and that the mechanisms by which AF sustains itself are diverse. Atrial fibrillation can be perpetuated by either a single source or by multiple sources. In Figure 1, different mechanisms of AF are presented schematically. Some of them are still hypothetical; others have been demonstrated experimentally or clinically.

Single-Source AF

An important characteristic of AF 'driven' by a single source is that the arrhythmia should terminate after elimination of the rapid source.

Automatic Focus: In this type of AF, an automatic focus is present, which fires so rapidly that the atria cannot follow 1:1 (fibrillatory conduction). Clinical studies have shown that the vast majority of these atrial ectopic foci are located in the myocardial 'sleeves' of the pulmonary veins.⁵

Mother Wave: This is a single stable macroreentrant circuit with a short cycle length and a fixed anatomically or functionally determined pathway. The high frequency of the reentrant circuit leads to fibrillatory conduction in the rest of the atria. Interruption of the circulating mother wave should terminate AF.

Fixed Rotor: AF can also be 'driven' by a stable fixed microreentrant circuit with a high frequency.

Moving Rotor: This is AF driven by a stable microreentrant circuit drifting through the atria. AF results because the rest of the atrium cannot follow the high frequency in a 1:1 fashion. Rotors may have preferential locations determined by anatomical and functional properties of the atria.

Single Source

Multiple Sources

Figure 1: Schematic representation of the different mechanism that can give rise to atrial fibrillation. See text for details.

Multiple-Source AF

Multiple Foci: Foci due to enhanced automaticity can be present at multiple sites. In this case the successful elimination of one of these foci will not restore sinus rhythm.

Multiple Circuits: Multiple reentrant circuits may co-exist in the atria. Their circular pathways can be anatomically or functionally determined. Because they are multiple, the circuits do not have to be stable in order to perpetuate AF.

Multiple Wavelets: The atria are activated with a high frequency by multiple wavelets wandering simultaneously through the atrial myocardium. The pathways of the wavelets are determined by the anatomical and functional properties of the atrial wall. While traveling through the atria, the wavelets often reenter the tail of refractoriness of other waves (random reentry). Incidentally they can also reenter their own refractory tail. However, stable rotors are not observed during this type of AF. The stability of AF depends on the number of wavelets that 'fit' on the atrial surface. The shorter the wavelength of the atrial impulse, the more wavelets will co-exist and the less likely AF will terminate. Prolongation of the wavelength by class III drugs reduces the number of wavelets and terminates AF.

Focus and Multiple Wavelets: It is not unlikely that different mechanisms of AF can be operative at the same time. If a rapid focus exists together with randomly re-

entering multiple wavelets, atrial fibrillation will be highly persistent or even permanent.

Aconitine-induced AF

Already in 1907, Winterberg⁶ proposed that AF can originate from a single automatic focus. Experiments by Scherf et al.⁷ confirmed this hypothesis. Local application of aconitine (a sodium channel opener) on canine atria induced a rapidly firing focus that, depending on the frequency, created either atrial flutter or fibrillation. When the focus was eliminated by clamping off the site of aconitine application, the arrhythmia stopped. This is illustrated in Figure 2. In this experiment performed by Moe and Abildskov,⁸ AF was induced by injecting aconitine in the tip of the atrial appendage. To investigate whether the focal discharge was responsible for perpetuation of AF, the focus was isolated from the rest of the atrium by clamping the atrial appendage. Indeed, after electrical isolation of the appendage, the arrhythmia was promptly terminated.

In a recent clinical study, Haissaguerre and co-workers⁵ demonstrated that in patients with paroxysmal AF, fibrillation was initiated by a rapidly firing focus in the pulmonary veins. Selective ablation of these foci strongly reduced the number of AF episodes; in many patients, anti-arrhythmic drugs could be discontinued. The ionic electrophysiological mechanism underlying the rapid focal activity is not clear. It is also not understood whether these foci act solely as the trigger to start AF or whether they also act as a 'driver' that perpetuates the arrhythmia. So far, only anecdotal reports have shown that chronic AF can be terminated by focal ablation inside the pulmonary veins.⁹ Circumferential radiofrequency ablation of the pulmonary veins has been reported to cardiovert long-lasting chronic AF in some patients.¹⁰ However, it is important to note that this rather extensive ablation procedure will not only isolate the trigger within the pulmonary veins but also affects the substrate of AF by markedly reducing the atrial tissue mass of the diseased left atrium (debulking). Although the clinical outcome of pulmonary vein ablation is often beneficial, complete and permanent electrical isolation of the veins is seldom accomplished.¹¹ This raises the question of whether alternative mechanisms like a reduction of atrial mass, autonomic denervation, or interruption of critical reentry crossroads may explain the clinical efficacy of pulmonary vein ablation.

Vagal AF

The autonomic nervous system, in particular the vagal nerves, has been recognized to play an important role in the initiation and perpetuation of atrial fibrillation. Already in 1897, Knoll¹² observed that AF often occurred during vagal stimulation in rabbits and dogs. In 1915, Rothberger and Winterberg¹³ showed that AF could easily be induced during vagal stimulation in normal canine atria. Figure 2 demonstrates the importance of the vagal nerves for the stability of AF. When the driver of AF (aconitine) was eliminated by clamping the atrial appendage, AF persisted when

A.

Figure 2A: Demonstration of AF 'driven' by a rapidly firing focus. Shown are electrograms recorded from the right atrium and ventricle. Injection of aconitine into the atrial myocardium resulted in the degeneration of atrial flutter into fibrillation. Isolation of the site of injection from the rest of the atrium by clamping the atrial appendage caused the arrhythmia to terminate. When the clamp was released, atrial fibrillation restarted.

B.

Figure 2B: Illustration of the effect of vagal stimulation on the stability of AF. Shown are the same tracings as in 2A. Aconitine injection into the right atrial appendage resulted in AF. When the vagal nerve was stimulated, the rate of fibrillation accelerated. Isolation of the aconitine focus by clamping at this point did not terminate the arrhythmia. When vagal stimulation was stopped, atrial fibrillation slowed down and sinus rhythm resumed. (From Moe and Abildskov⁸)

the vagal nerves were stimulated. To demonstrate that vagal stimulation was responsible for the maintenance of AF, vagal stimulation was stopped (see arrow in Figure 2). This led to prompt termination of AF.

Induction of AF by vagal stimulation, either by direct stimulation of the vagal nerve or by intravenous infusion of cholinergic agents, is an established way to induce AF. The main underlying electrophysiological mechanism is a shortening of the atrial refractory period and possibly an increase in the spatial dispersion of refractoriness.¹⁴ When a premature impulse encounters an area of prolonged refractoriness, the wavefront will be blocked locally, while in other directions (with shorter refractory periods) it will be propagated. When the time required for the impulse to travel around the area of block exceeds the refractory period of the cells proximal to the area of block, these cells will be re-excited by the returning wavefront and a re-entrant arrhythmia will be initiated. Not only the induction but also the persistence of fibrillation is facilitated by vagal stimulation. When the refractory period shortens, the length of the fibrillation waves will decrease and AF may become sustained because more wavelets can now circulate simultaneously in the atria. The multiple wavelet hypothesis of Moe⁸ was experimentally confirmed by Allesie et al.¹⁵ in isolated canine atria perfused with acetylcholine. Using a multi channel mapping system, they recorded the electrical activity from several hundred atrial sites. It was found that a critical number of four to six wavelets was required to sustain AF. The experiments by Wang et al.¹⁶ are in line with this observation. In a canine model of vagal AF, they demonstrated that class IC drugs terminated AF by decreasing the number of wavelets. In 1992, Schuessler et al.¹⁷ demonstrated that, at high dosages of acetylcholine, a single rotor with a very short cycle length of ~40 ms caused fibrillatory conduction in the rest of the atria. The small functionally determined re-entry circuit was either stationary or drifted slowly through the atrial wall. In addition, these authors demonstrated that in specific regions activation of the epicardium was discordant from the endocardial surface.¹⁸ The role of anatomical heterogeneities for the pattern of atrial activation during AF was further supported by Wu et al.¹⁹ In isolated canine atria, they found that pectinate muscles were involved in lines of conduction block, thus allowing stationary reentry. Also, in isolated perfused sheep heart, Skanes et al.²⁰ demonstrated that in the presence of acetylcholine, single sources of micro-reentry of very rapid rates caused loss of 1:1 conduction in other parts of the atria. Mandapati et al.²¹ demonstrated that these rotors were predominantly located in the posterior left atrium. However, evidence that such a mechanism is responsible for clinical AF is as yet lacking.

Although the above mentioned experiments have contributed significantly to our understanding of the mechanism of AF, one must be cautious in extrapolating these observations to clinical arrhythmias. First of all, it is important to note that contrary to most clinical cases of AF, no structural abnormalities are present in the atria in most animal studies. It is also questionable whether the extreme shortening of atrial refractoriness by vagal stimulation is encountered in patients. After the first description of vagal AF by Coumel et al.,²² several studies addressed this issue. In most studies, the neurohumoral activity was evaluated by analysis of heart rate variability.

Figure 3: The goat model of AF. Upper panel: Induction of AF by burst pacing. An atrial bipolar electrogram was continuously analyzed by a custom-made fibrillation pacemaker. As soon as sinus rhythm occurred a burst of stimuli was given to reinduce AF. In this way AF could be maintained 24 hr a day, 7 days a week. Left lower panel: Time course of the development of sustained AF. In the example shown, AF became persistent (>24 hours) after 7 to 10 days of artificially maintained AF. Lower right panel: AF-induced atrial electrical remodeling. After 6 to 24 hr of AF, the refractory period had shortened and the physiological rate adaptation was lost. (From Wijffels et al.⁵⁸).

No consistent picture regarding the onset of AF can be derived from these studies. While in some studies vagal tone was found to be increased,^{23,24} other studies demonstrated a predominance of sympathetic activity.^{25,26} Also, the circadian pattern of onset of paroxysmal AF does not support the concept of vagally induced AF. Since vagal tone is higher during the night, one would expect most episodes of AF to occur during the night. In contrast, Irwin et al.²⁷ and Kupari et al.²⁸ found that most paroxysms of AF actually occurred during the day.

AF-induced Atrial Remodeling

Clinical studies have shown that atrial fibrillation is progressive in nature. In many patients with paroxysmal AF the arrhythmia tends to become persistent with time.²⁹

Figure 4: Left panel: Voltage current relationships of I_{Ca} during control (P0) and after 1, 7, and 42 days of rapid atrial pacing in the dog. The density of I_{Ca} was progressively reduced with the duration of rapid pacing. Right panel: Action potentials recorded at 0.1 (●) and 2 Hz (◆) in control atrial cells (P0) and after 42 days of rapid atrial pacing (P42). Addition of nifedipine (C) mimicked the effects of electrical remodeling, whereas the Ca-agonist BayK 8644 restored the plateau phase of the action potential (D) (From Yue et al.³³).

In 1995, two studies helped to explain these clinical observations.^{30,31} Morillo et al.³⁰ subjected dogs to 6 weeks of continuous rapid atrial pacing (400 bpm) and observed a shortening of the atrial refractory period by about 15%. In chronically instrumented goats, Wijffels et al.³¹ maintained AF by a fibrillation pacemaker that automatically delivered bursts of stimuli (1 sec, 50 Hz) as soon as sinus rhythm was detected (Figure 3). Using this method, AF could be maintained 24 hr a day, 7 days a week. After 2 to 4 days of AF, the atrial refractory period had shortened from ~150 to ~80 ms (-45%). In addition, the normal physiological rate dependency of the refractory period was lost. Restoration of sinus rhythm completely restored the normal refractory period within 2 to 3 days (reverse electrical remodeling). In both studies, the rapid atrial rate led to a progressive increase in the duration of AF paroxysms. After 6 weeks of rapid atrial pacing in the dog, 82% of the paroxysms of AF lasted longer than 15 min. In the goat model, as a result of 2 to 3 weeks of repetitive induction of AF, 90% of the animals developed persistent AF.³¹ Both in the dog model of Morillo et al.³⁰ and the goat model of Wijffels et al.³¹ it was hypothesized that the mechanism of increased susceptibility for AF was based on a shortening of atrial refractoriness and wavelength. Morillo et al.³⁰ observed a significantly shorter atrial fibrillation cycle length in the left atrium compared to the right. Cryoablation of the area with the highest dominant frequency terminated AF in 9 of 11 dogs.

The ionic mechanisms underlying AF-induced electrical remodeling have been extensively studied.³² Yue et al.³³ were the first to demonstrate that the L-type Ca^{2+} current (I_{CaL}) was reduced in cardiomyocytes of electrically remodeled atria (Figure 4). Inhibition of I_{CaL} by nifedipine produced a shortening of the action potential, comparable to the changes caused by prolonged atrial tachycardia. On the other hand, an

increase of the L-type Ca^{2+} current by BayK8644 could partly reverse the shortening of the action potential by rapid atrial pacing. These data strongly suggest that a reduction of I_{CaL} underlies the tachycardia-induced shortening of the refractory period. In additional studies it was shown that AF also reduces the transient outward current (I_{to}) and the ultrarapid delayed rectifier current (I_{Kur})^{33,34}. The ionic remodeling resulting from prolonged high atrial rates or atrial fibrillation are reviewed by Bosch and Nattel.³²

Electrical remodeling of the atria also occurs in humans. As far back as 1982 Attuel et al.³⁵ observed a shortening and abnormal rate adaptation of atrial refractoriness in patients with AF. In 1986, Boutjdir et al.³⁶ found that atrial action potentials were shorter in patients with AF compared to those with normal sinus rhythm. Franz et al.³⁷ demonstrated that after electrical cardioversion of atrial flutter or fibrillation, the human atrial action potential was shortened. The time course of reverse electrical remodeling was studied by Yu et al.³⁸ In 19 patients, the change in atrial refractoriness was monitored daily after electrical cardioversion of chronic AF. It was found that the shortening of the refractory period was completely reversible within 3 to 4 days of sinus rhythm.

After the initial description of the AF-induced shortening of atrial refractoriness, several studies have tried to prevent tachycardia-induced electrical remodeling. First, it was reported that administration of the L-type Ca^{2+} antagonist verapamil could delay the AF-induced shortening of refractoriness.^{39,40} In other studies, initially a protective effect was described for angiotensin II antagonists,⁴¹ Na^+/H^+ exchanger inhibitors,⁴² anti-oxidants (ascorbate),⁴³ mibefradil (T-type Ca^{2+} channel blocker)⁴⁴ and amiodarone.⁴⁵ However, most of these studies addressed the short-term effects (hours) of atrial tachycardia. When the atria were paced for longer periods of time (days) most drugs failed to prevent electrical remodeling.^{46,47} Only mibefradil⁴⁴ and amiodarone⁴⁵ retained their protective effects during 7 days of rapid pacing and could prevent the development of persistent AF in dogs. Recently it was found that the class III action of I_{Kr} -blockers like *d*-sotalol, ibutilide and dofetilide was strongly reduced after 48 hr of AF.^{48,49} In contrast, the class III effect of AVE 0118, a substance blocking early activated K^+ currents (I_{Kur} and I_{to}), was *enhanced* after electrical remodeling. If these observations are reproduced in humans, such 'early' class III drugs may become very useful in the cardioversion and prevention of AF.

From clinical studies, it is known that the atrial contractile function is impaired after successful cardioversion of AF.⁵⁰ The degree of contractile dysfunction seems to be related to the duration of AF. In patients with lone AF, Sanfillippo et al.⁵¹ observed an increase in atrial volume of ~40% after 20.6 months of AF. After restoration of sinus rhythm atrial contractile function can recover, although this may take several months.⁵² Schotten et al.⁵³ hypothesized that the reduction in I_{CaL} during AF-induced electrical remodeling may also underlie atrial contractile dysfunction. To test this hypothesis, they chronically instrumented goats with pairs of piezoelectric crystals on both atria and a pressure catheter in the cavity of the right atrium (Figure 5). With the atrial volume-pressure loops obtained in this way, the external work performed by the right atrium could be measured chronically. During the first 5 days

Figure 5: Contractile remodeling during the first 5 days of AF. Upper left: A pair of piezoelectric crystals sutured on the right atrium. Right atrial pressure was measured by an implanted pressure transducer. LA, left atrium; RA, right atrium; CV, superior caval vein; Ao, Aorta. Upper right: Simultaneous recording of right atrial diameter and pressure. AF was induced by 1 sec (50 Hz) of burst pacing. Lower left: Right atrial pressure-volume loops recorded at baseline and after 12 and after 48 h of AF. Lower right: Time course of electrical and contractile remodeling and their reversibility in seven goats (From Schotten.⁵³).

of AF, the strength of the atrial contractions markedly declined. After 2 days the pressure-volume loop already showed that atrial contractile function was almost completely abolished. The atrial work index decreased from 16 to 2 mm × mmHg. The time course of electrical and contractile remodeling was the same. Also, during reverse remodeling, the time courses of restoration of the refractory period and atrial contractile function were the same; after 2 days of sinus rhythm, the pressure-diameter loop returned to control values. These findings indicate that during the first 5 days of AF, electrical and contractile remodeling are closely linked. They are probably both the result of the same mechanisms, i.e., a downregulation of the L-type Ca^{2+} current.

Apart from inducing electrical and contractile remodeling, AF also changes the structure of the atrium. The first experimental study in which this was demonstrated was done by Morillo et al.³⁰ Dogs subjected to 6 weeks of rapid atrial pacing revealed a marked disarray of atrial myofilaments, increased number and size of mi-

Myolysis

Sinus Rhythm

Atrial Fibrillation

Connexin 40

Figure 6: Structural remodeling of caprine atria by 4 months of AF. Top: Light microscopy shows enlarged atrial cells with severe myolysis and accumulation of glycogen. Bottom: Immuno labeling of Cx40 showing a clear reduction in Cx40 expression. (From Ausma et al.⁵⁴ and van der Velden et al.⁹⁰).

tochondria and disruption of the sarcoplasmic reticulum. Ausma et al.⁵⁴ found similar changes in the atria of goats in which AF was maintained for 9 to 23 weeks. The atrial myocytes were markedly increased in size, and changes in cellular structure were characterized by loss of myofibrils, accumulation of glycogen, reduction in connexin 40, changes in mitochondrial shape and size, fragmentation of sarcoplasmic reticulum, and dispersion of nuclear chromatin (Figure 6). It was suggested that these ultrastructural alterations closely resembled the changes observed in ventricular myocardium due to low flow ischemia, termed 'hibernation'. Hiber-

nating atrial myocytes were heterogeneously distributed, with some cells strongly affected and neighbouring cells virtually normal.

In two recent studies, the reversibility of structural atrial remodeling was evaluated. Everett et al.⁵⁵ found no recovery of structural remodeling 2 weeks after restoration of sinus rhythm in dogs subjected to 2 months of AF. Ausma et al.⁵⁶ investigated the reversibility of structural changes in the atria of goats that had been in AF for 4 months. Four months after restoration of sinus rhythm structural abnormalities were still present. Although by at that time cell size, connexin 40, and myocytes with severe myolysis were normal again, 25% of atrial myocytes still showed a moderate degree of myolysis. Also, the duration of induced AF episodes was still prolonged.

Atrial Dilatation

The impact of atrial dilatation on the propensity for AF is being studied in several experimental models. In most models the *acute* effects of atrial dilatation were studied. In the Langendorff perfused rabbit heart, Ravelli et al.⁵⁷ found that acute atrial stretch was associated with a shortening of the atrial action potential and refractory period. In contrast, other studies have reported no change⁵⁸ or a prolongation of atrial refractoriness⁵⁹ after acute volume overload. In a recent study, Eijsbouts et al.⁶⁰ evaluated the effects of acute atrial dilatation on atrial conduction. As a result of dilatation, atrial conduction slowed and the number of areas with local conduction delays increased significantly. In combination with a short atrial wavelength, such stretch-induced lines of intra-atrial conduction block may serve as sites of wavebreak which can initiate AF.

Although the models of acute dilation have provided important information about the electrophysiological effects of acute atrial stretch, atrial dilatation develops more gradually in patients. As early as 1981 Boyden et al.⁶¹ evaluated the effects of *chronic* atrial dilatation on atrial electrophysiology in dogs. Right atrial dilatation was created by cutting the chordae tendineae of the septal cusps of the tricuspid valves and partly occluding the pulmonary artery. After a mean follow-up of 93 days, the right atrial volume had increased by 39%. The propensity for atrial arrhythmias had clearly increased and the incidence of AF episodes lasting >10 min was significantly higher. However, *in vitro* recorded atrial action potentials did not differ in duration from action potentials recorded from non-dilated atria.⁶² An increased trabeculization of the right atrium was observed and atrial histology was characterized by hypertrophy and fibrosis. Cox et al.⁶³ developed a canine model of chronic mitral regurgitation by cutting the chordae tendineae of the mitral valve. After 3 months of regurgitation, AF could easily be induced by burst pacing. Multisite mapping revealed multiple activation patterns: ranging from the simplest pattern, in which a single re-entrant circuit was present, to the most complex cases, in which no consistent pattern of activation could be identified'. Of note, animals with sustained AF demonstrated the highest degree of dilatation. The most complex AF activation patterns were observed in these animals. Similar observations were made by

Figure 7: Effects of atrial dilatation on the electrophysiological properties of the atria in 6 goats with chronic AV-block. Left: Relative changes in atrial diameter and duration of induced AF episodes during 4 weeks of complete AV block. Right: Representative examples of unipolar endocardial atrial electrograms. Under control conditions (upper panels), the median AF interval shortened within the first 2 days of AF from 170 to 118 ms. After chronic AV block (lower panels), 2 days of AF still shortened the AERP (not shown) but no longer shortened the AF cycle length. (From Neuberger et al.⁹¹)

Verheule et al.⁶⁴ In dogs with 1 month of mitral regurgitation, the inducibility of AF was strongly increased. Atrial conduction did not change, but atrial refractory periods in the right and left atria were prolonged. Structural alterations in the atrial myocardium were confined to the left atrium and consisted of increased glycogen accumulation and interstitial fibrosis.

Recently Neuberger et al.⁶⁵ investigated the relation between the time course of atrial dilatation and the stability of AF in goats with chronic atrio-ventricular (AV) block. Endocardial screw-in leads with sonomicrometer ultrasound crystals were chronically implanted in the right atrium. In this way, atrial electrophysiology and atrial size could be monitored simultaneously. After His bundle ablation, a progressive increase in atrial size was observed; after 4 weeks, atrial diameter had increased by 12%. Before the creation of this AV-block, episodes of AF induced by burst pacing were generally brief (lasting seconds). After 4 weeks of AV-block, the duration of AF paroxysms gradually increased. In goats with the largest degree of dilatation, AF now lasted longer than 1 hr (Figure 7). As in the study of Boyden et al.⁶² no changes in atrial refractoriness were observed. Detailed mapping of the right and left atria revealed an increased heterogeneity in conduction. Whereas maintenance of AF still resulted in shortening of the atrial refractory period, the AF cycle length did not shorten accordingly. This suggests that, in this model of atrial dilatation, structural conduction defects are involved in the reentrant process.

Clinical data on the effects of chronic atrial stretch on atrial electrophysiology are scarce. Sparks et al.^{66,67} evaluated the effects of chronic VVI and DDD pacing on atrial size and electrophysiology in patients requiring permanent pacing. In this prospective randomized study, it was found that VVI pacing increased atrial dimension,⁶⁶ and that this was associated with a prolongation of the atrial effective refractory period (AERP).⁶⁷ No changes, either in atrial size or electrophysiological properties were observed after DDD pacing. Although a prolongation of atrial

refractoriness can be expected to prevent arrhythmias by prolonging the atrial wavelength, VI pacing clearly increased the propensity for AF compared to DDD pacing. The underlying mechanisms are unknown, but it is likely that changes in atrial conduction due to an increase in connective tissue play a role in the genesis of AF in patients with dilated atria.

Inflammation

In 1986, the group of Waldo developed a new canine model of atrial flutter.⁶⁸ After a thoracotomy, both atria were dusted with sterile talc and a gauze dressing was left on them. Electrodes were sutured to the atria and the chest was closed. During the first week after surgery, susceptibility for atrial arrhythmias was evaluated daily. In more than 90% of the animals, atrial flutter could be induced by programmed electrical stimulation on at least one of the first four postoperative days. With time, the susceptibility for arrhythmias declined; 1 week after surgery, flutter could be induced in only one of four animals. The mechanism of this temporarily high atrial vulnerability is still unclear. Atrial refractoriness and conduction velocity were not different in susceptible or non-susceptible dogs. In subsequent studies also atrial fibrillation was induced. Extensive mapping of electrical activation showed that one to four unstable reentrant circuits of short cycle length were responsible for the maintenance of AF.⁶⁹ Reentrant circuits involved the septum, right atrial free wall, pulmonary veins and inferior and superior caval veins. Importantly, Bachmann's bundle was involved in most of these reentrant circuits. Radiofrequency catheter ablation of this 'critical crossroad' completely abolished AF in this model. It is of interest to note that the time-course of inducibility of atrial flutter and fibrillation observed in the model shows striking similarities with the incidence of post-operative AF in patients. AF/atrial flutter occurs in 70% of patients during the first 4 days after surgery.⁷⁰ The canine model of sterile pericarditis can thus be useful in elucidating the mechanisms of postoperative atrial arrhythmias in humans. In a recent study, Waldo's group evaluated the anti-arrhythmic effects of steroids in the canine model of pericarditis.⁷¹ Administration of oral prednisone completely prevented atrial arrhythmias during the first 4 days after surgery. Histological examination revealed that the degree of inflammation was clearly reduced in steroid-treated animals. In an earlier clinical study, Yared et al.⁷² showed that, in patients undergoing elective coronary or valvular heart surgery, administration of a single dose of dexamethasone reduced the incidence of postoperative AF from 32.3 to 18.9%. These studies clearly indicate a link between postoperative AF and inflammatory responses.

Congestive Heart Failure

In clinical practice it is well known that heart failure often leads to AF.⁷³ On the other hand, AF can also worsen ventricular function. Not surprisingly, AF and heart failure coexist in many patients. To elucidate how heart failure increases the vulnerability for AF, Nattel and co-workers⁷⁴ subjected dogs to rapid ventricular pacing (220 to

Figure 8: Different kinds of atrial remodeling in canine atria. Left: crowding of isochrones was observed only in dogs with congestive heart failure, indicating local slowing of conduction. Right: Histological examination revealed severe atrial fibrosis in dogs with congestive heart failure, whereas prolonged rapid atrial pacing had no significant effect on the amount of connective tissue. (Modified from Li et al.⁷⁴).

240 bpm) for a period of 3 weeks. The animals progressively developed clinical and hemodynamical signs of heart failure. In 10 of 18 dogs, sustained AF could be induced. AF was characterized by long AF cycle lengths and a relatively low degree of polymorphism of the fibrillation electrograms. Atrial mapping suggested that AF was maintained by a small number of reentrant waves. Unlike the case in the rapid atrial pacing model, the increased stability of AF was not based on a shortening of the atrial wavelength. No changes in refractoriness, conduction velocity, or wavelength were noted. However, when the local conduction properties were studied in more detail, discrete regions of slow conduction were found. Histological analysis revealed a marked increase in fibrous tissue, which was held responsible for the local disturbances in atrial conduction (Figure 8). Characterization of the cellular electrophysiological changes showed a decrease in I_{to} , I_{CaT} , and I_{Ks} and an increase in the Na^+/Ca^{2+} exchange current.⁷⁵ Thus, in the setting of congestive heart failure (CHF), an increase in atrial fibrosis and tissue anisotropy may promote AF by the fa-

cillation of AF by inducing microreentry. Of note, the structural abnormalities showed striking similarities with the histological findings observed in atrial myocardium of the elderly⁷⁶ and in patients with rheumatic heart disease.⁷⁷

Sanders et al.⁷⁸ recently investigated the atrial electrophysiological properties in patients with CHF. Using electroanatomic mapping, they found that right atrial conduction velocity was markedly slowed and that the number of electrograms with fractionated potentials was increased. Although no clear differences in the inducibility of AF was observed, the duration of induced AF paroxysms was clearly prolonged.

Since the renin-angiotension system is thought to play a pivotal role in the development of cardiac fibrosis, Li et al.⁷⁹ investigated whether the angiotensin converting enzyme (ACE) inhibitor enalapril could prevent AF in the canine CHF model. After 5 weeks of rapid ventricular pacing, drug-treated animals showed a lower level of angiotensin II in the atrial wall. At the same time, the duration of induced AF episodes was significantly shorter than in the placebo group. ACE inhibition attenuated the development of interstitial fibrosis and could partly prevent the development of intra-atrial conduction abnormalities. Recent clinical data suggest that ACE-inhibitors may have beneficial effects in patients with atrial fibrillation. In the TRACE study,⁸⁰ it was found that trandolapril reduced the incidence of AF during a 2 to 4 year follow up in patients with reduced left ventricular function. Similar results were obtained in the SOLVD trial.⁸¹ Recurrences of atrial fibrillation after electrical cardioversion were significantly less in patients treated with amiodarone and irbesartan (an angiotensin I type 1 blocker) vs. patients treated with amiodarone alone.⁸² However, it remains to be determined whether the observed anti-arrhythmic action of ACE-inhibition is based on an anti-fibrotic effect. Other effects such as lowering of the atrial pressure might also explain a reduction in susceptibility for AF.

Ischemia

Myocardial infarction is an independent risk factor for the development of AF.³ Up to 21% of patients admitted with an acute myocardial infarction suffer from atrial fibrillation/flutter during their hospital stay.⁸³ The pathophysiological mechanisms of ischemia related AF are not well understood. Most of our knowledge of the arrhythmogenic mechanisms of ischemia has been acquired in the ventricles. Both a marked heterogeneity in refractoriness and areas of slow and discontinuous conduction may lead to a substrate of reentrant arrhythmias. It is unclear whether the same electrophysiological effects also occur in ischemic atria. Only recently, the effects of coronary occlusion on atrial arrhythmias have been studied. In a dog model, Jayachandran et al.⁴² observed an acute shortening of the atrial refractory period after occlusion of the proximal right coronary artery. In a study of Sinno et al.⁸⁴ no effects on atrial refractoriness were observed during the first hours after ligation of a branch of the right coronary artery (right intermediate atrial artery). In ischemic atrial tissue, the pacing threshold was increased, conduction was slowed, and the duration of induced AF episodes increased significantly. Miyauchi et al.⁸⁵ investigated

Figure 9: The early computer model of AF by Moe and Abildskov. Left: Each unit of a 992-unit matrix was assigned one of five states of excitability. State 1: absolutely refractory. State 2 to 4: relatively refractory with decreased conduction velocity in case of activation. State 5: fully recovered excitability. Right: Several simultaneously propagating wavefronts differing in size and direction of propagation. Arrows indicate progress of wavefronts. (From Moe et al.⁸⁷).

the long-term effects of occlusion of the left descending artery below the first diagonal branch in dogs on the susceptibility of the atria for AF. After 8 weeks occlusion, atrial electrophysiological measurements were performed. Both the inducibility and stability of AF were increased after myocardial infarction; in the control group, the duration of induced AF lasted for only 3 sec, after myocardial infarction atrial fibrillation lasted for an average of 41 sec. Mapping of atrial activation during application of premature stimulation showed epicardial wavebreaks degenerating into AF. No differences in the duration of atrial action potentials or refractoriness were found after myocardial infarction. However, at fast pacing rates, a higher spatial dispersion in the duration of monophasic action potentials was found. The slope of the restitution curve was also steeper. Immunocytochemical staining of sympathetic nerves showed an increased density and marked heterogeneities in different parts of the atria. The amount of connexin 40 was reduced and it was also more heterogeneously distributed.⁸⁶ No signs of atrial ischemia or fibrosis could be found. Thus it appears that ventricular infarction *itself* leads to the creation of a substrate for AF, possibly due to nerve sprouting, atrial stretch, or modulation of the autonomic nervous system.

Computer models of AF

In the early 60s Moe and co-workers⁸⁷ developed the first computer model of AF, consisting of a two-dimensional matrix of 992 units. Each unit could have five different states of excitability, ranging from fully excitable to absolutely refractory (Figure 9, left panel). Spatial dispersion in refractoriness was simulated by varying the duration of the lowest excitable state in different units. The 'turbulent activity' within the matrix was sustained by multiple wandering wavelets varying in both number and size (Figure 9, right panel). When the refractory period was increased or the surface area reduced, the activity was terminated. The creation of internal obstacles resulted in a circus movement flutter. The growing knowledge of cellular electrophysiology

led to the design of more sophisticated computer models implementing various ionic currents and concentrations, subcellular compartments, membrane capacity, and cell to cell coupling. One example is the model recently developed by Zou et al.⁸⁸ These authors simulated cell grids of varying size in the presence of spatially different acetylcholine concentrations, causing different patterns of spatial dispersion of refractoriness. In almost all cases, AF was sustained by a single spiral wave rotating around an area of low acetylcholine concentration. When the grid became too small, AF was not sustained. Since, above this critical size, the behavior of AF was qualitatively the same in an 11-fold increase in atrial grid size, these authors stated that the emphasis on tissue mass as an important determinant of AF stability may have been overstated.⁸⁸ Virag et al.⁸⁹ developed a model of AF based on the anatomy of human atria. An ion-based membrane model was implemented in an anatomical structure derived from segmented magnetic resonance images. However, anisotropy and differences in wall thickness were not implemented in this model, in which burst pacing induced atrial fibrillation even when the membrane properties were uniform. As expected, shortening of the refractory period increased the duration of AF.

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CHAPTER 2

Inhibitors of the Na⁺/H⁺ Exchanger Cannot Prevent Atrial Electrical Remodeling in the Goat

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ABSTRACT

Introduction: It has been suggested that blockade of the Na^+/H^+ exchanger (NHE1) can prevent atrial fibrillation (AF)-induced electrical remodeling and the development of AF.

Methods and Results: AF was maintained by burst pacing in 10 chronically instrumented conscious goats. Intravenous and oral dosages of two NHE1 blockers (EMD87580 and EMD125021) resulted in plasma-levels several magnitudes higher than required for effective NHE1 blockade. Shortening of atrial refractoriness immediately after 5 minutes of AF was not prevented by NHE1 blockade. In remodeled atria, increasing dosages of EMD87580 and EMD125021 did not reverse the shortening of the atrial refractory period or reduce the duration of AF episodes. The cycle length during persistent AF was also not affected. Oral pre-treatment with EMD87580 (8 mg/kg b.i.d) starting 3 days before AF could not prevent electrical remodeling. After 24 and 48 hours of remodeling, the duration of AF paroxysms was respectively 47 ± 32 and 135 ± 63 seconds compared to 56 ± 17 and 136 ± 52 seconds in placebo treated animals ($p > 0.8$), respectively.

Conclusion: In the goat model of AF, the Na^+/H^+ exchanger inhibitors EMD87580 and EMD125021 did not prevent or revert AF-induced electrical remodeling. This indicates that activation of the Na^+/H^+ exchanger is not involved in the intracellular pathways of electrical remodeling. This does not support the suggestion that blockers of the Na^+/H^+ exchanger may be beneficial for prevention and treatment of AF.

INTRODUCTION

Atrial fibrillation (AF) induces electrophysiological changes that are characterized by a shortening of the atrial refractory period and a loss of its physiological rate adaptation. This electrical remodeling has been shown to play an important role in the development of persistent AF.¹ Studies on cellular electrophysiology revealed that the abbreviation of the action potential was mainly due to a reduction of the L-type Ca²⁺ current ($I_{Ca,L}$).² Down-regulation of $I_{Ca,L}$ is believed to be due to AF-induced Ca²⁺ overload. One of the factors which may enhance Ca²⁺ overload is intracellular acidosis. A metabolic mismatch during AF may lead to an accumulation of protons which is known to activate the Na⁺/H⁺ exchanger (NHE1).³ Extrusion of H⁺ from the cell increases the intracellular Na⁺ concentration, which may activate the reverse mode of the Na⁺/Ca²⁺ exchanger. This, in its turn, will increase the intracellular Ca²⁺ concentration of the cell.⁴

Inhibition of the Na⁺/H⁺ exchanger would interrupt this cascade and thus might be able to attenuate AF-induced electrical remodeling. The first studies addressing this possibility have produced inconsistent results. Jayachandran et al. showed that blockade of the NHE1 indeed prevented shortening of atrial refractoriness during 5 hours of rapid pacing.⁵ In contrast, Shinagawa et al. showed no effect of NHE1 inhibition after 7 days.⁶ Thus, although blockade of the Na⁺/H⁺ exchanger seems to be able to prevent short-term electrical remodeling, this beneficial effect is not maintained. The present study was designed to evaluate the effects of NHE1 inhibitors on electrical remodeling in the goat during both *short-term* and *long-term* atrial fibrillation. Because NHE1 blockers have few side effects,^{7,8} prevention or reversal of electrical remodeling by these drugs could be of great potential for the treatment of AF.

METHODS

Goat Model of Atrial Fibrillation

Ten female goats (weight 54 ± 4 kg) were used for this study. The animals were handled according to the European Directive for Animal Research and the study protocol was approved by the Animal Investigation Committee of the University of Maastricht. During general anaesthesia, the heart was exposed and a double row of electrodes (inter-electrode distance 7 mm) was wrapped around the AV groove. Another band of electrodes (triple row; inter-electrode distance 5 mm) was pulled through the space between the aortic root and Bachmann's bundle (Figure 1). In addition, a small plaque with 3 electrodes was sutured on the free wall of the left ventricle. A silver plate was implanted subcutaneously to serve as indifferent electrode. The electrode leads were tunnelled to the neck and exteriorized by four 30-pole connectors. The experiments were started 3 weeks after surgery. AF was induced by repetitive burst pacing as described by Wijffels et al.¹ The duration of AF paroxysms progressively increased during the first days, then AF became persistent

Figure 1 Top: Diagram of the chronically implanted atrial electrodes. A long strip containing a double row of electrodes was wrapped around the atrio-ventricular groove and sutured to the lateral walls of the right and left atrial appendages. Another strip with three rows of electrodes was positioned along Bachmann's bundle and sutured to the anterior wall of the atrial appendages. Pairs of electrodes at the right atrium, left atrium and Bachmann's bundle were used for pacing and measurement of atrial refractoriness. Bottom: induction of AF by burst pacing (50Hz). During sinus rhythm the episodes of AF lasted <10 seconds. Continuous re-induction of AF progressively prolonged AF duration until after 1 week AF became persistent. BB = Bachmann's bundle, LA = left atrium, RA = right atrium, PV = pulmonary veins, IVC = inferior caval vein, SVC = superior caval vein.

after 1-2 weeks (lower panel Figure 1). During AF, an approximately 1.5 fold increase in ventricular rate was observed (from 110 bpm during sinus rhythm to 170 bpm during AF). The condition of the animals was checked regularly and no noticeable clinical signs of heart failure (shortness of breath, oedema or changes in body weight) were observed.

Figure 2: Dose response curves of NHE1 inhibition by EMD87580 and EMD125021 in the goat. An in vitro platelet swelling was used to measure the activity of the Na⁺/H⁺ exchanger. The curves show the platelet swelling rate constant ($\times 0.001/s$) at different concentrations for each compound. Data-points are the average values of 2 goats

Figure 3: Schematic representation of the three experimental protocols. In protocol I, the effects of EMD87580 (10 mg/kg) on the changes in atrial refractoriness after 5 minutes of AF were studied. Protocol II consisted of three parts, which studied the effects of cumulative dosages of EMD125021 and EMD87580 in non-remodeled atria (A), electrically remodeled atria (B, and during persistent AF (C). Protocol III evaluated the possible preventive effects of oral treatment with EMD87580 on AF-induced electrical remodeling.

Na⁺/H⁺ Exchange Inhibitors (EMD87580 and EMD125021)

Two selective inhibitors of the isoform 1 of the Na⁺/H⁺ exchanger were used: EMD87580 (N-[2-Methyl-4,5-bis(methylsulfonyl)-benzoyl]-guanidine hydrochloride) and EMD125021 (N-[5-Methanesulfonyl-2-methyl-4-trifluoro-methoxy-benzoyl]-guanidine methanesulfate) (Merck KGaA).⁹ To demonstrate effective inhibition of the NHE1 in the goat, a platelet swelling test was performed.¹⁰ In this test, propionic acid is added to platelet rich plasma. This leads to acidification of platelets and activation of the NHE1. As a result, intracellular Na⁺ concentration increases, leading to osmotic swelling. The concentration-response curves for EMD125021 and EMD87580 are shown in Figure 2. The concentrations of EMD125021 and EMD87580 which produced a 50% reduction of platelet swelling were 15 and 269 nM, respectively. These values correspond to a mass-concentration of respectively 7 and 105 ng/ml. Because protein binding of EMD87580 has been shown to be very weak, this value can be regarded as the free plasma concentration. A similar situation can be assumed for EMD125021. The dosages given in the present study produced plasma levels well above these effective dosages.

Experimental Protocol

Three different protocols were performed (Figure 3). In the first study, the effects of NHE1 blockade on the shortening of the atrial refractory period following 5 minutes of AF was evaluated. Atrial refractoriness was measured during sinus rhythm, before and after AF, both during infusion of saline and after intravenous EMD87580 (10 mg/kg). The second protocol assessed the effects of NHE1 blockade on atrial refractoriness and conduction velocity and consisted of three sub-studies (sinus rhythm, 24 hours AF, persistent AF). Two different NHE1 blockers were administered intravenously in incremental dosages of 1, 3, 10 and 30 mg/kg (EMD87580), and 0.1, 0.3, 1 and 3 mg/kg (EMD125021) at 90-minute intervals. The two compounds were studied in random order, with a washout period of at least 3 days in between. The third protocol was designed to test the hypothesis that oral administration of NHE1 blockers can prevent electrical remodeling. Capsules with EMD87580 (8 mg/kg) were given twice daily starting three days before the induction of AF. Atrial refractory period, conduction velocity and duration of AF were measured both before and 24 and 48 hours after induction of atrial fibrillation. In each goat this protocol was performed with EMD87580 and placebo in random order. A period of at least 7 days was allowed between the experiments.

Electrophysiological Measurements

Atrial effective refractory periods (AERP) were measured at three sites, during a range of pacing frequencies (S_1 - S_1 interval 400-200 ms). A single premature stimulus was interpolated at every eight basic interval and, starting well within the refractory period, the coupling interval (S_1 - S_2) was increased in 2ms steps. The longest

Figure 4: Absence of an effect of EMD87580 (10 mg/kg i.v.) on shortening of atrial refractoriness by 5 minutes of AF. Data are mean values $\pm$ SEM of five goats.

S_1 - S_2 interval which did not capture the atria was taken as the AERP. The transient change in atrial refractoriness immediately after spontaneous termination of AF was measured as follows.¹¹ Synchronized premature stimuli (4x threshold) were delivered at the right atrium after every six sinus beats incrementing the coupling interval in 2ms steps. In this manner the AERP could be determined three to five times per minute. To collect sufficient data, these measurements were performed twice.

Atrial conduction velocity was calculated from the conduction times along Bachmann's bundle during pacing from the right atrium at cycle lengths of 400,300,250 and 200ms. Conduction parallel to Bachmann's bundle was checked by similar activation times along the three rows of electrodes. The total distance over which the conduction velocity was measured ranged from 6 to 7.5cm.

The effects of EMD87580 and EMD125021 on the stability of AF were evaluated by measuring the duration of at least 10 consecutive AF paroxysms. In case of persistent AF, changes in the median AF cycle length (AFCL) were monitored (300-500 consecutive fibrillation intervals). The effects on the ventricles were monitored by measuring the R-R interval, QRS duration and QT time from a left ventricular electrogram.

Statistical Analysis

Data are given as mean $\pm$ SEM. Differences between groups were evaluated by the paired Student's *t*-test with the Bonferroni correction for multiple comparisons. Serial measurements of the AERP were analyzed using ANOVA for repeated measures. A *p*-value of <0.05 was considered as statistically significant.

Figure 5: Measurement of the atrial refractory period during cumulative dosages of EMD87580 and EMD125021 during normal sinus rhythm and after 24 hours of AF. Three pacing rates were used (S_1 - S_1 interval: 400,300 and 200 ms). Blockade of the Na^+ / H^+ exchanger had no effect on AERP in both non-remodeled and electrically remodeled atria. Data points are mean values $\pm$ SEM of three atrial sites in five goats.

RESULTS

Short-term Rate Adaptation

The effect of NHE1 inhibition on shortening of the atrial refractory period following 5 minutes of AF was evaluated in 5 goats. The median atrial cycle length during sinus rhythm was 564 ± 41 ms whereas during AF the median cycle length was 150 ± 7 ms. Adaptation of the atrial refractory period after 5 minutes of AF is shown in Figure 4. Immediately after termination of AF the AERP was shortened from 170 ± 10 to 149 ± 9 ms. NHE1 blockade by EMD87580 did not affect the transient shortening of atrial refractoriness after termination of short-lasting AF. Both with and without EMD87580 the AERP returned to baseline within less than 2 minutes.

Effects of NHE1 Blockade on Atrial Electrophysiology

Figure 5 shows the effects of EMD87580 and EMD125021 on steady-state atrial refractory periods during pacing at a cycle lengths of 400,300 and 200ms both during sinus rhythm and after 24 hours of AF. The data points represent the average AERP at three atrial sites (right and left atrium and Bachmann's bundle) in 5 goats. As expected, AF shortened the AERP at all pacing rates. NHE1 blockers did not affect atrial refractoriness in either non-remodeled and remodeled atria. In addition, no statistically significant effect on atrial conduction velocity was observed (Table).

Figure 6: No effect of EMD87580 and EMD125021 on the duration of AF paroxysms after 24 hours of electrical remodeling. Upper panel: Representative example in which the duration of each consecutive AF paroxysm is plotted individually. Lower panels: Median duration of AF paroxysms at increasing dosages of EMD125021 and EMD87580 are plotted for five goats (mean $\pm$ SEM).

Effects of NHE1 Blockade on Paroxysmal and Persistent AF

The effect of EMD87580 and EMD125021 on the duration of AF paroxysm is shown in Figure 6. In the upper panel, an example is shown in which more than 300 episodes of AF were induced consecutively at increasing dosages of EMD125021. During saline infusion, the duration of AF paroxysms ranged from 3 to 73 seconds (median 9). EMD125021 did not shorten the duration of AF paroxysms. Even at the highest concentration (3mg/kg) the median AF duration was still 12 seconds (range 2-42). The absence of an effect of EMD125021 and EMD87580 on duration of AF paroxysms is shown for all goats in the middle and lower panels of Figure 6 and in the Table.

The effect of NHE1 blockers on *persistent* AF (duration 35 ± 10 days, range 21-70) was evaluated in five goats. A representative example is shown in Figure 7. AFCL was monitored continuously for 7 hours. Every 90 minutes, a bolus of EMD87580 (dosage increasing from 1 to 30 mg/kg) was injected intravenously. No effect on AFCL was observed. In addition, EMD125021 had no effect on AFCL (Ta-

Figure 7: Unipolar atrial electrogram recorded from the right atrium during persistent AF. Infusion of EMD87580 up to a dosage of 30 mg/kg did not change AFCL. Bottom panel: Median AFCL in the same goat during the whole experiment of 7 hours.

Figure 8 Effects of oral pretreatment with EMD87580 (8 mg/kg b.i.d.) on AF-induced electrical remodeling. Left: No effect of EMD87580 on AF-induced shortening of AERP. Middle: No effect on conduction velocity (CV). Right: No effect on AF paroxysms.

ble). None of the drugs converted AF. No statistically significant changes in RR interval, QRS duration or QT interval were observed during persistent AF (Table).

Prevention of Electrical Remodeling by Oral NHE1 Blockade

Prevention of electrical remodeling by oral treatment with EMD87580 (8 mg/kg b.i.d.) was studied in five goats. Oral administration was started 3 days prior to induction of AF, resulting in a plasma concentration of 683 ± 67 ng/mL. The Na^+/H^+ exchanger inhibitor completely failed to prevent AF-induced electrical remodeling (Figure 8). Both AERP and conduction velocity did not differ from placebo-treated goats. Not surprisingly, the increase in duration of AF paroxysms due to electrical remodeling was not prevented by EMD87580. After 24 and 48 hours of AF, the duration of AF paroxysms was 47 ± 32 and 135 ± 63 seconds compared to 56 ± 17 and 136 ± 52 seconds in placebo treated animals ($p = \text{NS}$). In one additional goat in which AF became persistent already within 48 hours of remodeling, oral treatment with EMD87580 did not prevent early development of persistent AF.

DISCUSSION

Metabolic Mismatch and Atrial Fibrillation

An appropriate balance between myocardial oxygen demand and coronary perfusion is required for optimal cardiac performance. In case of increased metabolic demand or reduced supply, compensatory mechanisms come into play. The high activation rates during AF increase the metabolic demand of atrial myocytes. White et al. demonstrated that immediately after induction of AF, the myocardial oxygen consumption increased three-fold.¹² To investigate whether this increased oxygen demand could be met by an increased coronary perfusion, Lau et al. measured the arteriovenous difference in lactate in patients without ischemic heart disease.¹³ Myocardial lactate extraction did not increase during acutely induced AF. No changes in atrial ATP and lactate were found after 5 minutes of AF in dogs.¹⁴ In the goat model of AF, a decrease in atrial phosphocreatine occurred, without a concomitant decrease in creatine and ATP.¹⁵ Goette et al.¹⁶ and Wijffels et al.¹⁷ evaluated whether the ATP-dependent K^+ channel is activated during AF. No effect of the ATP-dependent K^+ channel blocker glibenclamide on AERP could be demonstrated. Thus, at present no clear evidence is available to support the hypothesis that atrial fibrillation leads to ischemia. However, this does not exclude that, in case of a reduced coronary reserve, a mismatch between oxygen demand and supply may occur in fibrillating atria.

The role of Atrial Acidosis in Electrical Remodeling

A factor that may contribute to atrial electrical remodeling is the development of intracellular acidosis during AF. In such a case, the Na⁺/H⁺ exchanger will start to exchange intracellular H⁺ for extracellular Na⁺ ions.³ The resulting increase in intracellular sodium will activate the reverse mode of the Na⁺/Ca²⁺ exchanger which will lead to an increase of intracellular Ca²⁺.⁴ In a recent study, Myata et al. demonstrated that selective blockade of the reverse mode of the Na⁺/Ca²⁺ exchanger by KB-R7943 indeed prevented electrical remodeling during 90 minutes AF.¹⁸ However, an increased intracellular Na⁺ concentration might not only result from an increase in NHE1 activity, but may be due to an increased influx through the Na⁺ channels, the Na⁺-HCO₃ cotransporter, the Na⁺/K⁺/2Cl⁻ cotransporter or the Na⁺-Mg²⁺ antiporter.¹⁹

Inhibition of the NHE1 has been shown to prevent ischemia-induced shortening of the ventricular action potential²⁰ and to reduce reperfusion arrhythmias.²¹ Administration of cariporide also led to spontaneous ventricular defibrillation.²² Because Ca²⁺ overload is believed to be involved in the intracellular signaling pathways leading to atrial electrical remodeling, it was hypothesized that blockade of the NHE1 should prevent AF-induced shortening of atrial refractoriness. Jayachandran et al. were the first to evaluate the effects of NHE1 inhibition on atrial electrical remodeling.⁵ In dogs subjected to 5 hours of rapid pacing, cariporide completely prevented the shortening of atrial refractoriness. In contrast, Shinagawa et al. found no beneficial effect of cariporide after 7 days of rapid pacing.⁶ In our present study, NHE1 inhibitors did not prevent shortening of atrial refractoriness after 5 minutes of AF, nor did it prevent electrical remodeling after 48 hours of AF. It is unclear why the results of Jayachandran et al. differ from the results obtained in the present study. However, there were several important differences in experimental conditions. In the study by Jayachandran et al., experiments were performed in anesthetized dogs under complete autonomic blockade, whereas in the present study awake goats without vagal or adrenergic blockade were used. It cannot be excluded that the NHE1 plays a different role in AF in different species. The extent to which the heart can meet the higher metabolic demand will not only depend on anesthesia and autonomic blockade but also on the degree by which the coronary blood flow can increase. Since different species show a wide variation in coronary reserve, the degree of intracellular acidosis during AF may vary among different animals. In addition, expression of the NHE1 in the heart may be genetically determined. Another explanation for the discrepant results of the different studies is that the compounds (EMD87580 and EMD125021) used in our study were less effective in blocking the Na⁺/H⁺ exchanger than cariporide. However, this seems not very likely since cariporide is only about three-fold more potent than EMD87580, but six-fold less potent than EMD125021. In addition, the attained plasma-levels of EMD87580 and EMD125021 in the goat were several magnitudes higher than the concentration required to block platelet swelling. Therefore, one must conclude that in the goat model of AF, activation of the Na⁺/H⁺ exchanger plays no role in the process

of AF-induced electrical remodeling. This does not mean that AF cannot cause a deficit between oxygen demand and supply. Metabolic mismatch can affect ion channels in different ways. Fearon et al. demonstrated that low O_2 levels inhibits the cardiac L-type Ca^{2+} current²³ whereas prevention of electrical remodeling by the anti-oxidant ascorbate suggests an important role for oxidative stress in AF.²⁴

Prevention of AF-induced Electrical Remodeling

Atrial electrical remodeling has been shown to occur in different species in response to rapid pacing, atrial flutter or fibrillation.^{1,25,26} Several studies pointed to an important role of the L-type Ca^{2+} current.^{2,27,28} Prevention of tachycardia-induced remodeling has been disappointing. L-type Ca^{2+} channel-blockers and ACE-inhibitors delayed the time course of remodeling^{16,29,30} but failed to prevent the long-term effect.^{6,31} Our present study shows that also NHE1 inhibitors did not prevent or revert long-term electrical remodeling.

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CHAPTER 3

Consequences of Atrial Electrical Remodeling for the Anti-Arrhythmic Action of Class IC and Class III Drugs

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ABSTRACT

Objective: Atrial fibrillation (AF) induces electrical and ionic remodeling of the atria. We investigated whether AF-induced remodeling alters the electrophysiological and anti-fibrillatory effects of class I (flecainide) and class III (*d*-sotalol, ibutilide) anti-arrhythmic drugs.

Methods: In 9 goats, the effects of flecainide (6 mg/kg) and *d*-sotalol (6 mg/kg) on atrial electrophysiology were measured both before and after 48 hours of electrically induced AF. During a 1-hour infusion period the atrial effective refractory period (AERP) and conduction velocity (CV) were measured both during slow and rapid pacing (interval 400 and 200 ms). In 8 other goats, the rate-dependent effects of ibutilide (0.12 mg/kg) on AERP were determined.

Results: The effects of flecainide on atrial conduction and refractoriness were not altered after 48 hours of AF. At a dose of 6 mg/kg flecainide reduced the CV200 by $19 \pm 5\%$ in normal atria and by $21 \pm 9\%$ after 48 hours of AF ($p=0.20$). The AERP200 was prolonged by $10 \pm 6\%$ and $8 \pm 7\%$ respectively ($p=0.40$). In contrast, the effect of *d*-sotalol on atrial refractoriness was markedly diminished. During control *d*-sotalol prolonged the AERP400 by $17 \pm 6\%$ compared to only $6 \pm 5\%$ after 2 days of AF ($p<0.01$). Also ibutilide lost much of its class III effect on the AERP by electrical remodeling (from 15 to 5%; $p<0.05$). The loss of class III action was less pronounced at rapid heart rates.

Conclusions: AF-induced atrial electrical remodeling in the goat did not modulate the action of flecainide on atrial conduction and refractoriness. In contrast, the class III effects of *d*-sotalol and ibutilide on the atria were strongly reduced after 2 days of AF. The prolongation of QT duration was not affected.

INTRODUCTION

Experimental and clinical studies have established that atrial fibrillation (AF) induces a shortening of atrial refractoriness (electrical remodeling).^{1,2} This AF-induced shortening of the atrial action potential has been shown to be due to downregulation of a number of ionic currents (ionic remodeling). The L-type Ca^{2+} ($I_{\text{Ca,L}}$), the transient outward current (I_{to}) and the ultrarapid delayed rectifier current (I_{Kur}) become reduced after prolonged rapid atrial pacing or AF.³⁻⁵ Whereas Lai et al. showed a reduced expression of HERG in humans with longstanding AF,⁶ others found no changes in HERG or I_{Kr} current density.^{3,7}

It remains unclear whether AF also induces depression of intra-atrial conduction and/or downregulation of I_{Na} current. In a canine model, rapid atrial pacing was associated with a slowing of intra-atrial conduction and reduction of the rapid Na^+ current (I_{Na}).⁸ In contrast, in patients with chronic AF, densities and biophysical properties of I_{Na} were found to be unaltered.⁹ Similarly, in the goat conduction velocity remained unaltered, suggesting no major changes in sodium current.²

If the relative contribution of different ionic currents to the atrial action potential changes by electrical remodeling, also the sensitivity for certain specific ion-channel blockers may be altered. This may explain why some antiarrhythmic agents lose their efficacy to cardiovert AF with time.¹⁰⁻¹²

In the present study we evaluated the changes in electrophysiological effects of class IC and class III drugs in the goat model of 'lone' AF. The effects of intravenous infusion of flecainide, *d*-sotalol and ibutilide on atrial refractoriness and conduction were measured both before and after 48-hours of atrial fibrillation.

METHODS

The Goat Model of Atrial Fibrillation

Seventeen goats (46 ± 14 kg) were used for this study. The experiments were carried out according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and approved by the Animal Investigation Committee of the University of Maastricht. Anesthesia was induced by administration of Nesdonal (15 mg/kg) and maintained by ventilation with halothane (1-2%) and a mixture of O_2 and N_2O . A thoracotomy was made to expose the heart and a teflon-felt plaque containing 30 electrodes (3×2.5 cm, inter-electrode distance 4 mm) was sutured on the free wall of each atrium. A 10 cm long plaque with three rows of electrodes (54 electrodes; inter-electrode distance 5 mm) was implanted on Bachmann's bundle (BB) from the tip of the right to the tip of the left atrial appendage. A small plaque with 3 electrodes was sutured on the left ventricle. Three silver plates were left under the skin of the thorax to record a precordial ECG and to serve as indifferent electrode. The leads were tunneled subcutaneously to the neck and exteriorized by four 30-pole

connectors. Postoperatively the animals received buprenorphine for 2-4 days. Experiments were started 3-4 weeks after surgery. Atrial fibrillation was induced by an automatic fibrillation pacemaker as described previously.² Custom-made software continuously analyzed the atrial rhythm of one of the unipolar atrial electrograms. As soon as sinus rhythm was detected, a 1 second burst of biphasic stimuli (duration 2 ms, interval 20 ms, 4x pacing threshold) was applied by a pacemaker (Medtronic SP3084) to reinduce AF. In this way atrial fibrillation could be maintained 24 hours a day, seven days a week. Previous experiments have shown that under these conditions atrial electrical remodeling is nearly complete after 48 hours.²

Electrophysiological Measurements

The atria were paced with biphasic stimuli of 2 ms duration (4x threshold) generated by a constant current generator. Atrial effective refractory periods (AERP) were determined at the free wall of the right (RA) and left atrium (LA) during bipolar stimulation at pacing cycle lengths between 400 and 200 ms. Single interpolated stimuli were applied after every 8th basic stimulus starting well within the refractory period. The longest S1-S2 interval that failed to capture the atria (increments 2ms) was taken as the AERP. Atrial conduction velocity was measured along BB during right atrial pacing with a cycle length of 400 and 200 ms. Longitudinal conduction along BB was checked by comparing the (similar) activation times of the three parallel rows of electrodes on Bachmann's bundle. Inducibility of AF was measured by applying single early premature stimuli to the free wall of the right or left atrium during regular pacing with a cycle length of 400 ms. AF was considered inducible if a premature stimulus induced a rapid irregular rhythm lasting > 1 second. This criterion was used to exclude induction of only one or a small number of extra beats. The duration of the QRS complex (QRSD) and the QT interval were determined from a unipolar ventricular electrogram during atrial pacing at a cycle length of 400 ms.

Experimental Protocol

The effects of flecainide and *d*-sotalol were investigated in 9 goats both before and after electrical remodeling during 48 hours of AF. These experiments were performed in random order and were separated by at least one week of normal sinus rhythm during which the atria completely recovered from an earlier episode of remodeling and drug administration.¹³ Before each drug, a baseline study was performed during 30-minutes of saline infusion. During this baseline period the refractory period of the right ($n=6$ goats) or left atrium ($n=3$ goats) and the conduction velocity along Bachmann's bundle were measured every 10 minutes during regular pacing with an interval of 400 and 200 ms. Then flecainide or *d*-sotalol were administered at a constant infusion rate of 0.1 mg/kg/min and the electrophysiological measurements were repeated every 10 minutes. After one hour of drug infusion the inducibility of AF was re-evaluated by applying single early premature stimuli at the RA ($n=6$) or LA ($n=3$). The electrophysiological action of ibutilide

was studied in a separate series of 8 goats: Ibutilide was infused at a rate of 0.002 mg/kg/min for 1 hour. To evaluate both the rate- and site-dependent action of ibutilide, the refractory period was measured at pacing intervals of 400, 350, 300, 250, 225 and 200ms both at the RA and LA.

The chosen drug dosages were based on previous experiments in the goat.¹⁴ At the dosages used the drugs were well tolerated and the electrophysiological effects were comparable to the effects of clinically used dosages. Clinically, flecainide (2 mg/kg) prolongs the QRS duration by 18%¹⁵ whereas *d*-sotalol (1.5 mg/kg) and ibutilide (2 mg/30 min) prolong the QT time by respectively 15% and 22%.^{16,17} In our experiments flecainide widened the QRS complex by 15-18% and *d*-sotalol and ibutilide prolonged the QT time by respectively 19-23% and 9-11%. Thus, although in the goat the dosages were higher than used clinically, the biological effects of flecainide, *d*-sotalol and ibutilide were comparable.

Statistical analysis

For the flecainide and *d*-sotalol experiments data of the right ($n=6$) and left atria ($n=3$) were pooled. The effects of ibutilide on RA and LA are given separately. Data are given as mean $\pm$ SD. Differences between groups were determined by the paired Student's *t*-test. Serial measurements were analyzed by ANOVA with repeated measures. A *p*-value of <0.05 was considered as statistically significant.

RESULTS

Flecainide

The effects of flecainide on atrial conduction before and after 48 hours of AF are shown in Figure 1. In the example in the left panel, flecainide lowered the conduction velocity along Bachmann's bundle during rapid pacing (interval 200 ms) from 121 to 95 cm/s in non-remodeled atria (-21%) and from 133 to 105 cm/s in remodeled atria (-21%). In the right panel the effects of flecainide on atrial conduction (S1-S1: 200 ms) are plotted for all goats ($n=9$). Before and after electrical remodeling, conduction velocity along Bachmann's bundle was similar (124 ± 12 versus 125 ± 14 cm/s; $p=0.79$). At a dosage of 6 mg/kg, flecainide depressed atrial conduction in normal atria by $19 \pm 5\%$ and after 48 hours of AF by $21 \pm 9\%$ ($p=0.20$). Also at lower pacing rates the effect of flecainide on atrial conduction and refractoriness was not affected by electrical remodeling (Table 1). During rapid pacing flecainide increased the AERP by 8-10% ($p=0.4$) and during slow pacing by 4-7% ($p=0.53$). Also the prolongation of the QRS complex by flecainide was not affected by 2 days of AF (15 vs. 18%; $p=0.72$) (Table 1).

Normal Atria

Remodeled Atria (48h AF)

CV₂₀₀ (cm/s)

Figure 1: Effects of flecainide on conduction velocity along Bachmann's bundle during rapid atrial pacing from the left atrial free wall (S₁-S₁:200 ms). Left: Electrograms recorded along Bachmann's bundle. Both before (normal atria) and after 48h of remodeling, flecainide (6mg/kg) significantly depressed atrial conduction velocity. Right: Equal effects of flecainide on atrial conduction during a 1 hour infusion (0.1 mg/kg/min) in 9 goats before (open circles) and after remodeling (closed circles). LAA = left atrial appendage, BBleft = left part of Bachmann's bundle, BBmid = middle part of Bachmann's bundle, BBright = right part of Bachmann's bundle, RAA = right atrial appendage. NS = non-significant

Normal Atria

Remodeled Atria

AERP₄₀₀ (ms)

Figure 2: Effects of d-sotalol on atrial refractoriness in normal and remodeled atria (S₁-S₁:400 ms). Left: In normal atria d-sotalol (6mg/kg) prolonged the AERP from 156 to 186 (+30 ms), whereas after 48h of AF the AERP lengthened only by 4 ms (from 74 to 78 ms). Induction of AF was not prevented by the high dosage of d-sotalol. Right: The average effects of d-sotalol on the AERP₄₀₀ in normal and remodeled atria (n=9). * p<0.01; Δ% normal versus remodeled.

Table 1 Effects of flecainide (6mg/kg), *d*-sotalol (6mg/kg) and ibutilide (0.12mg/kg) in normal and remodeled Atria.

	QRSD or QT (ms)	AERP400 (ms)	AERP200 (ms)	CV400 (cm/s)	CV200 (cm/s)
Flecainide					
Normal	37±1	144±9	138±6	132±9	124±12
Flecainide	43±1	149±10	151±5	119±15	101±13
Δ%	+15±2%	+4±9%	+10±6%	-10±8%	-19±5%
Remodeled	37±5	83±16	93±20	135±14	125±14
Flecainide	43±4	88±16	100±20	118±14	99±16
Δ%	+18±13%	+7±7%	+8±7%	-13±7%	-21±9%
<i>d</i> -sotalol					
Normal	229±18	146±13	142±7	128±17	123±14
<i>d</i> -sotalol	273±28	171±19	165±8	125±16	117±14
Δ%	+23±7%	+17±6%	+17±5%	-2±4%	-5±10%
Remodeled	221±20	81±14	87±17	133±15	128±12
<i>d</i> -sotalol	257±27	85±16	99±19	132±15	126±14
Δ%	+19±7%	+6±5% (**)	+13±5%	-1±1%	-2±4%
Ibutilide					
Normal	218±17	158±16	145±15	130±10	118±2
Ibutilide	241±23	182±23	158±13	128±11	111±15
Δ%	+11±5%	+15±7%	+12±4%	-1±3%	-7±5%
Remodeled	213±17	93±17	114±15	127±17	122±11
Ibutilide	233±26	98±19	126±18	127±18	115±14
Δ%	+9±6%	+5±7% (*)	+10±7%	+0±4%	-6±6%

* $p < 0.05$, ** $p < 0.01$; Δ% normal versus remodeled, QRSD: duration of the QRS complex

d-Sotalol

The class III effects of *d*-sotalol before and after electrical remodeling are shown in Figure 2. In non-remodeled atria *d*-sotalol (6 mg/kg) clearly prolonged the atrial refractory period. In the given example the AERP₄₀₀ at the RA prolonged from 156 to 186 ms (+30 ms). As expected, after 48 hours of AF atrial refractoriness was abbreviated and the AERP₄₀₀ had shortened to 74ms. Single premature stimuli now induced paroxysms of AF. Administration of the same dosage of *d*-sotalol (6 mg/kg) prolonged the AERP₄₀₀ of the RA by only 4 ms (from 74 to 78 ms) and induction of AF was not prevented. In the right part of figure 2, the effects of *d*-sotalol on the

Figure 3: Effects of ibutilide (0.12 mg/kg) on atrial refractoriness at different heart rates before and after remodeling (n=8; pooled data of right and left atrium). In normal atria (left) ibutilide prolonged the AERP in a reverse frequency-dependent way. In remodeled atria (right) the class III effect was reduced, especially at slow heart rates. As a result the reverse frequency-dependence of ibutilide was changed into a slight frequency-dependence.

Figure 4: Effects of flecainide, d-sotalol and ibutilide on atrial refractoriness and inducibility of AF by single premature stimuli in electrically remodeled atria (48h of AF). After 48h of AF the atrial refractory period had shortened by 45% and premature stimuli induced AF in 100% of the cases. All three drugs prolonged the AERP only slightly (5-7%) and none of them could prevent initiation of AF.

AERP₄₀₀ are plotted for all 9 goats. At a dosage of 6 mg/kg, *d*-sotalol prolonged the AERP₄₀₀ in normal atria by $17 \pm 6\%$ and by $6 \pm 5\%$ after 2 days of AF ($p < 0.01$). As shown in Table 1, this loss of class III effect was less at higher pacing rates. During pacing at a cycle length of 200 ms *d*-sotalol still prolonged the refractory period in remodeled atria by $13 \pm 5\%$ compared to $17 \pm 5\%$ during control ($p = 0.10$). The prolongation of the QT time by *d*-sotalol was the same before and after 48 hours of atrial fibrillation (23% vs 19%; $p = 0.20$).

Ibutilide

In a separate series of 8 goats the site-dependent class III effect of ibutilide was studied before and after 2 days of AF. Whereas in normal atria ibutilide (0.12mg/kg) prolonged the AERP₄₀₀ of the right and left atrium respectively from 161 ± 17 and 156 ± 17 ms to 186 ± 25 ($15 \pm 8\%$) and 180 ± 24 ms ($16 \pm 9\%$), in remodeled atria the class III effect was reduced to 4 ± 7 and $6 \pm 8\%$ ($p < 0.05$). In figure 3 and Table 1, the average effects on the right and left atrial refractory period are given. The rate-dependence was evaluated by measuring the effects on atrial refractoriness at different cycle lengths between 400 and 200 ms. In non-remodeled atria a normal physiological rate adaptation was present and the AERP shortened from 158 ± 16 to 145 ± 15 ms. Ibutilide (0.12mg/kg) prolonged the atrial refractory period less at faster than at slower pacing rates (24 ± 12 vs. 16 ± 5 ms at 400 and 200 ms interval), resulting in a slight increase in the slope of the normal rate-adaptation curve. In electrically remodeled atria the physiological rate adaptation was lost and became slightly inverted (right part figure 3). Note that at low pacing rates the AERP was now shorter (93 ± 17 ms) than during rapid pacing (114 ± 15 ms). In remodeled atria the class III effect of ibutilide was reduced at all pacing rates. However, since this loss was more marked at slower rates, 2 days of AF converted the normal reverse rate-dependence of ibutilide into a moderate rate-dependent action. At a cycle length of 400 ms the AERP was prolonged by 4 ± 6 ms compared to 12 ± 9 ms during pacing with a cycle length of 200 ms ($p < 0.05$).

Effects of Flecainide, *d*-Sotalol and Ibutilide on Inducibility and Duration of AF

In figure 4 the effects of flecainide, *d*-sotalol, and ibutilide on the inducibility of AF are given. After 48 hours of atrial electrical remodeling, flecainide, *d*-sotalol and ibutilide prolonged the AERP by only 5-7%. Compared to normal atria, the class III effect of *d*-sotalol and ibutilide was significantly decreased (Table 1). In non-remodeled atria premature stimuli induced atrial fibrillation in 33% of the animals. Due to the shortening of atrial refractoriness, in remodeled atria single premature stimuli induced AF in 100% of the cases. Even at high dosages none of the drugs could prevent the induction of AF (still 100%). Administration of flecainide, *d*-sotalol, or ibutilide also did not shorten the median duration of AF paroxysms. With and without drugs, paroxysms of AF lasted respectively 4 (range 1-44) and 7 seconds (2-52)

(flecainide), 4 (1-59) and 3 seconds (1-62) (*d*-sotalol) and 4 (2-500) and 3 seconds (1-8) (ibutilide); all $p=NS$).

DISCUSSION

Main findings

In the present study we evaluated the effects of 2 days of AF-induced electrical remodeling on the electrophysiological action of one class IC (flecainide) and two class III agents (*d*-sotalol and ibutilide). The effect of flecainide on atrial conduction and refractoriness was not changed by electrical remodeling. In contrast, the class III effect of *d*-sotalol and ibutilide were strongly reduced. All three drugs failed to prevent induction of atrial fibrillation in electrically remodeled atria by single premature beats.

Importantly, the effects on the QRS complex and QT duration were similar before and after atrial remodeling, suggesting that the biological availability of the drugs were not different. The effective dosages in our experiments were comparable to the clinically used dosages since the prolongation of the QRS complex and QT interval was similar to the changes observed clinically.¹⁵⁻¹⁷

Consequences of Electrical Remodeling for the Action of Class IC Drugs

Class IC anti-arrhythmic agents are often used for termination of atrial fibrillation. However, various clinical studies have shown that the anti-fibrillatory efficacy of class IC drugs declines when AF persists for a longer period of time.^{10,11} Crijns et al. showed that flecainide cardioverted AF in 74% of patients if given less than 24 hours after the start of the arrhythmia. In contrast, when AF had persisted for longer than 24 hours, flecainide did not terminate AF in any of the patients.¹¹

The failure of flecainide to cardiovert AF of >24 hours can be explained either because electrical remodeling makes the fibrillatory process more resistant to class I drugs,¹⁸ or because it diminishes the class I action of the drug itself. The latter possibility could be excluded in the present study by showing that 48 hours of AF did not alter the electrophysiological action of flecainide on atrial conduction and refractoriness. This supports the concept that failure of class IC drugs to cardiovert AF must be due to a higher resistance of AF, rather than to a lower sensitivity of remodeled atrial myocardium to sodium channel blockers.

Our finding that the effect of flecainide on atrial conduction was unaltered in remodeled atria is in agreement with other studies. Sato et al. showed that slowing of conduction by the class IC agent pilsicainide was not affected by 14 days of rapid atrial pacing in the dog.¹⁹ Wijffels et al. observed that flecainide still markedly depressed atrial conduction in goats after more than 4 weeks of persistent AF.²⁰ Like in our previous studies we observed only a moderate but consistent rate dependent prolongation of the AERP by flecainide.^{14,20} In the present study we observed that this

prolongation was not affected by electrical remodeling. This moderate prolongation is different from studies by Wang et al. who found that propafenone caused a marked and use-dependent prolongation of refractoriness in canine atria.²¹ In human atria, Le Grand et al. showed that flecainide only increased the action potential duration and effective refractory period if the transmembrane potentials had a long plateau preceded by a notch.²²

It still remains to be determined whether the preserved electrophysiological effects of flecainide on atrial conduction means that the I_{Na} current is unaltered in remodeled atria. Whereas conduction velocity and I_{Na} current density were found to be reduced in remodeled canine atria,^{8, 23} in the goat, intra-atrial conduction was not altered after several weeks of AF.² Also in patients with chronic AF the density of I_{Na} current was not changed.⁹

Consequences of Electrical Remodeling for the Action of Class III Drugs

Although class III agents are often used to prevent or terminate atrial fibrillation,²⁴ several observations suggest that they become less effective in the setting of electrical remodeling. Tieleman et al. showed that class III antiarrhythmic drugs could not prevent early recurrences of AF after successful electrical cardioversion.²⁵ Similarly, in the SAFIRE-D study the probability to maintain sinus rhythm for at least 7 days after electrical cardioversion was not different in patients treated with dofetilide or placebo. Only after a follow up of 1 year dofetilide turned out to be slightly superior to placebo in preventing AF recurrences.²⁶ The efficacy of class III drugs to terminate AF clearly declines when AF is present for a longer period of time. Whereas ibutilide terminated AF of recent-onset in approximately 70% of patients, this success rate dropped to 30% in cases of long-lasting AF.^{10,12}

In the present study we tested the hypothesis that AF-induced electrical remodeling reduces the electrophysiological effects of class III drugs. We found that the prolongation of atrial refractoriness was significantly reduced after 2 days of atrial fibrillation. This loss of class III action was the same in the right and the left atrium. Neither *d*-sotalol or ibutilide could prevent re-induction of AF by single premature beats in remodeled atria (inducibility 100%). These findings imply that class III drugs can not be expected to prevent early recurrences of AF in remodeled atria. It has been shown that most relapses occur during the first days after cardioversion of AF when the atrial refractory period is still short.²⁵ It may take several days before the atria are recovered from electrical remodeling and the normal electrophysiological action of I_{Kr} blockers is restored.

Our findings are in agreement with other studies. Li et al. showed that the ability of dofetilide to prolong atrial refractoriness and to cardiovert AF was lower in dogs subjected to prolonged rapid atrial pacing compared to dogs with congestive heart failure and normal AERPs.²⁷ Also in humans there are indications that the action of class III drugs is affected by electrical remodeling. Tse et al. reported a reduced effect of *dl*-sotalol on atrial refractoriness in patients with previous episodes of atrial fibrillation.²⁸ Opposite results were reported by Shiroshita-Takeshita et al. who

observed a preserved effect of the I_{Kr} blocker E4031 and the I_{Kr}/I_{Ks} blocker azilide in dogs subjected to 14 days of rapid atrial pacing.²⁹ However, in the latter study the degree of electrical remodeling was quite moderate and the AERP₄₀₀ was only shortened by 27 ms after 14 days of rapid pacing.

The mechanisms responsible for the reduced effect of class III drugs in electrically remodeled atria remain speculative. Both *d*-sotalol and ibutilide prolong the action potential mainly by blocking the rapid component of the delayed rectifier K^+ current (I_{Kr} blockade).³⁰ Still only limited information is available regarding the effects of AF on I_{Kr} . Whereas Lai et al. showed a reduced expression of HERG in humans with longstanding AF,⁶ others found no changes in HERG or I_{Kr} current density.^{3,7} Theoretically a reduced HERG expression (or I_{Kr} current density) would lead to a greater efficacy of I_{Kr} blockade. The reduced availability of I_{Kr} channels reduces the repolarization reserve and will render the atria more vulnerable to blockade of repolarizing currents. The fact that we observed a lower rather than a higher efficacy of I_{Kr} blockers in remodeled atria raises some doubts about the significance of the reported changes in HERG expression. An alternative explanation for the reduced class III effect of I_{Kr} blocking agents might simply be that the contribution of this current to atrial repolarization is reduced. When the action potential is shortened by a reduction of the plateau phase due to down-regulation of the $I_{Ca,L}$, the membrane will be repolarized by earlier activated potassium currents like I_{to} , I_{KACh} and I_{Kur} . Because I_{Kr} is activated later during the action potential, the actual contribution of this current may diminish. Under these conditions, blockade of I_{Kr} channels obviously will exert less effect. This latter explanation, proposed by the group of Nattel, is based on a mathematical model of the human atrial action potential in which inhibition of the I_{Kr} current produced less prolongation of the action potential when the cells were 'electrically remodeled'.³¹ These investigators stated that: 'because I_{Kr} is activated relatively late during the action potential, the inhibition does not disturb the delicate balance of currents during phase 1 repolarization or during the initial phase of the action potential plateau'. These computer simulations were recently supported by experimental evidence from the goat showing that the reduced efficacy of I_{Kr} blockers could be immediately and completely restored by restoration of the plateau phase of the remodeled atrial action potentials.³²

Limitations

An important limitation of the present study is that no in-vitro studies were performed. For this reason, the cellular mechanisms underlying the loss of class III effect of I_{Kr} blockers remain speculative. Obviously, additional studies will be required to determine the ionic basis of the changed action of class III drugs in electrically remodeled atria.

Although we did not verify plasma concentrations, the effects of flecainide, *d*-sotalol and ibutilide on QRS and QT duration were the same before and after electrical remodeling. It therefore seems highly unlikely that the reduced class III action was the result of a lower effective dosage. All drugs were infused at a single

constant rate during 1 hour. Steady state dose-dependent effects were not measured. On the other hand, since the effective dosage during a 1 hour infusion period gradually increases, we can exclude that our observations are limited to a single or narrow range of dosages.

Extrapolation of experimental results to the human situation should always be done with great caution. Marked differences exist in the distribution of cardiac ionic currents between species. In humans the duration of the action potential is longer than in goats and also the degree of AF-induced electrical remodeling is less.¹ It is therefore quite possible that in humans the reduced class III effect of I_{Kr} blockers is less marked than in goats. In addition, electrophysiological measurements were only performed at the free wall of the right and left atrium. We cannot exclude that in other parts of the atria (especially with longer action potentials) the action of class III drugs is affected differently. Opposite to our findings Li et al. demonstrated marked regional differences in action potential duration between the right and left atrium of the dog.³³ Differences in species and experimental conditions may explain these discrepancies.

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CHAPTER 4

'Early' Class III Drugs for the Treatment of Atrial Fibrillation: Efficacy and Atrial Selectivity of AVE0118 in Remodeled Atria of the Goat

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ABSTRACT

Background: Currently available anti-arrhythmic drugs are only moderately effective against atrial fibrillation (AF) and may cause ventricular pro-arrhythmia. AVE0118 is a blocker of atrial specific early K^+ currents (I_{Kur}/I_{to}).

Methods and Results: Effects of intravenous AVE0118 and dofetilide on atrial effective refractory period (AERP) and inducibility of AF were measured before and after 48-hours of AF-induced electrical remodeling in the goat. During persistent AF (53 ± 19 days), the cardioversion efficacy and effects on atrial wavelength of AVE0118, dofetilide, and ibutilide were evaluated. QT durations were measured during atrial pacing and persistent AF. After 48 hours of AF, the effect of dofetilide on AERP was reduced and induction of AF was not prevented. In contrast, the class III action of AVE0118 was enhanced and AF inducibility decreased from 100% to 32% ($p < 0.001$). At 1, 3 and 10mg/kg/h AVE0118 terminated persistent AF in respectively 1/8, 3/8 and 5/8 goats. Dofetilide and ibutilide terminated AF in 1/5 and 2/7 goats. AVE0118 0.5, 1.5, and 5mg/kg prolonged the AERP during AF and increased the fibrillation wavelength from 6.7 ± 0.6 to 8.5 ± 0.5 , 9.7 ± 0.5 and 11.2 ± 0.9 cm ($p < 0.01$). Whereas dofetilide and ibutilide prolonged QT duration, AVE0118 had no appreciable effect on QT duration.

Conclusions: AVE0118 markedly prolongs the atrial refractory period during AF without affecting QT duration. Cardioversion of AF was due to an almost twofold increase of the wavelength of the fibrillation waves. Atrial selective class III drugs like AVE0118 may be a promising new option for safe and effective cardioversion of AF.

INTRODUCTION

Anti-arrhythmic drugs have been widely used for prevention and termination of atrial fibrillation (AF). Unfortunately, their clinical efficacy has been disappointing and the risk of pro-arrhythmia is a significant concern.^{1,2} The antifibrillatory action of commonly used class III drugs is based on prolongation of the action potential by blockade of the delayed rectifier K^+ current (I_{Kr}). Clinical studies have shown that these class III drugs can terminate persistent AF in ~30% of the cases.^{3,4} Their therapeutic range is limited because they prolong the QT duration and induce torsade de pointes arrhythmias.² The development of atrium-specific drugs therefore would be a major step forward.

Nattel and colleagues showed that an early ultra-rapid component of the delayed rectifier (I_{Kur}) contributes significantly to repolarization of the human atrial action potential.⁵ Because I_{Kur} is present in atrial but not in ventricular myocytes, they postulated that: "It is a potentially promising target for the development of drugs that prevent atrial reentrant arrhythmias without a risk of ventricular pro-arrhythmia".⁶

Several experimental I_{Kur} blockers are recently under investigation.^{7,8} One of them (AVE0118), which, in addition to I_{Kur} also blocks the I_{to} current, is evaluated in the present study. We hypothesized that in electrically remodeled atria, blockade of the early repolarizing currents restores the plateau of the action potential, thereby exerting an anti-fibrillatory effect. This hypothesis was tested in the goat model of AF in which the electrophysiological and anti-fibrillatory effects of AVE0118 were compared with the action of conventional class III drugs (dofetilide and ibutilide).

METHODS

The Goat Model of Atrial Fibrillation

Thirteen female goats weighing 52 ± 2 kg were used. The animals were handled according to the European Directive for Animal Research and the study protocol was approved by the local Animal Investigation Committee. Goats were instrumented as described previously.⁹ Briefly, during general anesthesia Teflon-felt plaques with multiple electrodes were sutured on the free wall of each atrium, Bachmann's bundle (BB) and the left ventricle. All leads were tunneled subcutaneously to the neck and exteriorized by four 30-pole connectors. Experiments were started 3 to 4 weeks after surgery. AF was induced by a fibrillation pacemaker as described previously.¹⁰

Electrophysiological Measurements

The atria were paced with biphasic stimuli of 2 ms duration and 4x threshold. The atrial effective refractory period (AERP) was measured at the free wall of the right and left atrium during regular pacing (interval 400 to 200 ms). Single interpolated

stimuli were applied after 8 basic stimuli, starting within the refractory period. The longest interval that failed to capture the atria (2 ms increments) was taken as the AERP. Atrial conduction velocity was measured along Bachmann's bundle during right atrial pacing. The distance over which conduction velocity was measured ranged from 3.5 to 5 cm.

The length of the fibrillation waves was determined at the right atrial free wall by measuring the refractory period (RP_{AF}) and conduction velocity (CV_{AF}) during AF. RP_{AF} was measured by slow, fixed-rate pacing (1 Hz) resulting in a series of single, randomly coupled, premature stimuli.⁹ Local capture of AF was evidenced by radial spread of activation from the pacing site and a short delay between stimulus and response. For each coupling interval, the percentage of capture was determined. The shortest interval capturing the atrium $\geq 50\%$ was taken as the RP_{AF} . CV_{AF} was determined with a mapping electrode containing 5 x 6 electrodes (interelectrode distance, 4 mm) from the local conduction vectors within areas of 3 x 3 electrodes. At least 50 AF cycles were used to determine the CV_{AF} .

Inducibility of AF was measured at the right and left atrium by single premature stimuli applied during regular pacing (400 ms). In case a premature beat induced a rapid irregular rhythm lasting > 1 second, AF was considered inducible. The AF cycle length (AFCL) was measured automatically by an algorithm detecting the negative intrinsic deflection of the fibrillation electrogram. The median value of 300 consecutive intervals was calculated. QT duration was measured during atrial pacing and persistent AF either from an epicardial electrogram or a precordial electrocardiogram. Since during AF the Bazett's formula cannot be applied, we used another approach to correct the QT duration. In each goat the relationship between the RR-interval and the QT duration was determined during 20 seconds of AF. The RR-QT relationship after drug administration was compared with the normal RR-QT relation. In some animals, the effects of class III drugs during persistent AF was evaluated by recording monophasic action potentials (MAP) under general anesthesia from the endocardium of the right atrium.

Experimental Protocol

The electrophysiological effects of AVE0118 ($n = 10$ goats) and dofetilide ($n = 6$) were measured before and after 48 hours (1 to 4 days) of AF. In five goats, both drugs were evaluated. Drug effects were studied on separate days with at least 10 plasma half-lives between the experiments. AVE0118 and dofetilide were administered intravenously over 1 hour at 3 mg/kg/h and 20 μ g/kg/h respectively. AERP, CV, QT duration and inducibility of AF were measured after 30 minutes of infusion.

In a total of 8 goats the effects of AVE0118 ($n = 8$), dofetilide ($n = 5$) and ibutilide ($n = 7$) were evaluated during persistent AF (53 ± 19 days). In 6 of these animals, the baseline studies had been performed. AVE0118 was infused in 5 dosages between 0.1 and 10 mg/kg/h. Dofetilide and ibutilide were given in a dosage of respectively 20 μ g/kg/h and 4 mg/h. Drugs were infused for 1 hour, during which the AFCL was monitored. After 30 minutes of infusion, RP_{AF} , CV_{AF} , median

RR-interval and QT duration were measured. Successful cardioversion was defined as termination of AF within ≤ 1 hour of drug administration. Plasma-concentrations of AVE0118 were determined by Aventis.

Statistical analysis

Differences between groups were evaluated by the paired Student's *t* test or by 2-way repeated ANOVA with post-hoc Bonferroni *t* test. McNemar's test was used to compare AF inducibility. Changes in corrected QT duration were evaluated by the 1-sample *t*-test (test value 0). Differences were considered statistically significant at $p < 0.05$. Results are presented as mean $\pm$ SEM.

RESULTS

Atrial Refractoriness and Conduction Velocity

Figure 1 shows the effects of dofetilide and AVE0118 before and after 48 hours of AF. In non-remodeled atria, dofetilide and AVE0118 prolonged AERP at all pacing rates. Both drugs showed a smaller class III effect at shorter cycle lengths (reverse frequency-dependence). After AF-induced electrical remodeling the effect of dofetilide was reduced, whereas the action of AVE0118 was enhanced. In figure 1 (right) the prolongation of atrial refractoriness (Δ -AERP) is plotted. The normal reverse frequency dependence of dofetilide was changed into a frequency-dependent effect after 48 hours of AF because of a loss of class III action at slow rates. The reverse frequency dependence of AVE0118 was reduced. At short cycle lengths (200 ms), the class III effect of AVE0118 was almost tripled (2.8), whereas at 400 ms, the prolongation of atrial refractoriness was enhanced by a factor of 1.4.

Before drug infusion a physiological rate-dependent slowing of conduction along Bachmann's bundle was observed from 125 ± 4 to 114 ± 4 cm/s (pacing interval 400 and 200 ms). After 48 hours of AF, conduction velocity was not changed. Dofetilide did not affect conduction velocity either before or after electrical remodeling (data not shown). At slow pacing rates (400 ms), AVE0118 had no effect on CV in either normal (126 ± 4 vs. 123 ± 5 cm/s, $p = \text{NS}$) or remodeled atria (128 ± 5 vs. 126 ± 5 cm/s, $p = \text{NS}$). During rapid pacing (200 ms) AVE0118 slightly slowed conduction from 116 ± 4 to 105 ± 5 cm/s in non-remodeled and from 120 ± 5 to 113 ± 6 cm/s in remodeled atria (both $p < 0.01$).

Prevention of AF by Dofetilide and AVE0118

Figure 2 shows the effects of dofetilide and AVE0118 on the inducibility of AF. In non-remodeled atria, single premature stimuli initiated AF in 15% of the cases (10 goats, 3 of 20 sites). After 48 hours of AF, the AERP had shortened from 160 ± 6 to 96 ± 5 ms and the inducibility of AF was 100%. After electrical remodeling,

Dofetilide

AVE0118

Figure 1: Effects of dofetilide and AVE0118 on atrial refractoriness at different pacing rates before and after 48 hours of AF. In contrast to dofetilide, the class III action of AVE0118 was significantly enhanced in remodeled atria. AERP: atrial effective refractory period; *** $p < 0.001$ vs. no drugs; † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$ vs. normal atria.

dofetilide prolonged the AERP only by 7 ± 2 ms and did not reduce atrial vulnerability (inducibility 100%; 6 goats, 12 of 12 sites). In addition, the median duration of AF paroxysms remained the same (3.5 vs. 2 seconds; $p = 0.1$). In contrast, AVE0118 prolonged AERP by 65 ± 5 ms and exerted a clear preventive effect on AF inducibility (32%; 10 goats, 6 of 19 sites; $p < 0.001$). The duration of induced AF episodes ranged between 2 and 1260 seconds (median, 6 seconds) before and 1 to 95 seconds (median, 2 seconds) after administration of AVE0118.

Cardioversion of Persistent AF by Dofetilide, Ibutilide and AVE0118

After 53 ± 19 days of persistent AF, attempts were made to cardiovert AF pharmacologically. Infusion of saline never terminated AF. Administration of dofetilide (20 $\mu\text{g/kg/h}$) or ibutilide (4 mg/h) restored sinus rhythm in 1 of 5 and 2 of 7 goats, respectively. The lowest dosage of AVE0118 (0.1 mg/kg/h) restored sinus rhythm in 1 of 6 animals. At higher dosages (1, 3 and 10 mg/kg/h), the cardioversion efficacy increased and AF was terminated in 1, 3, and 5 of 8 goats, respectively.

Figure 2: Effects of dofetilide and AVE0118 on inducibility of AF. Unipolar electrograms recorded from the right atrial free wall during application of single premature stimuli. In remodeled atria, dofetilide only slightly prolonged atrial refractoriness with no effect on the inducibility of AF. In contrast, AVE0118 markedly prolonged the atrial refractory period and reduced inducibility of AF.

The effects of dofetilide, ibutilide and AVE0118 on AFCL are shown in figure 3. Dofetilide and ibutilide had a moderate effect on AFCL, which was prolonged from 105 ± 5 and 100 ± 4 ($t=0$) to 121 ± 5 and 115 ± 5 ms ($t=60$; $p<0.01$). AVE0118 exerted a much stronger effect on AFCL. After 60 minutes infusion at 0.1, 0.3, 1, 3 and 10 mg/kg/h, the AFCL prolonged by respectively 5 ± 1 , 16 ± 2 , 30 ± 4 , 52 ± 4 and 60 ± 5 ms (all $p<0.01$).

In 4 goats with persistent AF, MAPs were recorded from the right atrium during administration of dofetilide ($n=1$), ibutilide ($n=1$) and AVE0118 ($n=2$; 3 mg/kg/h). During control, the fibrillation MAPs were characterized by a short duration and an absent plateau. The degree of repolarization showed beat-to-beat variation, and fractionation occasionally occurred. Apart from the prolongation in AF cycle-length, dofetilide and ibutilide exerted no clear effects on the duration and shape of the action potentials. AVE0118 caused a significant prolongation of AFCL which was associated with restoration of the plateau-phase and increase in duration of the action potential. This demonstrates that the effects of AVE0118 on AFCL are primarily due to a prolongation of the atrial action potential.

Effects on Refractory Period and Conduction Velocity during AF

During cardioversion of persistent AF, RP_{AF} and CV_{AF} were measured after 30 minutes infusion of dofetilide (20 μ g/kg/h; $n=4$), ibutilide (4 mg/h; $n=4$) and AVE0118 (1, 3, 10 mg/kg/h; $n=5$). Figure 4 shows a representative example of

Figure 3 Left: effects of dofetilide ($n = 5$), ibutilide ($n = 7$) and AVE0118 ($n = 8$) on AFCL during 1-hour infusion. Right: examples of the effects of dofetilide, ibutilide, and AVE0118 on atrial MAPs during AF. MAPs were recorded from the right atrial free wall. In contrast to dofetilide and ibutilide, AVE0118 markedly prolonged AFCL due to the increase in MAP duration.

the effects of ibutilide and AVE0118 on RP_{AF} . During control, the shortest coupling interval capturing AF in 50% of the cases was 84 ms. The two tracings show a stimulus with a coupling interval of 84 ms which in one case captured and in the other case did not capture the fibrillating atria. Ibutilide slightly prolonged the RP_{AF} to 95 ms. AVE0118 exerted a much stronger effect, and RP_{AF} was prolonged to 119 and 150 ms at dosages of 0.5 and 5 mg/kg. Figure 5 shows the results of all goats. Shown is the percentage of capture of AF at different coupling intervals before and after drug administration. The S-shaped curves indicate that the RP_{AF} is probabilistic rather than deterministic. Both dofetilide and ibutilide shift the curves slightly to the right, indicating that they prolonged the RP_{AF} only slightly. In contrast, AVE0118 caused a marked rightward shift of the curve because the RP_{AF} was significantly prolonged.

The table gives results for all goats. Dofetilide and ibutilide prolonged the RP_{AF} from 88 ± 3 and 81 ± 6 ms to 95 ± 6 and 95 ± 8 ms respectively (dofetilide: $p=0.09$; ibutilide: $p<0.05$). AVE0118 increased the RP_{AF} up to 144 ± 6 ms at 5 mg/kg ($p<0.001$). The conduction velocity during AF was measured at the free wall of the right atrium. In figure 6 the histograms of the local conduction velocities are shown during control and after AVE0118 (1.5 mg/kg). No effect on atrial conduction was observed. Also at higher dosages AVE0118 caused no changes in CV_{AF} .

Figure 4: Representative examples of effects of ibutilide (2mg) and AVE0118 (0.5 and 5mg/kg) on RP_{AF}. RP_{AF} was defined as the shortest coupling interval that captured AF in 50% of cases. Whereas ibutilide prolonged the RP_{AF} only slightly, AVE0118 prolonged RP_{AF} in a dose dependent manner from 84 to 150 ms.

The atrial wavelength during AF ($WL_{AF} = RP_{AF} \times CV_{AF}$) was increased only slightly by dofetilide or ibutilide. AVE0118 markedly prolonged the wavelength during AF. At dosages of 0.5, 1.5 and 5 mg/kg, the WL_{AF} increased by 28 ± 7 , 53 ± 10 and $60 \pm 7\%$ respectively ($p < 0.01$). The corresponding plasma-levels of AVE0118 were 0.6 ± 0.1 , 1.7 ± 0.4 and 4.9 ± 0.8 $\mu\text{g/ml}$.

Atrial selectivity of AVE0118

During atrial pacing (400 ms), dofetilide significantly prolonged the QT duration before and after 48 hours of AF (from 252 ± 4 to 266 ± 3 ms and from 239 ± 7 to 259 ± 3 ms, respectively; $p < 0.05$). AVE0118 (1.5 mg/kg) did not cause any changes in QT duration (259 ± 7 vs. 261 ± 8 ms and 239 ± 7 vs. 246 ± 5 ms; $p > 0.2$). The class III action on the ventricles was also evaluated during persistent AF. Figure 7 shows a representative example of the effects of ibutilide and AVE0118 (1.5 and 5 mg/kg) on the QT duration during AF. Because the ventricular rhythm was irregular, the QT duration showed significant beat-to-beat variations. In general, shorter RR-intervals were followed by a shorter QT duration and longer RR-intervals by a longer QT duration. Infusion of dofetilide prolonged the QT duration more during long RR-intervals (reverse frequency dependence). At a dosage of 1.5 mg/kg, AVE0118 had no effect on the median QT duration and RR-interval. In this

Figure 5: Percentage of capture by premature stimuli of different coupling intervals before and after administration of dofetilide ($n = 4$), ibutilide ($n = 4$) and AVE0118 (0.5 and 5 mg/kg; $n = 5$). AVE0118 caused a marked rightward shift of the curve, indicating an increase in refractory period during AF.

Figure 6: Histograms of local atrial conduction velocities during AF before and after administration of AVE0118 (1.5mg/kg). No effects on CV_{AF} were observed.

Figure 7: Representative examples of the relation between RR-interval (RR_{AF}) and QT duration during AF (QT_{AF}) before and after administration of ibutilide (2mg) and AVE0118 (1.5 and 5mg/kg). Right bottom: Average changes in corrected QT duration during AF by dofetilide, ibutilide and AVE0118. ** $p < 0.01$.

example, 5 mg/kg AVE0118 clearly prolonged the median RR-interval from 268 to 357 ms. The QT duration prolonged according to its normal rate adaptation. This finding shows that AVE0118 did not cause an independent prolongation of QT duration. In figure 7 (lower right), effects of the different drugs on the corrected QT duration during AF are given for all goats. Dofetilide and ibutilide prolonged the corrected QT duration by 17 ± 2 and 18 ± 2 ms ($p < 0.01$), whereas AVE0118 did not prolong the corrected QT duration ($p > 0.25$).

DISCUSSION

In the present study, a novel I_{Kur}/I_{to} blocker (AVE0118) was evaluated in the goat model of AF. It was found that AVE0118 markedly prolonged atrial refractoriness without affecting corrected QT duration. Whereas the class III effect of I_{Kr} blockade (dofetilide) was reduced after 48 hours of AF, the action of AVE0118 was enhanced after atrial electrical remodeling. AVE0118 suppressed the inducibility of AF, whereas dofetilide failed to prevent induction of AF paroxysms. During persistent AF, AVE0118 prolonged the atrial wavelength in a dose-dependent way. In 5 of 8 goats persistent AF was cardioverted by intravenous administration of AVE0118.

Blockade of Ion Channels by AVE0118

The effects of AVE0118 on I_{Kur} and I_{to} have been investigated in CHO-cells expressing hKv1.5 (I_{Kur}) and hKv4.3 plus KChIP2S (I_{to}) and in atrial myocytes of the pig.⁸ In CHO-cells, half maximal inhibition of I_{Kur} and I_{to} occurred in the micromolar range (0.9–3.5 μ M). Voltage clamp experiments in pig myocytes revealed a 50% reduction of I_{Kur} and I_{to} at comparable concentrations. The I_{KACh} current was blocked at an IC_{50} of 2.6 μ M.⁸ Additionally, in human atrial myocytes I_{Kur} and I_{to} were blocked by low concentrations of AVE0118.¹¹ Although these data are still preliminary they indicate that AVE0118 is a blocker of I_{Kur} , I_{to} and I_{KACh} .

The Effects of Electrical Remodeling on 'Early' and 'Late' Class III drugs

It has become clear recently that AF leads to a down-regulation of several ionic currents including $I_{Ca,L}$, I_{Kur} and I_{to} .^{12–14} Ionic remodeling has important implications for the action of anti-fibrillatory drugs. It was recently demonstrated that the action of the I_{Kr} blockers *dl*-sotalol was reduced in patients with long-lasting AF.¹⁵ In the present study, a reduced action of dofetilide was found. This suggests that in electrically remodeled atria, the late activated I_{Kr} current contributes less to atrial repolarization. In a mathematical model of the human atrial action potential, the effect of I_{Kr} blockade on the duration of the action potential was reduced in remodeled atrial cells.¹⁶ During a short action potential, less I_{Kr} was activated; in this setting, the net prolongation by I_{Kr} blockade therefore was minimal. The opposite was true for I_{Kur} blockade, which exerted an enhanced effect in remodeled atria. This finding was rather surprising because this current is down-regulated in remodeled atria.^{13,14} A possible explanation for this paradoxical effect is that the $I_{Ca,L}$ is reduced to a greater extent than I_{Kur} , resulting in a larger contribution of I_{Kur} to atrial repolarization. Wirth et al.¹⁷ showed that AVE0118 prolonged atrial refractoriness more at sites with shorter refractory periods. Nagasawa et al.⁷ reported that the I_{Kur} blocker NIP-142 became more effective when the action potential was shortened by vagal stimulation.

Table I Effects of dofetilide, ibutilide and AVE0118 on atrial electrophysiology during atrial fibrillation

	Dofetilide (20mg/kg/h)		Ibutilide (4mg/h)		AVE0118 (1mg/kg/h)		AVE0118 (3mg/kg/h)		AVE0118 (10mg/kg/h)	
	Control	30min	Control	30min	Control	30min	Control	30min	Control	30min
AFCL (ms)	105±4	117±6	100±7	115±8	107±6	135±6	104±3	151±7	106±3	169±8
Refractory Period (ms)	88±3	95±6	81±6	95±8	85±5	111±6	82±4	130±5	85±4	144±6
Conduction velocity (cm/s)	76±2	75±3	78±2	79±2	78±1	77±3	78±3	75±2	82±3	78±5
Wavelength (cm)	6.6±0.2	7.1±0.5	6.3±0.6	7.5±0.7	6.7±0.4	8.5±0.5	6.4±0.4	9.7±0.5	7.0±0.6	11.2±0.9

Numbers represent mean ± SEM. AFCL: atrial fibrillation cycle length. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control

Antifibrillatory Action and Atrial Selectivity of AVE0118

During slow pacing, we measured a prolongation of the atrial refractory period by 70% in remodeled atria. Also during AF, the refractory period was prolonged, while no changes in conduction velocity were observed. As a result, the wavelength of the fibrillating waves increased from 7 to 11 cm. This prolongation in atrial refractory period and wavelength can adequately explain the antifibrillatory effects of AVE0118. The increase in size of the intra-atrial reentrant pathways and a consequent reduction in number of fibrillation waves increase the likelihood that the fibrillation waves will die out simultaneously and fibrillation terminates.

Most class III drugs used for the treatment of AF are I_{Kr} inhibitors. Unfortunately, blockade of the I_{Kr} current also prolongs the ventricular action potentials and QT duration. This increases the chance of torsade de pointes arrhythmias.² Since I_{Kur} is absent in the ventricles, I_{Kur} blockers may have a selective action on the atria.⁶ As shown in the dog by Nattel et al., 4-aminopyridine (a blocker of I_{Kur} and I_{to}) increased atrial refractoriness without affecting the ventricular refractory period.¹⁸ In the goat, AVE0118 also did not prolong ventricular repolarization while exerting a strong class III effect on the atria. Thus, compared to the I_{Kr} blocker dofetilide, AVE0118 was highly atrial selective. In pigs, no prolongation of ventricular refractoriness and QT duration was found after administration of AVE0118.¹⁷

Clinical Implications and Limitations

Despite the development of various ablation techniques for the treatment of AF, the development of a safe and effective drug for prevention and termination of AF would have major impact on the management of AF. Our present study suggests that blockers of early K^+ currents (I_{Kur} and I_{to}) may have a high clinical potential. The strong class III effect in remodeled atria, together with the absence of an effect on

QT duration, offers hope that these drugs can be used effectively without the risk of ventricular arrhythmias. However, caution is warranted in extrapolating our results to humans. One important difference is that the atrial action potential is shorter and the degree of electrical remodeling is higher in the goat than in humans.¹⁹ Therefore it is hard to predict whether the beneficial effects of I_{Kur}/I_{to} blockade, as observed in the goat, will also occur in humans. Another important caveat is that the goat model lacks the pathological changes seen in atria of patients with AF. Hypertension, valvular and coronary artery disease, and heart failure all cause structural changes in the atria such as interstitial fibrosis, apoptosis and dilatation. Therefore, it remains to be seen whether early class III drugs like AVE0118 will be safe and effective in humans.

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CHAPTER 5

The Combination of I_{K_{UR}}/I_{to} - and I_{K_r}-Blockers for Effective Cardioversion of Persistent Atrial Fibrillation in the Goat

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ABSTRACT

Background: In electrically remodeled atria the class III action of I_{Kr} blockers is reduced, whereas the efficacy of early class III drugs ($I_{Kur}/I_{to}/I_{KACh}$) is enhanced. In the present study we evaluated the antifibrillatory action of the combination of $I_{Kur}/I_{to}/I_{KACh}$ and I_{Kr} blockade on persistent atrial fibrillation (AF) in the goat.

Methods and Results: The effects of separate and combined administration of AVE0118, dofetilide and ibutilide on atrial refractoriness (AERP) and conduction velocity were determined before and after 48-hours of AF-induced electrical remodeling. The prolongation of AERP₄₀₀ by dofetilide and ibutilide was clearly reduced after 48 hours of AF (from 22 ± 2 to 6 ± 2 ms and 25 ± 5 to 5 ± 2 ms ($p < 0.01$). Pre-treatment with AVE0118 restored the normal prolongation of refractoriness by dofetilide or ibutilide (from 6 ± 2 to 20 ± 3 ms and 5 ± 2 to 25 ± 2 ms respectively; $p < 0.01$). This effect was atrial selective since the prolongation of the QT interval was not changed. The anti-fibrillatory effect was evaluated in 10 goats with 46 ± 17 days of persistent AF. Infusion of dofetilide ($20 \mu\text{g/kg/h}$) or ibutilide (4 mg/h) alone restored sinus rhythm in 20% of the animals. In contrast, in combination with AVE0118 (1, 3 and 10 mg/kg/h), dofetilide or ibutilide terminated AF in respectively 56%, 80% and 100% of the cases. The effects on AF cycle length were synergistic.

Conclusions: In electrically remodeled caprine atria blockade of $I_{Kur}/I_{to}/I_{KACh}$ restores the class III action of I_{Kr} blockade. The efficacy of the combination of AVE0118 and dofetilide/ibutilide for cardioversion of persistent AF was up to 100%. AVE0118 did not affect the prolongation in QT duration by dofetilide or ibutilide. These findings warrant exploration of a combination therapy of early and late class III drugs in patients with persistent AF.

INTRODUCTION

Class III drugs (sotalol, ibutilide, dofetilide) used for the treatment of atrial fibrillation (AF) prolong the atrial action potential mainly by blockade of the rapid component of the delayed rectifier K^+ current (I_{Kr}). Clinical studies have demonstrated that the success rate of pharmacological cardioversion decreases with the duration of AF.¹⁻³ A possible explanation for this observation is that the class III effect of I_{Kr} blockade diminishes when the action potential is shortened due to AF-induced electrical remodeling.^{4,5}

AVE0118 is a novel I_{Kur}/I_{to} blocker presently under development for the treatment of AF.⁶ In animal experiments,^{4,7} this drug caused an atrial selective prolongation of the action potential and terminated persistent AF in 70% of the cases. Interestingly, in remodeled atria the class III action of AVE0118 was enhanced.⁴ A possible explanation for this observation is that due to abbreviation of the plateau of the action potential (electrical remodeling), the relative contribution of early activated K^+ currents (I_{Kur}/I_{to}) becomes more important. We hypothesized that under these conditions prolongation of the plateau-phase by I_{Kur}/I_{to} blockade restores the contribution of the I_{Kr} current to atrial repolarization.

The aim of the present study was to evaluate whether in the goat model of AF, I_{Kur}/I_{to} blockade increases the class III effect of I_{Kr} blockade and whether this combination is more effective to cardiovert persistent AF.

METHODS

The Goat Model of AF

Thirteen female goats (weight 53 ± 2 kg) were used for the present study. Experiments were performed according to the European Directive for Animal Research and the study protocol was approved by the Animal Investigation Committee of the University of Maastricht. Animals were instrumented as described previously.⁸ During general anesthesia teflon-felt plaques with multiple electrodes were sutured on the free wall of both atria and on Bachmann's bundle (BB). All leads were tunneled subcutaneously to the neck and exteriorized by 30-pole connectors. The study protocol was started 3 to 4 weeks after surgery.

Electrophysiological Measurements

All experiments were performed in conscious animals. The atrial effective refractory period (AERP) was measured at the free wall of the right and left atrium during regular pacing (2 ms pulse duration, 4x threshold) with intervals ranging between 400 and 200 ms. Single interpolated stimuli were applied after every 8th basic stimuli. The longest coupling interval that failed to capture the atria (increments 2 ms) was taken as the AERP. Inducibility of AF was evaluated by single early premature stimuli

applied to the free wall of the right or left atrium during regular pacing (400 ms). AF was considered inducible if the premature stimulus evoked a rapid irregular atrial rhythm lasting > 1 second.

Atrial conduction velocity was measured along BB over a distance of ranging from 3.5 to 5 cm during right atrial pacing at a cycle length of 400 and 250 ms. During persistent AF the median cycle length (AFCL) of 300 consecutive intervals was measured by an algorithm detecting the negative intrinsic deflections of a left or right atrial unipolar fibrillation electrogram. QT duration was measured during atrial pacing (cycle length 400 ms) from a precordial electrocardiogram or from a left ventricular unipolar electrogram.

In some animals, during general anesthesia monophasic action potentials were recorded from the high right atrium with a Franz MAP-catheter.

Experimental Protocol

The electrophysiological effects of dofetilide, ibutilide, AVE0118, and the combination of AVE0118 with dofetilide or ibutilide were studied before and after 48 hours of AF (range: 1-4 days) maintained by burst pacing.⁹ Programmed electrical stimulation was used to measure atrial refractory periods, conduction velocity and inducibility of AF. All drugs were infused intravenously with a rate of respectively 20 $\mu\text{g/kg}$ (dofetilide), 0.12 mg/kg (ibutilide) and 3 mg/kg (AVE0118) per hour. Measurements were performed before and after 30 minutes of infusion. Since previous studies have shown that after 30 minutes of AVE0118 infusion the AERP reaches a steady state, the combination of AVE0118 and I_{K_r} blockade was studied by infusing dofetilide or ibutilide after 1 hour administration of AVE0118. Measurements were made 30 minutes after AVE0118 alone and after 30 minutes of the combination. The drug studies were performed in random order with at least 5 plasma half lives between experiments.

The effects on persistent AF were studied by infusion of dofetilide and ibutilide at a rate of respectively 20 $\mu\text{g/kg/h}$ and 4 mg/h. AVE0118 was administered at different rates of 0.1, 0.3, 1, 3 and 10 mg/kg/h. During drug infusion the AFCL was monitored continuously. In case of cardioversion the total infusion time and cumulative dosages of the drugs were noted.

Statistical Analysis

Differences between groups were evaluated by the paired Student's *t*-test or by 2-way repeated ANOVA with post-hoc Bonferroni *t*-test. McNemar's test was used to compare AF inducibility. Differences were considered statistically significant when *p*-value was < 0.05 . Results are presented as mean $\pm$ SEM.

RESULTS

Effects of 'Early' and 'Late' Class III Drugs in Remodeled and Non-Remodeled Atria

Experiments with the combination of AVE0118 and dofetilide were performed in six goats and the combination with ibutilide was tested in seven animals. Figure 1 shows the separate and combined effects of AVE0118 and dofetilide or ibutilide on the atrial refractory period before and after 48 hours of AF. In non-remodeled atria AVE0118, dofetilide and ibutilide all prolonged the AERP in a reverse frequency-dependent way. At all pacing rates the combination of AVE0118 with dofetilide or ibutilide exerted an additive effect on the refractory period. Whereas AVE0118 and dofetilide alone increased the AERP_{400ms} by 51 ± 5 and 22 ± 2 ms, the combination resulted in a prolongation of 75 ± 6 ms. This was not different from the sum of their separate effects (73 ± 7 ms; $p = \text{NS}$). The same was true for the combination of AVE0118 and ibutilide.

After 48 hours of AF (lower panels) the AERP was markedly shortened and the physiological rate adaptation was lost (open circles). The class III action of dofetilide and ibutilide was markedly reduced at slower pacing rates (400, 350 and 300 ms). In contrast, the effect of AVE0118 on AERP (open squares) was enhanced at all cycle lengths and the normal rate-adaptation was restored. The combination of AVE0118 and dofetilide or ibutilide had a larger effect on the AERP than the sum of their separate effects (synergism). The combined administration of AVE0118 and dofetilide or ibutilide prolonged the AERP₄₀₀ by respectively 92 ± 6 and 94 ± 9 ms whereas the sum of the separate effects was 78 ± 5 ms ($72 \pm 5 + 6 \pm 2$) and 68 ± 8 ms ($64 \pm 5 + 5 \pm 2$) (both $p < 0.01$).

Figure 2 shows the class III effects of dofetilide and ibutilide, given alone and in the presence of AVE0118 (ΔAERP). In non-remodeled atria dofetilide and ibutilide increased the AERP equally with or without AVE0118. However, after 48 h of AF the effect of I_{Kr} blockade on the AERP was stronger in the presence of AVE0118. This was especially true at longer cycle lengths when the class III action of I_{Kr} -blockade was most reduced by electrical remodeling. In fact, pre-treatment with AVE0118 completely restored the normal action of dofetilide and ibutilide as seen in non-remodeled atria.

Dofetilide and ibutilide exerted no effect on intra-atrial conduction velocity both before and after 48 hours AF. In non-remodeled atria AVE0118 slowed down atrial conduction at slow and fast pacing rates (400 and 250 ms): respectively 123 ± 4 to 120 ± 5 cm/s and 117 ± 4 to 110 ± 4 cm/s, $p < 0.01$). In remodeled atria, AVE0118 slowed down conduction at fast pacing rates (from 121 ± 5 to 115 ± 4 cm/s, $p < 0.01$) but not at slow rates (126 ± 4 to 124 ± 4 cm/s, $p = 0.15$). The combination of AVE0118 and dofetilide did not slow conduction both during slow and fast pacing in normal and remodeled atria. AVE0118 and ibutilide did not affect conduction in non-remodeled atria at slow rate (123 ± 7 to 120 ± 5 cm/s) but at fast rates conduction slowed from 120 ± 5 to 114 ± 5 cm/s ($p = 0.03$). In remod-

Figure 1: Upper panels: Effects of separate and combined effects of AVE0118 and dofetilide (left) and ibutilide (right) on the atrial refractory period before 48 hours of AF (non-remodeled atria). The datapoints represent the mean AERP of the right and left atrium. Lower panels: AERP after drug administration in electrically remodeled atria (after 48 hours AF). * $p < 0.001$ versus control, † $P < 0.001$ versus AVE0118.

Figure 2: Increase in atrial refractory period (Δ AERP) after administration of dofetilide (left) and ibutilide (right) either given alone (open dots) or in the presence of AVE0118 (closed dots). In non-remodeled atria (upper panels), no differences were found between the effects of I_{K_r} -blockers alone or after AVE0118. In electrically remodeled atria, the Δ AERP was enhanced in the presence of AVE0118. * $p < 0.001$.

Control

AVE0118 (3mg/kg)

AVE0118 + Dofetilide (5μg/kg)

Dofetilide

Figure 3: Unipolar right atrial electrograms recorded during separate and combined infusion of dofetilide and AVE0118. Dofetilide alone only slightly prolonged the AFCL. AVE0118 caused a much larger increase in AFCL. When dofetilide were infused in addition to AVE0118 the prolongation of the AFCL was much larger than expected from the effects of the drugs separately. AFCL: Atrial fibrillation cycle length.

Figure 4 Left: Atrial fibrillation cycle length (AFCL) during 1 hour infusion of AVE0118 (0.3, 1, 3, 10 mg/kg/h) followed by additional dofetilide (20μg/kg/h) or ibutilide (4 mg/h). Increase in atrial fibrillation cycle length (ΔAFCL) after infusion of dofetilide (5 μg/kg) and ibutilide (1mg) after pre-treatment with AVE0118 (different dosages). A synergistic action was observed using AVE0118 pre-treatment in dosages of 1, 3 and 10 mg/kg/h). * $p < 0.05$ versus dofetilide or ibutilide alone (AVE0118: 0 mg/kg/h).

eled atria, the combination decreased conduction velocity at slow rates from 124 ± 6 to 119 ± 6 and from 119 ± 6 to 110 ± 5 cm/s at fast pacing rates (both $p < 0.01$).

The inducibility of AF was measured by applying single premature stimuli at the free wall of the right and left atrium. In non-remodeled atria AF could be induced in 15% of the cases. After 48 h of electrical remodeling single premature stimuli induced AF in all animals. Whereas administration of dofetilide or ibutilide did not reduce the inducibility of AF in remodeled atria (still 100%), AVE0118 alone reduced the inducibility to 31 to 33%. The combination and ibutilide or dofetilide caused a further reduction of atrial vulnerability, respectively 23 and 8%.

Effects on AF Cycle Length

The effects of the separate and combined administration of AVE0118 and dofetilide or ibutilide were evaluated in 10 goats with persistent AF of 46 ± 17 days (range 5–181). Figure 3 shows an example of fibrillation electrograms recorded during administration of dofetilide and AVE0118 alone and in combination. In a dosage of 5 μ g/kg dofetilide increased the median AFCL from 98 to 110 ms (+12 ms). Administration of 3 mg/kg AVE0118 increased the median AFCL by 44 ms (from 96 to 140 ms). When the drugs were given together the AFCL was prolonged by 77 ms. Thus in the presence of AVE0118 the class III effect of dofetilide was markedly stronger than the effect of dofetilide alone (Δ AFCL: 12 versus 33 ms).

In figure 4 the effects of different dosages of AVE0118 followed by dofetilide or ibutilide are given for all goats. Infusion of AVE0118 at a rate between 0.3 and 10 mg/kg/h increased the AFCL in a dose dependent way from 103 ± 3 (saline) to 167 ± 6 ms (10 mg/kg/h). After 60 minutes the infusion of dofetilide or ibutilide was started. The effect of dofetilide and ibutilide on AFCL was clearly dependent on pre-treatment with AVE0118. During 30 minutes of dofetilide or ibutilide the slope of the effect on AFCL was steeper at higher concentrations of AVE0118. In the right panels the average effects of dofetilide (5 μ g/kg) and ibutilide (1 mg) is plotted for all dosages of AVE0118. Dofetilide and ibutilide alone (no AVE0118) prolonged the AFCL by 10 ± 2 and 12 ± 4 ms. At increasing dosages the effect of I_{Kr} blockade became progressively enhanced. At ≥ 3 mg/kg/h the class III effect of dofetilide and ibutilide was more than 3 times higher than without AVE0118 (respectively $p=0.01$ and $p=0.02$).

To elucidate the mechanism of the prolongation of AF cycle length by (the combination of) AVE0118 and I_{Kr} blockers we recorded right atrial monophasic action potentials during pharmacological cardioversion. In Figure 5 the effects of AVE0118 alone and in combination with dofetilide are shown. During control, the fibrillation MAPs were triangular shaped without a plateau phase. In addition, the amplitude and take off potentials of the action potential varied on a beat to beat basis. The absence of a diastolic baseline potential (phase 4) suggests that no excitable gap was present. Infusion of AVE0118 prolonged the AFCL which was associated with the appearance of a plateau-phase in the action potential. Infusion of additional dofetilide caused a further prolongation of the fibrillation cycle length and a marked

Monophasic Action Potentials

Figure 5: Illustration of the effects of combined administration of AVE0118 and dofetilide on persistent atrial fibrillation. Monophasic action potentials were recorded from the right atrial free wall during infusion of AVE0118 followed by additional administration of dofetilide.

Figure 6: Cumulative cardioversion percentages during infusion of saline and AVE0118 (1, 3 and 10 mg/kg/h) for a 1-hour period followed by additional infusion of I_{Kr} -blocker (dofetilide and ibutilide).

prolongation of the action potential was observed, especially during phase III of the action potential. Shortly before cardioversion the cycle-length had prolonged to 311 ± 8 ms and the action potential duration at 70% repolarization was 227 ± 5 ms. This demonstrates that the prolongation of the AFCL by the combination of

Figure 7: Example of the effects of AVE0118 and ibutilide on the stability of atrial fibrillation (AF). AVE0118 (3mg/kg/h) could terminate AF, but burst pacing easily reinduced AF paroxysms. Additional infusion of ibutilide resulted in a progressive shortening of the duration of AF-episodes and eventually AF could not be reinduced.

Figure 8 Left: Representative examples of electrocardiograms showing the effects of separate and combined infusion of dofetilide and AVE0118 on the QT duration measured during atrial pacing (cycle length 400 ms). When given alone, dofetilide prolonged the QT duration but AVE0118 did not. When dofetilide was given in addition to AVE0118 the increase in QT duration was equal to when the drug was given alone. The right panels show the data of all goats for combined administration of AVE0118 and dofetilide (upper panel) or ibutilide (lower panel). Measurements were performed both before and after 48 hours of AF. * $p < 0.01$ versus control.

AVE0118 and I_{Kr} blockers is primarily due to a prolongation of the atrial action potential.

Success Rate of Cardioversion

Figure 6 shows the cumulative cardioversion success rates shown for all goats during infusion of AVE0118 (1, 3 and 10 mg/kg/h) and additional dofetilide ($n = 5$) or ibutilide ($n = 5$) (46 ± 17 days of persistent AF). AVE0118 alone terminated AF in a dose dependent manner ranging from 11 (1 mg/kg/h) to 60% (10 mg/kg/h). After 60 minutes infusion, additional dofetilide or ibutilide was administered for another 30 minutes. In the presence of AVE0118, the I_{Kr} blockers restored sinus rhythm in 56 (1 mg/kg/h) and 100% (10 mg/kg/h) of the animals. Of note, both dofetilide and ibutilide alone terminated AF in 1 of 5 goats (20%).

Figure 7 shows an example of the effects of separate and combined infusion of ibutilide and AVE0118 on the stability of persistent AF. AVE0118 (3 mg/kg/h) could restore sinus rhythm, but AF was easily reinduced by burst pacing. Combined infusion of AVE0118 and ibutilide exerted a very strong anti-fibrillatory effect and AF was completely suppressed (lower tracing). The lower tracing shows that during burst pacing an irregular atrial response was provoked, but after resumption of pacing AF could not persist.

Effects on QT duration

The effects of the separate and combined administration of dofetilide, ibutilide and AVE0118 on QT duration (atrial pacing at 400 ms) were determined before and after 48 hours AF (figure 8). As expected, the QT duration increased following infusion of dofetilide (5 μ g/kg) and ibutilide (0.06 mg/kg) whereas AVE0118 (1.5 mg/kg) did not change the QT interval.⁴ The combination of AVE0118 and dofetilide or ibutilide prolonged QT duration to a similar extent as dofetilide or ibutilide alone. In the right panels the effects on QT duration are shown for all goats. Both before (sinus rhythm) and after 48 hours of AF the prolongation in QT interval by the combination of AVE0118 and I_{Kr} blockade was the same as by dofetilide and ibutilide alone ($p > 0.1$). Thus, in contrast to atria, in the ventricles the class III effect of dofetilide and ibutilide were not enhanced by combined administration with AVE0118.

DISCUSSION

Main Findings

The present study shows that in electrically remodeled atria, prolongation of the refractory period by the I_{Kur}/I_{To} blocker AVE0118 restored the class III action of I_{Kr} blockers. No synergistic prolongation of the QT duration was observed. Intravenous

administration of the combination of AVE0118 with dofetilide or ibutilide cardioverted persistent AF of long duration (46 ± 17 days) in 100% of the cases.

Modulation of the Effects of Class III drugs by Electrical Remodeling

It was recently demonstrated in experimental and clinical studies that the class III action of I_{Kr} -blockade is reduced by AF-induced electrical remodeling.^{4,5,10} In the goat, the prolongation of left and right atrial refractoriness by *d*-sotalol, dofetilide and ibutilide was reduced by $\sim 75\%$.^{4,10} In humans, *dl*-sotalol had a reduced effect in patients with long-lasting AF.⁵ After internal electrical cardioversion *dl*-sotalol prolonged the AERP at the distal coronary sinus and high right atrium by ~ 25 ms whereas in a matched control group (no AF) this was ~ 40 ms. Also the reverse frequency dependence of *dl*-sotalol was lost in remodeled atria.⁵ These observations suggest that the contribution of the I_{Kr} to atrial repolarization is reduced after AF-induced remodeling. Courtemanche et al.¹¹ studied the effects of blockade of the various K^+ channels in a mathematical model of the human atrial action potential. It was found that in electrically remodeled cells blockade of the I_{Kr} caused a smaller prolongation of the action potential than in normal atrial cells. This was explained by the relatively late activation of I_{Kr} during the action potential. Because of this, the contribution of the I_{Kr} current is reduced when the action potential is shorter. Also in ventricular myocytes the effect of I_{Kr} blockade was shown to be dependent on the shape of the action potential.¹² Acceleration of the repolarization by constant current injection showed a reduced effect of the I_{Kr} blocker E-4031.¹² In contrast to this reduced effect of I_{Kr} blockade, the action of AVE0118 (I_{Kur}/I_{Io}) was enhanced after electrical remodeling.⁴ This was true despite the fact that the current densities of I_{Kur} and I_{Io} were down-regulated in remodeled atria.¹³⁻¹⁵ However, due to the loss of the plateau-phase, the relative contribution of the early (I_{Io} and I_{Kur}) may yet be increased.

In the present study we found that the loss of action of I_{Kr} blockers by electrical remodeling was restored by AVE0118. This finding is in agreement with computer modeling showing that the combined inhibition of I_{Kur} and I_{Kr} results in a larger prolongation of the APD than expected from the sum of the separate effects.¹¹ Blockade of I_{Kur} prolonged the plateau of the action potential which in turn increased the contribution of I_{Kr} to atrial repolarization. This may explain the synergism between AVE0118 and I_{Kr} blockers.

Effects on QT duration

In the present study, the combined administration of AVE0118 with dofetilide or ibutilide did not prolong the QT interval to a larger extent than I_{Kr} blockers alone. Since the expression of I_{Kur} is large in the atrium and absent in the ventricles, inhibition of I_{Kur} thus not affect ventricular repolarization.^{16,17} In the ventricles, I_{Kr} is the major repolarizing current and blockade of this channel markedly prolongs the action potential.¹⁸ The role of I_{Io} for the shape of the action potential is less clear and re-

markable differences between species exist.¹⁹ In species with short triangular action potentials (mouse and rat) blockade of the current markedly prolongs the action potential.^{20,21} In larger animals, I_{to} only plays a role during the early phase of the repolarization (phase I). In normal canine ventricle I_{to} blockade did not affect the duration of the action potential.²² Computer modeling showed no effect.²³ On the other hand, it has been suggested that the prolongation of the ventricular action potentials in failing hearts is due to reductions in I_{to} .²⁴ In the present study, the lack of effect on the QT interval suggest that blockade of the I_{to} and I_{Kur} alone or in combination with I_{Kr} blockade, does not affect the action potential of the goat ventricle.

Possible Clinical Implications

Currently available anti-arrhythmic drugs are moderately effective in converting persistent AF. Class IC drugs such as flecainide are only effective in cardioverting recent onset AF. In patients in which AF persisted for >24h, class I drugs are of no value for pharmacological cardioversion.^{25,26} The cardioversion efficacy of the class III drug ibutilide was only 16% in of patients with AF of longer than 30 days.¹ In addition to this low efficacy, also the risk of serious ventricular pro-arrhythmia is a concern.^{27,28}

In the present study we found that the combined administration of AVE0118 and dofetilide or ibutilide during persistent AF in the goat caused a marked dose dependent increase in the AF cycle length leading to restoration of sinus rhythm in 100% of the cases. Ventricular arrhythmias were not observed. Blockade of I_{Kur}/I_{to} had no effect on the QT interval whereas the combination of AVE0118 with dofetilide and ibutilide caused the same (slight) QT prolongation as caused by the I_{Kr} blockers alone. If this combination would yield the same effect in humans then this may have important implications for the management of AF in patients.

Obviously, caution is warranted in extrapolating the results of the present study to humans with AF. Important differences exist in K^+ currents between species.^{29,30} Therefore, it is possible that the electrophysiological effects of AVE0118, but also of I_{Kr} blockers may be different in man. In addition, the substrate of AF is very different in patients than in the goat. Patients often have underlying heart diseases that contributes to the persistence of the arrhythmia by changing the electrophysiological, structural and contractile properties of the atrium.

Limitations

A major limitation of the present study is that the underlying ionic mechanisms of the action of AVE0118 and dofetilide/ibutilide were not addressed. At the moment only computer modeling data is available on the effects of combined blockade of I_{Kur}/I_{to} and I_{Kr} on atrial electrophysiology. It is important that the data of the modeling study of Courtemanche et al.¹¹ is backed up by additional in vitro experiments.

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CHAPTER 6

Long-Term Changes in Sequence of Atrial Activation and Refractory Period: No Evidence for 'Atrial Memory' in the Goat

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ABSTRACT

Objectives: The purpose of this study was to test whether the spatial distribution of the atrial refractory period (AERP) and the vulnerability to atrial fibrillation (AF) are altered by long-term changes in the sequence of atrial activation.

Background: The spatial distribution of the AERP plays an important role in AF. Changes in the activation sequence have been postulated to modulate atrial repolarization ("atrial memory").

Methods: Six goats were chronically instrumented with epicardial atrial electrodes to determine activation time and AERP at 11 different areas of the right (RA) and left (LA) atrium and the Bachmann's bundle. Activation time and AERP were measured during sinus rhythm and during prolonged RA and LA pacing (1 week RA pacing, 2 weeks LA pacing, 1 week RA pacing; 150 bpm). Inducibility of AF was determined by the number of atrial sites where single premature stimuli induced AF paroxysms >1 second.

Results: During sinus rhythm (106 ± 4 bpm), AERP was longest at the Bachmann's bundle and shortest at the LA free wall (185 ± 6 ms and 141 ± 5 ms, $P < .001$). In five of six goats, an inverse correlation between local activation time and AERP was found during sinus rhythm ($r = -0.53 \pm 0.05$; $P < .05$). The increase in atrial rate during RA and LA pacing caused an overall shortening of AERP from 167 ± 6 ms to 140 ± 6 ms ($P < .001$). However, a switch between long-term RA and LA pacing did not significantly change AERP at any of the 11 atrial regions and had no significant effect on AF inducibility.

Conclusions: During sinus rhythm, an inverse relationship exists between the sequence of atrial activation and the local refractory period. However, long-term changes in the sequence of atrial activation do not alter the spatial distribution of AERP or the inducibility of AF.

INTRODUCTION

Spatial differences in the atrial effective refractory period (AERP) play an important role in the initiation and perpetuation of atrial reentrant arrhythmias.¹⁻³ Regional differences in AERP have been attributed to differences in ionic currents and action potential duration (APD).⁴ In isolated canine atria Spach et al. found an inverse relationship between the atrial APD or AERP and the distance from the sinus node.^{5,6} Whether this spatial distribution in APD and AERP is the long-term result of the sequence of activation during sinus rhythm or whether it is due to intrinsic electrophysiological differences between atrial myocytes in different parts of the atria is unclear.

Local activation time and APD are inversely related in the ventricles.⁷ A change in ventricular activation results in prolongation of the action potential in early activated and a shortening in later activated areas.⁸ Rosenbaum et al. introduced the term 'cardiac memory' to describe this capability of myocardial cells to adjust their repolarization to alterations in sequence of activation.⁹ Electrocardiographically, cardiac memory manifests itself as a change in morphology of the T wave, persisting for a considerable time after the normal sequence of activation is restored. Also in the atria, a change in the so-called 'atrial gradient' occurs on the surface ECG after prolonged ectopic atrial activation, indicative of changes in the atrial T(a)-wave.¹⁰ It has been proposed that the duration of the action potential in the atria, as in the ventricles, is modulated by the sequence of activation ('atrial memory').¹⁰ It has also been speculated that tachycardia-induced electrical remodeling may incorporate aspects of cardiac memory.^{10,11} However, data on changes in atrial APD and AERP in response to long-term alterations in sequence of activation are sparse and limited to a small number of atrial sites.^{10,12,13}

In the present study, we investigated whether long-term changes in atrial activation lead to changes in the spatial distribution of the atrial refractory period. In chronically instrumented goats, AERP was measured at 11 atrial sites (right atrium (RA), left atrium (LA) and Bachmann bundle (BB)) both during sinus rhythm and after 1-2 weeks of right and left atrial pacing.

METHODS

Animal model

Six goats were used for this study. The investigation conformed with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Anaesthesia was induced with Thiopental (15mg/kg) and maintained by ventilation with Isoflurane and a mixture of O₂ and N₂O. After left intercostal thoracotomy, the pericardium was opened and a teflon felt strip measuring 10 x 1.3 cm and containing 54 electrodes (diameter 2 mm) was pulled through the sinus transversus between the atria and the aortic root

Figure 1: Upper left: Schematic drawing of the chronically implanted epicardial electrodes. Electrophysiological measurements were performed at 11 atrial regions. Upper right: Unipolar electrograms illustrating the change in sequence of activation during pacing from the right (RA₁; RA-pacing) and left atrial appendage (LA₁; LA-pacing). Bottom: Experimental protocol. Electrophysiological studies were performed during sinus rhythm and were repeated after 1 hour, 1 day and 1 week of RA-pacing and after 1 hour, 1 day, 1 week and 2 weeks of LA-pacing.

and sutured to the right and left atrial appendage. Two teflon felt plaques of 1 x 1.5 cm (12 electrodes) were sutured on the free wall of the right and left atrium (Fig 1). A subcutaneously implanted silver plate served as indifferent electrode.

All leads were tunneled subcutaneously to the neck and exteriorized by three 30-pin connectors. Buprenorphin and ampicillin were given pre-operatively and during three days after surgery. The experimental protocol started after 4 weeks when the animals had recovered from the implantation procedure.

All experiments were performed in the fully conscious state. Electrophysiological studies were performed both during normal sinus rhythm and during 3 consecutive periods of right and left atrial pacing. A conventional pacemaker (Kappa SR703, Medtronic) was used for continuous atrial pacing at a rate of 150/min. The external device was connected to a pair of electrodes at the right or left atrial appendage. The pacemaker was programmed in the AAI mode to avoid induction of atrial arrhythmias. Stimulus strength was set at twice threshold at a pulse duration of 1.5 ms. The device counter for sensed and paced atrial beats was used to verify a high percentage of atrial pacing (>99%). Atrial tachyarrhythmias were automatically detected and stored with an atrial electrogram strip if more than 4 consecutive atrial beats at a rate of ≥ 220 /min occurred. Figure 1 illustrates the experimental protocol. After baseline measurements were obtained during sinus rhythm, the right atrial appendage was paced continuously for 1 week (RA-pacing). Then the left atrial appendage was paced during 2 weeks (LA-pacing) followed again by 1 week of RA-pacing.

This protocol was designed to measure changes in AERP (and their reversibility) resulting from long-term changes in sequence of atrial activation without a change in pacing rate.

Electrophysiological Measurements

Electrophysiological studies were performed during sinus rhythm and after 1 hour, 1 day and 7 days of RA and LA-pacing. In case of LA-pacing measurements were done after 14 days of pacing. The local activation time and refractory period were determined at 11 atrial regions together with the inducibility of AF by single premature stimuli (Figure 1). In each area the average value at three neighboring electrodes was measured. Electrode selection was based on signal quality and stimulation threshold. The local activation time was determined by the steepest negative deflection in the unipolar electrograms. During sinus rhythm the earliest onset of the P-wave in the extremity-leads was defined as the first moment of activation. During pacing the stimulus artefact was taken as time zero. The AERP was measured by single premature stimuli (2 ms, 2x threshold). An extra-stimulus (S_2) was interpolated at every 8th interval during RA- or LA-pacing (S_1 - S_1 400 ms) synchronized to the local atrial activation (A_1). Starting well within the refractory period, the A_1 - S_2 coupling interval was increased in steps of 2 ms. The shortest coupling interval resulting in a propagated response was taken as the AERP. The spatial dispersion in refractory periods was defined as the difference between the longest and shortest refractory period of the 11 regional AERPs. The local atrial recovery time was determined by the sum of the local activation time and the AERP. Atrial vulnerability was measured by the percentage of pacing sites where atrial fibrillation could be induced by administration of single premature stimuli.

Statistical Analysis

Data are expressed as mean $\pm$ SEM. A one-way repeated-measures ANOVA with a Tukey Kramer post test was used to analyze regional differences in AERP during sinus rhythm and to compare the dispersion in AERP, the dispersion in recovery time and AF-inducibility between RA- and LA-pacing. A two-way repeated-measures ANOVA with a Bonferroni post test was used to compare AERPs at different timepoints (sinus rhythm, RA- and LA-pacing). A one sample *t*-test was used to evaluate whether local changes in AERP during RA- and LA-pacing differed from zero. A *p*-value <0.05 was considered to be statistically significant.

The Pearson correlation coefficient was calculated to evaluate the relationship between local activation time and AERP during sinus rhythm. A correlation coefficient of < -0.50 or >0.50 with a *p*-value of <0.05 was considered as statistically significant.

Figure 2: Sequence of activation (top) and spatial distribution of atrial refractory periods (bottom) in 6 goats during normal sinus rhythm. Refractory periods were shorter at late activated regions. *** $p < 0.001$;

RESULTS

Sinus Rhythm

The sequence of atrial activation and the spatial distribution in refractoriness during normal sinus rhythm are illustrated in Figure 2. Sinus rate ranged between 90 and 123 bpm with an average of 106 ± 4 bpm. In all animals the earliest activation was recorded at RB₁ or RB₂. The latest activation was seen at the free wall of the left atrium (LFW). The AERP was longest at the mid portion of Bachmann’s bundle (MB: 185 ± 6 ms) and was significantly shorter at the left and right atrial appendage and free wall (LA₁: 159 ± 9 ms; RA₁: 154 ± 7 ms; LFW: 141 ± 5 ms; RFW: 153 ± 10 ms; $p < 0.05$ to 0.001). Differences between right and left atrial refractory periods were not statistically significant. The AERP values from all atrial sites are given in Table 1. In 5 of 6 goats an inverse correlation existed between the local activation times and the AERP ($r = -0.53 \pm 0.05$; $p < 0.05$ to 0.001). In one goat no correlation was found.

Figure 3: Electrophysiological measurements at a left atrial site (LA₂). Left: Electrograms recorded at LA₂ during determination of the refractory period in one goat. Right: Local (LA₂) activation times and refractory periods from 6 goats. The increase in atrial rate during RA- and LA-pacing was associated with a shortening of the AERP compared to sinus rhythm ($p < 0.01$). A shift in pacing site from the right to the left atrial appendage and back caused large changes in the relative moment of activation, but had no significant effect on the AERP.

Figure 4: Changes in activation time and refractory period in 11 atrial regions after switching from RA- to LA-pacing (left) and back (right). A change in pacing site caused large changes in the local activation time (top) but had no significant effect on the AERP in any of the 11 atrial regions (bottom).

Table 1 Local activation time and AERP during sinus rhythm and after right atrial (RA-) and left atrial (LA-) pacing.

Atrial Site	Sinus Rhythm		After 1 week of RA-Pacing		After 2 weeks of LA-Pacing		After 1 week of RA-Pacing	
	Activation Time (ms)	AERP (ms)	Activation Time (ms)	AERP (ms)	Activation Time (ms)	AERP (ms)	Activation Time (ms)	AERP (ms)
RFW	38 ± 1	153 ± 10	26 ± 4	130 ± 8	86 ± 5	126 ± 9	20 ± 1	125 ± 9
RA1	33 ± 3	154 ± 7	9 ± 1	132 ± 6	81 ± 3	136 ± 9	8 ± 2	131 ± 8
RA2	28 ± 5	155 ± 5	21 ± 4	129 ± 7	69 ± 3	136 ± 7	16 ± 3	129 ± 7
RB1	21 ± 4	169 ± 4	31 ± 3	138 ± 5	58 ± 4	142 ± 6	28 ± 3	140 ± 5
RB2	23 ± 4	178 ± 4	41 ± 3	144 ± 5	48 ± 4	144 ± 6	39 ± 3	144 ± 5
MB	31 ± 4	185 ± 6	52 ± 3	154 ± 5	42 ± 4	147 ± 7	50 ± 3	150 ± 5
LB2	34 ± 3	184 ± 8	61 ± 3	157 ± 6	34 ± 4	152 ± 6	60 ± 4	149 ± 5
LB1	40 ± 3	182 ± 10	71 ± 4	161 ± 6	25 ± 3	152 ± 8	71 ± 4	145 ± 7
LA2	47 ± 4	165 ± 10	77 ± 3	148 ± 8	19 ± 4	144 ± 9	78 ± 4	137 ± 8
LA1	51 ± 4	159 ± 9	82 ± 2	150 ± 7	8 ± 3	149 ± 7	82 ± 2	141 ± 6
LFW	58 ± 3	141 ± 5	90 ± 3	123 ± 4	28 ± 3	124 ± 7	90 ± 8	122 ± 6
Average	-	167	--	143	--	142	--	140

Right and Left Atrial Pacing

The increase in atrial rate from 106 ± 4 bpm during normal sinus rhythm to 150 bpm during atrial pacing was associated with a shortening in the AERP (Table 1). The average AERP shortened from 167 ± 6 ms during sinus rhythm to 143 ± 5 ms after 1 week of RA-pacing ($p < 0.001$). In contrast, switching from RA- to LA-pacing (and back) did not cause an overall change in the atrial refractory period (142 ± 7 ms after 2 weeks of LA-pacing; $p > 0.05$).

Figure 3 illustrates local changes (LA_2) in AT and AERP. Whereas the transition from sinus rhythm to atrial pacing was associated with a shortening in the AERP (due to the higher rate), the switch between long-term RA- and LA-pacing did not cause a change in the local refractory period, despite the large change in the relative moment of activation. The change in local activation time that was caused by switching from right to left atrial pacing was largest at the atrial appendages (Figure 4). The bottom panel demonstrates the absence of any significant effect of the long-term change in sequence of atrial activation on the refractory period at 11 atrial sites.

Atrial Tachyarrhythmias

No spontaneous atrial arrhythmias were detected by the monitoring function of the pacemaker during the 4 weeks of atrial pacing, nor were they observed during the electrophysiological studies. The inducibility of AF by single premature stimuli

Figure 5: Although not statistically significant, there was a trend that the inducibility of AF was less during left (LA-) compared to right atrial (RA-) pacing. This was associated with a lower spatial dispersion in local recovery times (AT+AERP), while the dispersion in AERP remained the same. ** $p < 0.01$; * $p < 0.05$ compared to RA-pacing.

tended to be lower during LA- than during RA-pacing. However, due to the high inter-individual variation this difference was not statistically significant. Although the spatial distribution and the dispersion in AERP were not altered by a change in atrial pacing site, the regional differences in repolarization time (AT+AERP) were significantly less during LA- than during RA-pacing (Figure 5). The increased synchronicity in atrial repolarization during left atrial pacing may explain the lower vulnerability for induction of atrial arrhythmias. However, it should be noted that these changes in atrial repolarization occurred immediately after a switch in pacing site and were not the result of 'atrial memory'. Long-term atrial pacing had no further effect on differences in repolarization or inducibility of AF.

DISCUSSION

Spatial Distribution of Atrial Refractoriness

During sinus rhythm, the AERP was longest at the mid portion of Bachmann's bundle and became shorter towards the tip of the right and left atrial appendage. Also at the right and left free wall the AERP was shorter than at BB. In general the shortest AERPs were measured at the left atrial free wall, but the differences in AERP between right and left atrium were variable and not statistically significant. Also in the dog the longest AERP was found at Bachmann's bundle,¹⁴ whereas the AERP in the left atrial wall was reported to be shorter than in the right atrium.^{4,14} It has been suggested that the shorter refractory period in the left atrium contributes to the preferential role of the left atrium in perpetuation of AF.^{4,15} In humans, Chen et al. measured the AERP at 16 atrial sites in a control group and in patients with paroxysmal AF.¹⁶ Again the AERP was longest in Bachmann's bundle. The shortest refractory periods were found distal in the pulmonary veins and in the inferior right atrium. There was a considerable spatial variation in AERP in each atrium with no overall difference between right and left atrial refractoriness.

Spach et al. mapped the local refractory periods in isolated canine right atria. The longest refractory periods were found close to the sinus node. At increasing distances from the sinus node the refractory period became shorter.⁶ In our present study we also observed an inverse relationship between local AERP and activation time during sinus rhythm. However, the spatial distribution of refractory periods was not an exact mirror image of the sequence of activation. Although the AERP was shortest at the latest activated atrial site (LFW), differences in activation time within the right atrium or between the right and left atrium did not correlate with differences in AERP. In addition, the earliest point of activation was measured at the right part of Bachmann's bundle, whereas the longest AERP was measured at the mid-portion of BB. Consequently, the inverse correlation between AT and AERP during sinus rhythm, although statistically significant in 5 of 6 goats, was rather weak ($r = -0.53 \pm 0.05$). In anaesthetized pigs, no consistent relationship was found in the right atrium between the relative time of activation during sinus rhythm and the duration of the monophasic action potential. Only in 2 of 10 animals a statistically significant (inverse) correlation was present.¹⁷

Definition of Cardiac Memory

The term 'cardiac memory' was introduced in 1982 by Rosenbaum and colleagues.⁹ They described persistent T-wave changes after a transient alteration in the sequence of ventricular activation. Changes in T-wave morphology started to appear after 10-15 minutes and reached a plateau within several days to weeks. After the normal sequence of activation was restored, the changes in T-wave morphology could persist for weeks. Based on these findings it was hypothesized that ventricular myocytes were able to adapt the duration of their action potential to changes in the

relative moment of activation. Because the effects were long-lasting, the ventricular cells were said to possess a 'memory' of the prevailing sequence of activation. Later experimental studies by the group of Rosen confirmed that the T-wave changes induced by right ventricular pacing resulted exclusively from an altered sequence of activation and were not due to changes in rate, hemodynamic load, or ventricular ischemia.¹⁸ It was also demonstrated that a change in sequence of ventricular activation modulates the duration of the ventricular action potential and refractory period.^{8,18-20} Costard-Jäckle et al. were the first to record monophasic action potentials at multiple ventricular sites during normal ventricular activation (atrial pacing) and during a transient period of right ventricular pacing.⁸ During the normal sequence of ventricular activation, local activation times and action potential duration were inversely related. At the onset of ventricular pacing this relationship was abruptly lost. However, during 2 hours of ventricular pacing the action potential gradually prolonged at early activated sites and became shorter at later activated regions. As a result, the spatial distribution of the action potential durations changed and the inverse relationship between moment of activation and action potential duration was re-established. Cardiac memory has been interpreted as a specialized form of electrical remodeling, not due to prolonged tachycardia but exclusively resulting from a change in sequence of activation.^{11,21}

The ionic mechanisms underlying cardiac memory are not precisely understood. Experimental studies point to a role of the transient outward current (I_{to}) and the L-type calcium current ($I_{Ca,L}$).²²⁻²⁴ In dogs, pacing-induced changes in the ventricular activation sequence have been shown to reduce the density of I_{to} and alter the kinetics of I_{to} and $I_{Ca,L}$.^{23,24} Blockade of these currents prevented the induction of cardiac memory.^{22,24} However, little is known about the involved intra-cellular signalling pathways. There are some indications that alterations in the myocardial stress-strain relationship and production of angiotensin II are involved.²⁵

Do the Atria Also Have a 'Memory'?

So far only three studies have evaluated the possible existence of an 'atrial memory'. Wood et al. investigated the effects of pacing at different atrial sites on the duration of the atrial action potential in the rabbit.¹² No consistent relationship was found between local activation time and action potential duration after 3 hours of pacing. The group of Rosen measured changes in the atrial electrocardiogram and refractory periods in chronically instrumented dogs with total AV-block.^{10,13} In their first study, a shortening in right atrial refractory periods was reported after 2 hours of left atrial pacing.¹⁰ In contrast, in a more recent investigation no changes in right or left atrial refractory periods (3 sites) were observed after prolonged right or left atrial pacing (4 weeks).¹³ However, still changes in the so-called spatial 'atrial gradient' (ratio between P and T(a) iso-areas in the X, Y, and Z axis) were observed, indicating that the sequence of atrial repolarization might have been changed.^{10,13}

In the present study we evaluated the long-term effects of right and left atrial pacing (1-2 weeks) in the goat. The AERP was measured in 11 atrial regions (free wall of

right and left atrium, right and left atrial appendages, Bachmann's bundle). No effect of long-term changes in atrial activation on the distribution of the AERP could be established. If we consider local changes in duration of the action potential or refractory period -induced exclusively by a change in sequence of activation- as the expression of cardiac memory, then no evidence for 'atrial memory' could be found. Our observations do also not support the concept that a change in atrial activation sequence contributes to atrial electrical remodeling by prolonged rapid atrial pacing or atrial fibrillation.

The Spatial Atrial Gradient

Compared to the ventricles, repolarization of the atria is more difficult to study from the surface electrocardiogram. Not only that the atrial T-wave is much smaller than its ventricular counterpart, but it is also superimposed by the end of the P-wave and the beginning of the ensuing QRS complex. Therefore, the Ta-wave can only be measured in the surface electrocardiogram during AV-block. To detect primary changes in the process of atrial repolarization, P and Ta voltage-time integrals (arithmetic sum of P and Ta isoareas) have been measured in the X, Y and Z plane using dogs with total AV-block, and the 'spatial atrial gradient' has been defined as the root-mean-square of the three P-Ta voltage time integrals.^{10,13,26} In the study by Herweg et al., the spatial atrial gradient was shown to increase after 1 hour of left atrial pacing. Changes were more marked at rapid pacing rates and showed accumulation by repeated episodes of left atrial pacing.¹⁰ Similar as in our experiments, Chandra et al. could not find changes in atrial refractory period during 4 weeks of left atrial pacing. Also the spatial atrial gradient did not change during prolonged left atrial pacing. The sole indication of 'atrial memory' was a decrease in the spatial atrial gradient occurring 2-3 weeks *after cessation* of atrial pacing.¹³

How should we interpret a change in the 'spatial atrial gradient' in the surface electrocardiogram without a measurable change in atrial action potential or refractory period? On the one hand, one may argue that the atrial gradient is a more sensitive parameter. The action potential and refractory period can not be measured at all atrial sites, whereas the spatial atrial gradient does represent the whole atrium. On the other hand, it is also possible that the atrial gradient is a less specific marker for atrial memory than direct evaluation of atrial repolarization and refractoriness. The fact that in the recent study by Chandra et al.¹³ the spatial atrial gradient did not change *during* the 4 weeks of left atrial pacing but only 2-3 weeks after restoration of the normal sequence of activation suggests that other factors (related to chronic AV-block and ventricular pacing) may have led to the observed changes in atrial gradient. Since measurement of the spatial atrial gradient was not possible in our study (no total AV-block), we could not compare the absence of changes in refractory period in the majority of the atria (right and left atrial free wall, right and left atrial appendages, Bachmann's bundle) with changes in the spatial atrial gradient.

Implications For Atrial Tachyarrhythmias

Spatial heterogeneity in atrial repolarization is an important determinant of reentrant arrhythmias.^{1,27} Animal and clinical studies have shown that an increased spatial dispersion in atrial refractoriness is associated with an increased vulnerability for AF.^{2,3,28} In our study, long-term changes in the sequence of atrial activation did not alter the spatial distribution and dispersion in AERP. Apart from spatial differences in AERP, also differences in local AT will determine the degree of synchronicity in repolarization. Several studies have shown that the dispersion in atrial recovery time (activation time + AERP or APD) and the inducibility of atrial tachyarrhythmias are lower during left atrial pacing.^{12,29,30} Also in the present study the dispersion in atrial recovery time and the inducibility of AF were lower during LA-pacing as compared to RA-pacing. However, since changes in atrial recovery time and AF vulnerability occurred immediately after the shift in pacing site, they are not related to 'atrial memory'.

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CHAPTER 7

Preferential Class III Action of Dofetilide on the Bundle of Bachmann. A Critical Determinant for the Initiation of Atrial Fibrillation in the Goat

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INTRODUCTION

Spatial heterogeneities in repolarization play an important role in the initiation and perpetuation of reentrant arrhythmias.¹⁻³ It is well known that marked heterogeneities in action potential duration and refractoriness are present in the heart.⁴⁻⁸ In a recent study, Duytschaever et al. showed that the distribution of the refractory period in the caprine atrium showed a consistent pattern characterized by significantly longer refractory periods at Bachmann's bundle than at the right and left atrial free wall.⁴ This spatial dispersion of refractoriness was associated with the initiation of atrial fibrillation (AF). Whereas premature stimuli delivered at the left or right atrial free wall induced AF, early stimuli at Bachmann's bundle never caused AF.

Recently it was demonstrated that the class III effect of blockers of the delayed rectifier current (I_{Kr}) on atrial refractoriness was reduced by AF-induced electrical remodeling.^{9,10} We hypothesized that the contribution of I_{Kr} to atrial repolarization decreased when the action potential is shortened due to AF. These observations are in agreement with recent findings in a computer model of the human atrial action potential in which it was shown that the class III effects of I_{Kr} blockade was much stronger in rectangular than triangular shaped action potentials.¹¹ Based on these observations, we postulated that blockers of the I_{Kr} current may exert a preferential effect at atrial sites with a long refractory period. If this is the case, I_{Kr} blockers will increase the dispersion of refractoriness. In the present study we evaluated the effects of the I_{Kr} blocker dofetilide on the atrial refractory period at multiple atrial sites in the goat model of AF. We showed that dofetilide increased the spatial dispersion of refractoriness because it exerted a preferential class III effect on Bachmann's bundle. In electrically remodeled atria this caused a widening of the window of inducibility of atrial fibrillation.

METHODS

The Goat Model of Atrial Fibrillation

Nine female goats were used for this study. The animals were handled according to the European Directive for Animal Research and the study protocol was approved by the Animal Investigation Committee of the University of Maastricht. During general anesthesia (halothane 1-2% and a mixture of O_2 and N_2O) the heart was exposed through a thoracotomy and a teflon-felt plaque containing 30 electrodes (3 x 2.5cm, inter-electrode distance 4mm) was sutured on the free wall of each atrium. A 10cm long plaque of three rows of electrodes (54 electrodes, inter-electrode distance 5mm) was implanted on Bachmann's bundle between the tips of the right and left appendages (figure 1). Three silver plates were left under the skin of the thorax to serve as indifferent electrode. The leads were tunneled subcutaneously to the neck and exteriorized by four 30-pole connectors. Experiments were started 3-4 weeks after surgery. Details of the goat model have been described elsewhere.¹²

Figure 1: Left: Epicardial instrumentation. Atrial refractory periods were measured at 7 atrial sites at the left and right atrial free wall (LA and RA), left and right atrial appendage (LAA and RAA) and the left, right and middle portion of Bachmann's bundle (LBB, RBB and MBB). Right: The study protocol. Electrophysiological measurements included determination of the atrial refractory period, window of inducibility and duration of AF.

Electrophysiological Measurements

The atria were paced with biphasic stimuli of 2 ms duration (4x threshold) generated by a constant current generator (Medtronic SP3111). The atrial effective refractory period (AERP) was measured at multiple atrial sites (right and left atrial free wall (RA and LA), right and left atrial appendage (RAA and LAA) and right, middle and left part of Bachmann's bundle (RBB, MBB and LBB)) during regular bipolar pacing with a cycle length ranging between 400 and 200 ms (Figure 1). Single interpolated stimuli were applied after each 8th basic stimulus starting well within the refractory period. The longest S_1 - S_2 interval that failed to capture the atria (increments 2 ms) was taken as the AERP.

The window of inducibility was determined at 3 atrial sites (RA, MBB, RA). During regular pacing (400 ms) a single premature stimulus, starting at a coupling interval of 250 ms, was given after a drive train of 30 beats. In case a premature stimulus evoked a rapid irregular atrial rhythm (cycle length ≤ 150 ms) lasting ≥ 1 second, AF was considered to be inducible. Each coupling interval was repeated 3 times and then decremented in steps of 5 ms. The upper and lower limit of inducibility were defined as respectively the longest and shortest coupling interval that induced AF in at least 2 of 3 attempts. The window of inducibility was defined as the difference between the upper and the lower limit of inducibility.

The effect of dofetilide on the stability of AF was evaluated by measuring the duration of at least 10 consecutive AF paroxysms induced by atrial burst pacing.

Conduction through Bachmann's bundle was determined during application of premature stimuli. During decremental pacing from either the right or the left atrial

free wall the A_1A_2 interval was determined at the right and left portion of Bachmann's bundle. The coupling interval that resulted in an A_1A_2 interval that was >10 ms longer than the S_1S_2 interval was taken as the upper limit for conduction delay in BB.

Experimental Protocol

The electrophysiological effects of dofetilide were measured before and after 48 hours of AF. Atrial fibrillation was induced by a fibrillation pacemaker as described previously.^{12,13} Measurements of atrial refractoriness, the window of inducibility and the duration of AF were performed before and after 30 minutes of infusion of dofetilide at a rate of 20 $\mu\text{g/kg/h}$ (figure 1).

Statistical analysis

Results are presented as mean $\pm$ SEM. Differences between groups were evaluated by the paired Student's *t*-test. Differences between groups were determined by the paired Student's *t*-test. Serial measurements were analyzed by ANOVA with repeated measures. Differences were considered statistically significant when $P < 0.05$.

RESULTS

Regional Effects of Dofetilide on Atrial Refractoriness

The effect of dofetilide on the atrial refractory period was measured at 7 atrial sites both before and after 48 hours of AF-induced electrical remodeling. As shown previously, the distribution of refractoriness was characterized by a long AERP at the mid portion of Bachmann's bundle and shorter AERP's at the right and left atrial free wall (figure 2: open circles).⁴ After 48 hours of AF this particular distribution was still present. The shortening of atrial refractoriness by AF-induced electrical remodeling was significantly larger at RA and LA than at MBB (respectively -64 ± 5 , -55 ± 5 and -34 ± 7 ms, $p < 0.05$). Dispersion of refractoriness, defined as the difference between the longest and shortest atrial refractory period increased from 44 ± 6 during control to 74 ± 9 ms after 48h of AF ($p < 0.001$).

Administration of dofetilide prolonged the AERP at all atrial sites both in non-remodeled and after 48 hours of AF (solid circles). However marked differences were observed at different atrial sites. In the lower panels of figure 2 the prolongation of atrial refractoriness (ΔAERP) are given at 7 atrial sites. In non-remodeled atria the degree of prolongation was most pronounced at Bachmann's bundle ($+26 \pm 2$ ms). This effect was statistically different from the effect at LA ($+19 \pm 2$ ms, $p < 0.05$) but not from RA ($+21 \pm 3$ ms, $p = 0.08$). Because of the stronger prolongation of the AERP at MBB, the spatial dispersion of refractoriness increased from 44 ± 6 to 55 ± 9 ms ($p < 0.001$). After 48 hours of AF the measurements were repeated and

Figure 2: Upper panels: Atrial refractory periods measured at 7 atrial sites in both normal (left) and remodeled atria (right), before (open dots) and after infusion of dofetilide (closed dots). In both normal and remodeled atria dofetilide increased the dispersion of refractoriness, due to a stronger prolongation of the AERP at the middle portion of Bachmann's bundle than at the atrial free walls (lower panels).

the prolongation of the AERP was again markedly stronger at MBB than at RA and LA (24 ± 2 compared to 6 ± 2 and 8 ± 2 ms, both $p < 0.01$). Because of this preferential action of dofetilide at MBB, the spatial dispersion of refractoriness increased from 74 ± 9 to 92 ± 8 ms ($p < 0.001$). When the effect of dofetilide on remodeled atria was compared to its effect in normal atria, the Δ AERP was reduced at the right and left atrial free wall but not at Bachmann's bundle.

To test the hypothesis that the contribution of I_{Kr} to atrial repolarization depends on the duration of the action potential, we determined the correlation between the AERP and the class III effect of dofetilide. In figure 3 the class III effect of dofetilide is plotted against the mean AERP at 7 atrial sites both in non-remodeled (open circles) and remodeled atria (closed circles). A strong positive linear relationship was found between the duration of the refractory period and the degree of AERP prolongation ($R = 0.85$, $p < 0.001$).

Rate Dependent Action of Dofetilide at RA, LA and MBB

It is well known that the class III effect of I_{Kr} blockers is more pronounced at slower than at fast pacing rates. However, in electrically remodeled RA and LA this so called reverse frequency-dependency was not preserved and turned into frequency-dependency or a flat frequency effect.^{9,10} To evaluate whether this was also the case in

Bachmann's bundle, the class III action of dofetilide was determined during pacing at cycle lengths between 400 and 200 ms. Figure 4 shows the rate-dependent effects of dofetilide on the AERP at RA, LA and MBB both in normal and remodeled atria. In non-remodeled atria, at all sites the prolongation of AERP was stronger at pacing cycle lengths of 400 than at 200 ms. After 48 hours of AF this reverse-frequency dependence was no longer present at RA and LA due to a loss of class III effect at slow pacing rates. In contrast, at BB the AERP still prolonged in a reverse-frequency dependent manner.

Figure 5 shows the effects of dofetilide on the rate-dependent dispersion of refractoriness in normal and electrically remodeled atria. Dispersion of refractoriness was clearly dependent on the pacing rate, i.e. the largest dispersion was observed at the slowest pacing rates. In normal as well as remodeled atria, the spatial dispersion in AERP increased after infusion of dofetilide at pacing rates >300 ms. At shorter pacing intervals, no changes were observed.

Conduction of Premature Beats along Bachmann's Bundle

Since the AERP was longest at the mid part of Bachmann's bundle it can be expected that early premature beats will have difficulty to conduct through Bachmann's bundle. In addition, since dofetilide preferentially prolongs the AERP at MBB, this drug may further increase conduction delay at BB. To evaluate this, we measured the conduction of premature beats from RA and LA through Bachmann's bundle before and after infusion of dofetilide ($n = 5$ goats). Shown in figure 6 are unipolar electrograms recorded from the right, left and mid portion of Bachmann's bundle during application of premature stimuli at the RA. Before drug administration, premature stimuli with a coupling interval >180 ms propagated along Bachmann's bundle without conduction delay, i.e. the A_1A_2 interval at RBB was equal to A_1A_2 at LBB. The electrograms at RBB, LBB and MBB showed steep biphasic deflections without fractionation. Shortening of the S_1S_2 coupling interval progressively prolonged the A_1A_2 interval at LBB but not at RBB. This was associated with an increased fractionation at MBB but not at RBB and LBB. This indicates that conduction was preferentially impaired at MBB. The upper limit of conduction delay at BB, defined as the longest S_1S interval resulting in ≥ 10 ms A_1A_2 interval was 165 ms. After infusion of dofetilide, fractionation of the electrogram of MBB already occurred at coupling intervals ≤ 220 ms. The upper limit of conduction delay was increased to 200 ms. On average, dofetilide increased the upper limit of conduction delay during right and left atrial pacing from 171 ± 7 and 168 ± 6 to respectively 199 ± 6 ms and 197 ± 5 ms (both $p < 0.001$).

Effects of Dofetilide on the Window of Inducibility

The window of inducibility was measured in 5 goats at 3 atrial sites (RA, LA and BB) both in non-remodeled and remodeled atria (48 hours of AF). A representative example of the measurements (and the effects of dofetilide) is given in figure 7. Shown

Figure 3: Relation between the atrial refractory period (AERP) measured at different atrial sites in normal (open dots) and remodeled atria (closed dots) and the increase in atrial refractory period by dofetilide (Δ AERP). The significant correlation demonstrates that the class III effect of dofetilide was strongly dependent on the AERP, i.e. the longer the AERP, the stronger the effect.

Figure 4: Effects of dofetilide on the rate adaptation of the atrial refractory period (AERP) at the right and left atrial free wall (RA and LA) and the mid portion of Bachmann's bundle (MBB). Measurements were performed in normal (solid line) and remodeled atria (dotted lines). In normal atria, the prolongation of the AERP by dofetilide was most pronounced at slow pacing rates (reverse-frequency dependence) at all sites. After 48 hours of induced electrical remodeling, the normal rate adaptation was lost at the RA and LA but not at MBB. The action of dofetilide on RA and LA was now no longer reverse frequency dependent. In contrast, at MBB the prolongation was still strongest at slow pacing rates.

Figure 5: Effects of dofetilide on the rate dependent dispersion of refractoriness in normal (left) and re-modeled atria (right). Dispersion was calculated as the difference between the longest and shortest AERP at the right and left atrial free wall and the mid portion of Bachmann's bundle.

Control

Dofetilide

Figure 6: Upper panels: Unipolar electrograms recorded at right, middle and left portion of Bachmann's bundle (RBB, MBB and LBB) during application of premature stimuli from the left atrial free wall. Right panel shows the A1A2 interval at RBB and LBB for different S1S2 intervals. Lower panels: Identical measurement after infusion of dofetilide. After drug administration, fragmentation in the MBB electrogram occurred at longer coupling intervals and the S1S2 interval that resulted in a conduction delay >10 ms increased from 165 to 200 ms.

Figure 7: Measurement of the window of AF-inducibility, before (left) and after (right) infusion of dofetilide. Dofetilide increased the window of inducibility from 65 ms to 80 ms. This was due to a stronger prolongation of the upper limit of inducibility (ULI) than the lower limit of inducibility (LLI), respectively +20 and +5 ms.

is a unipolar electrogram recorded from the right atrial free wall during application of premature stimuli at RA. During control, coupling intervals ≥ 175 ms captured the atrium, but did not induce AF. However, at a coupling interval of 170 ms AF was induced (=upper limit of inducibility). Subsequently, the coupling interval was decremented in steps of 5 ms until the AERP was reached (100 ms). The shortest coupling that induced AF was 105 ms and was defined as the lower limit of inducibility. The window of inducibility was defined as the difference between the upper and lower limit of inducibility (= 65 ms). In the right part of figure 7 the same measurements are shown after administration of dofetilide. Dofetilide caused a stronger increase in the upper than the lower limit of inducibility. As a result, the window of inducibility increased to 80 ms. In non-remodeled atria, AF was hardly inducible and in only 1 goat the window of inducibility could be determined at the right atrium (15 ms). After infusion of dofetilide, the window of inducibility was 5 ms. In electrically remodeled atria, the inducibility of AF had increased markedly and premature stimuli evoked AF in 100% of the cases (at both RA and LA). At MBB, AF was never induced. No differences were observed in the window of inducibility at the RA and LA (68 ± 5 and 61 ± 9 ms, $p=0.5$). Infusion of dofetilide increased the window of inducibility at both RA and LA to respectively 80 ± 4 ($p<0.01$) and 78 ± 9 ms ($p<0.05$). This was due to a larger prolongation of the upper limit of inducibility than the lower limit of inducibility. Dofetilide increased the upper limit, at respec-

Figure 8: Unipolar electrograms and mapping during initiation of AF by a premature stimulus from the right atrium. See text for explanation.

Figure 9 Left: Unipolar electrograms recorded along Bachmann's bundle (BB) showing initiation of AF. Application of a premature beat from the left atrium was associated with impaired conduction and fragmented electrograms at the mid portion of BB (electrode 8-10). The first spontaneous beat of AF was seen in electrode 1, suggesting that AF originated in that area. However, after amplification of the signals (right panel) small biphasic deflections could be observed in electrode 3-8. Mapping of the activation pattern was suggestive of an excitation wavefront traveling from MBB to the LAA (arrow).

tively the RA and LA, by 18 ± 2 and 24 ± 3 ms whereas the lower limit increased by only 6 ± 1 and 8 ± 3 ms (both $p < 0.05$).

Effects of Dofetilide on the Stability of Atrial Fibrillation

The effect of dofetilide on the stability of AF was evaluated by measuring the median duration of at least 10 AF-episodes induced by burst pacing. In non-remodeled atria, AF was short-lasting with a median duration of 8 seconds (range 3 to 26). Infusion of dofetilide shortened the duration of AF to a median duration of 3 seconds (range 2 to 13) ($p = 0.04$). After 48 hours of AF the duration of AF episodes was increased significantly to 32 seconds (range 3 to 136) ($P < 0.01$). In this situation, dofetilide infusion shortened the median duration of AF to 11 seconds (2.5 to 54) ($p = 0.01$).

Mapping of Initiation of AF

The epicardially implanted electrodes were used to map the activation pattern during induction of AF. In figure 8 an example is shown of the initiation of AF by a single premature stimulus delivered at the RA. In the left panel electrograms are shown recorded from RA, RAA, RBB, MBB, LBB, LAA and LA. The right panels show the corresponding activation maps during pacing with 400 ms, application of the premature beat and, the first beat of AF. During regular pacing the wavefront propagated along Bachmann's bundle without conduction delay. Application of a premature stimulus unmasked fractionation at the MBB recording site. The activation map demonstrated a marked local conduction delay (> 30 ms) at the mid portion of BB. Mapping of initiation of AF demonstrated that the earliest excitation originated from the RBB area, just proximal to the area of local conduction delay. The combination of local conduction delay and early excitation of the area proximal to the site of block suggests that the bundle of Bachmann played a critical role in the initiation of AF.

Initiation of AF by Bachmann's Bundle Reentry

In two goats we could demonstrate that the initiation of AF was due to reentry within BB. Shown in the left panel of figure 9 are electrograms recorded along Bachmann's bundle during pacing from the LA. Application of a premature stimulus resulted in fragmented potentials in electrodes 9 and 10, indicative of local conduction block at the mid portion of Bachmann's bundle. In this case the earliest activation of the first AF beat was seen at LAA (electrode 1), suggesting that AF originated close to the pacing site and far from BB. However, when electrograms from BB are magnified (right panel) small but distinct biphasic deflections can be seen in the MBB, LBB and LAA electrograms. The sequence of the small potentials showed that the pacemaker impulse actually traveled back from MBB to LAA (arrow). AF thus did not originate at the LAA area, but from the mid portion of Bachmann's bundle. Obviously, during propagation of the premature beat, a small muscle bundle in the left

part of BB was not activated (longitudinal dissociation) and was available as a return pathway to the site of stimulation. The fact that so far this phenomenon was observed in only 2 goats does not imply that it is a rare phenomenon. Given the low amplitude of the potentials and the various return pathways, it is quite likely that, due to the low spatial resolution of the implanted electrodes reentry in BB was not detected in the other animals.

DISCUSSION

Main Findings

In the present study we evaluated the effects of the I_{Kr} blocker dofetilide on atrial refractoriness at multiple atrial sites in non-remodeled atria and after 48 hours of AF. It was demonstrated that the class III action of dofetilide was stronger at areas with a longer refractory period. As a result, the spatial dispersion of refractoriness increased due to preferential prolongation of the AERP at Bachmann's bundle. In remodeled atria this caused a widening of the window of inducibility, which was due to a stronger prolongation of the upper than the lower limit of inducibility. Premature beats were associated with local conduction delays at the mid portion of Bachmann's bundle. After infusion of dofetilide these critical conduction delay already occurred at longer coupling intervals, because of the prolonged refractory periods at Bachmann's bundle. Mapping of initiation of AF suggested that longitudinal dissociation in Bachmann's bundle was the mechanism of initiation of AF.

Action Potential Dependence of Dofetilide

Recently we demonstrated that AF-induced electrical remodeling alters the atrial sensitivity for K^+ channel blockers. Wijffels et al. were the first to show that after cardioversion of persistent AF in the goat, *d*-sotalol failed to prolong the atrial refractory period.¹⁴ More recently it was established that this loss of efficacy was lost in remodeled atria.^{9,10} In addition, the well known reverse frequency dependent action of I_{Kr} blockers was lost in remodeled atria. Tse et al. showed that this also occurs in humans.¹⁵ *DL*-sotalol prolonged atrial refractoriness less after electrical cardioversion in patients compared to in normal sinus rhythm. Also the reverse-frequency dependency of *dl*-sotalol was lost in AF-patients.

These observations can be explained by the hypothesis that the action of I_{Kr} blockers is dependent on the duration of the action potential. In the present study we evaluated this hypothesis by measuring the effects of dofetilide on the atrial refractory period at multiple atrial sites, before and after electrical remodeling. In agreement with the hypothesis we observed that the prolongation of the AERP by dofetilide was stronger at sites with long AERPs (mid Bachmann's bundle). Also the reverse frequency dependence of dofetilide was preserved at BB but not at RA or LA. This may

be explained by the fact that, contrary to RA and LA, in remodeled atria the refractory period was at Bachmann's bundle still longer at slow than at fast pacing rates.

The ionic mechanisms of these observations are not completely understood. In a mathematical model of the human atrial action potential it was demonstrated that inhibition of I_{Kr} caused a smaller prolongation of the remodeled action potential than the normal action potential (+105 versus 32 ms).¹¹ Interestingly, it was also shown that the net effect of I_{Kr} blockade was dependent on the shape of the action potential. In spike-and-dome like action potentials, blockade of I_{Kr} caused a much stronger prolongation of the action potential duration than in triangular action potentials.¹¹

Only a few studies have looked at regional effects of I_{Kr} blockers (or other drugs) in the atria. Li et al. showed that in the dog dofetilide caused a larger increase in ERP in the left than in the right atrium.¹⁶ This was believed to be due to a larger I_{Kr} current in left atrial cells. In the present study we did not observe differences in class III effect in the right or left atrium. The reason for this discrepancy is unclear but may be due to important differences in K^+ currents between species.^{17,18} In contrast to the dog,¹⁶ in the goat also the refractory period in the left and right atrium are not different.

Arrhythmogenic Properties of Bachmann's Bundle

The present study revealed some interesting aspects of Bachmann's bundle. First of all, again we demonstrated that the caprine atria exhibit a distinct regional distribution of refractoriness, characterized by short refractory periods at the right and left atrial free walls and a longer refractory period at the mid-portion of BB.⁴ This was true both in non-remodeled as well as remodeled atria (after 48 hour of AF). Also in other species this has been described. Hara et al. showed that in the dog the action potential recorded from Bachmann's bundle had a longer duration than in cells of right atrial trabecular cells.¹⁹ Whereas right atrial trabecular action potentials were triangular in shape, at Bachmann's bundle typical spike-and-dome potentials were seen. Also in humans, Chen et al. found the longest AERP at Bachmann's bundle.²⁰

We observed that the degree of AF-induced shortening of atrial refractoriness was significantly less at Bachmann's bundle than in the right or left atrial free wall. At Bachmann's bundle the normal rate adaptation of the refractory period was maintained after 48 hours of AF. This is in agreement with the study of Fareh et al. who found less electrical remodeling at Bachmann's bundle in the dog.²¹ It is not clear why AF-induced electrical remodeling is different at BB. One explanation could be that the ionic remodeling (downregulation of $I_{Ca,L}$ ²²) is less. It is also possible that the long AERP at Bachmann's bundle causes some degree of entrance block of fibrillation waves entering from the right or left atrial free walls. Because of this, the rate of fibrillation at BB may be lower than at of the right or left free wall.

Bachmann's bundle has also unique structural properties. Besides, getting inputs from the right and left atrium and the atrial septum, it exhibits a high degree of tissue anisotropy.²³ It can be postulated that the fractionation of the electrograms recorded from Bachmann's bundle during premature beats, is caused by electrical

dissociation of the parallel oriented muscle bundles. Dolber and Spach showed that in contrast to longitudinal conduction along Bachmann's bundle which was associated with smooth biphasic extracellular potentials, during transverse conduction polyphasic potentials with multiple small peaks and troughs were recorded.²³

The combination of a long refractory period together with a high degree of tissue anisotropy make Bachmann's bundle a potentially important factor in the initiation and perpetuation of AF. With regard to the initiation of AF, we found that premature stimuli were associated with critical conduction at Bachmann's bundle and initiation of AF. When the AERP at Bachmann's bundle was prolonged by dofetilide, the upper limit of inducibility increased accordingly. The role of Bachmann's bundle in the perpetuation of AF is still less clear. However, in a recent study we observed that cardioversion of persistent AF by the class IC drugs was associated with a reduction in the degree of fragmentation at Bachmann's bundle.²⁴ It was hypothesized that slowing of the rate of fibrillation (without affecting refractoriness) increases the chance that parts of the atria with longer refractory period can also follow the fibrillation frequency in a 1:1 fashion. When this occurs, AF may convert either to sinus rhythm or to atrial flutter. The observation in the present study that dofetilide shortened the duration of AF, despite preferential prolongation of the AERP at Bachmann's bundle, appears to contradict this hypothesis. However, at the higher pacing rates (200 ms) the degree of AERP prolongation was not different at the RA, LA or BB. Thus at the pacing cycle length closest to the rate of AF, dofetilide did not increase the dispersion of refractoriness.

Limitations

An important limitation of the present study is that we did not record the electrical activity of the atrial septum. Therefore activation maps during initiation of AF do not allow distinction between macro-reentry involving the atrial septum or micro-reentry within Bachmann's bundle.

We would like to emphasize that the findings cannot be directly translated to the human situation. There are important differences in atrial electrophysiology between the goat and humans. In humans the action potential is longer than in the goat and also the degree of electrical remodeling is less.^{12,25} This may be explained by the different distribution of cardiac ion channel between species.^{17,18} Therefore it is quite likely that the observed drug effects may be different in man than in the goat.

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CHAPTER 8

Future Perspectives

WHY RHYTHM CONTROL?

In 2002, the results of two large randomized clinical trials were published in which two treatment strategies for AF were compared.^{1,2} One strategy was aimed at restoring and maintaining sinus rhythm (rhythm control). In the other, the goal was to reduce symptoms by lowering the ventricular heart rate during AF (rate-control). In both the North American AFFIRM-Study (Atrial Fibrillation Follow-Investigation of Rhythm Management)¹ and the Dutch RACE- (RAte Control versus Electrical cardioversion)² it was demonstrated that the overall mortality was not different between rate and rhythm control. In the AFFIRM-trial there was a trend towards increased mortality in the rhythm control arm. The results of these studies showed that at present, rate control is (at least) equally effective in treating AF as rhythm control. In a recent guideline of the American Academy of Family Physicians and the American College of Physicians published in the *Annals of Internal Medicine* it was stated that 'Rate control with chronic anticoagulation is the recommended strategy for the majority of patients with atrial fibrillation'.³ As a result, the number of patients referred for pharmacological cardioversion of AF has clearly decreased. This is illustrated in figure 1. Shown is the number of patients referred for chemical cardioversion between 2001 and august 2003 in a phase III study which evaluated the prophylactic efficacy of a new anti arrhythmic drug. After the simultaneous publication of the RACE and AFFIRM studies (arrow) there was a drop in the number of patients referred for cardioversion.

However in the aftermath of these trials several important issues have been raised. In both the RACE and AFFIRM trial it became clear that the current treatment strategies for maintaining sinus rhythm are far from optimal. In RACE, despite the use of anti-arrhythmic drugs, the prevalence of sinus rhythm was only 39% after 2.3 years of follow up. Anti-arrhythmic drugs were also often associated with adverse effects, causing drug discontinuation. In a sub-study from the AFFIRM-study it was found that after one year treatment 12% of patients on amiodarone, 11% on sotalol and 28% of patients using class I drugs stopped taking their drugs due to side effects.⁴ In light of these findings it seems reasonable to raise the question whether the results of the AFFIRM and RACE could have yielded different results with a safer and

Number of patients referred for pharmacological cardioversion of AF. Between 2001 and february 2002 approximately 25 patients underwent a pharmacological cardioversion each month. After the publication of RACE and AFFIRM (arrow) the number of patients decreased markedly. (Courtesy H Crijns)

more effective rhythm control strategy. It is interesting to note that in patients in whom sinus rhythm could be effectively restored and maintained, quality of life improved and long term survival was actually better.^{1,5,6} In a non-randomized retrospective analysis, Pappone et al. showed that patients who underwent successful catheter ablation of AF by circumferential isolation of the pulmonary veins had a lower rate of cardiovascular death when compared to patients receiving anti-arrhythmic drugs.⁷ Thus, although the RACE and AFFIRM study have clearly shown that at this point in time, rhythm control is not superior, this should not discourage the development of better drugs for rhythm control. The AFFIRM investigators concluded that 'if an effective method for maintaining sinus rhythm with fewer side effects were available, this might improve survival'.⁶

Clinical Implications of Atrial Selective Drugs

In 1996, Li et al. from the group of Nattel investigated the expression of K^+ currents in human explanted hearts.⁸ They demonstrated that the ultra-rapid delayed rectifier current (I_{Kur}) was present in the atrium, but absent in the ventricle. The possible clinical relevance was stressed by the statement: "Since I_{Kur} plays an important role in human atrial repolarization and is absent in human ventricle, it is a potentially promising target for the development of drugs that prevent reentrant atrial arrhythmias without a risk of ventricular proarrhythmia".⁸

At present, several pharmaceutical companies are developing compounds that block the I_{Kur} current.⁹⁻¹¹ Of note, none of these compounds is a pure I_{Kur} blocker, other K^+ currents are affected as well. In the present thesis we describe the pre-clinical results of AVE0118 in the goat model of AF. This drug was synthesized by Aventis

pharma Germany and in-vitro studies demonstrated a selectivity for the repolarizing currents I_{Kur} , I_{to} and I_{Kach} .¹⁰ In the beginning of 2002 the first experiments with AVE0118 were performed in a chronic goat model of persistent AF. Infusion of AVE0118 caused a marked prolongation of the atrial fibrillation cycle length, the arrhythmia terminated and the drug was well tolerated. In subsequent experiments we observed the following: first of all, AVE0118 has an atrial selective action, in the atrium the refractory period was markedly prolonged, in the ventricle the QT interval was not changed. Secondly, AVE0118 exerted an enhanced action in remodeled atria (Chapter 3). This was rather surprising, since we previously demonstrated that *d*-sotalol and ibutilide, blockers of the 'late' activated I_{Kr} current, actually had a reduced effect after AF (Chapter 2). Thirdly, in goats with persistent AF lasting for more than 1 month, AVE0118 still exerted a strong class III effect and AF was terminated in up to 70% of the animals. Finally, we showed that combined administration of AVE0118 could restore the action of I_{Kr} blockers in electrically remodeled atria and in goats with persistent AF, the arrhythmia was easily terminated (Chapter 4).

Altogether these properties make AVE0118 a very promising drug for the treatment of atrial fibrillation. In May 2004 the first clinical phase II study with AVE0118 has been started. In this multi-center trial (principal investigator Harry Crijns) the safety and cardioversion efficacy of AVE0118 is evaluated.

Although the pre-clinical results in the goat (Chapter 3) and in the pig¹² suggest that AVE0118 may be a very potent anti-arrhythmic agent, there are several reasons why the efficacy may be different in man. First of all, important differences exist in atrial electrophysiology between the goat and man. For example, the right atrial refractory period in man is ~ 250 ms^{13,14} whereas in the goat it is only 150 ms. After AF-induced electrical remodeling, AERP shortens to ~ 200 ms^{13,14} in man whereas in the caprine atria the AERP will become as short as 100 ms. Since we observed the strongest effects of AVE0118 at short AERPs, it is uncertain whether the same effects will be seen in patients with long AERPs. Another important difference is that the substrate of atrial fibrillation is very dissimilar in man than in goats. In the present study we used healthy goats without underlying pathology. Of note, contrary to the human situation, in none of these animals spontaneous episodes of AF occurred. Only after a prolonged period of repetitive burst pacing, the animals developed persistent AF. This is very different from the human situation where atrial fibrillation is often seen in patient with concomitant underlying heart disease. The atria of these patients are very prone to AF, and the first episode may already cause persistent AF. In this respect, the results of the clinical trial will also tell us something about the value of the goat model of 'lone' AF for evaluating the clinical efficacy of novel anti-arrhythmic drugs.

Pending the results of the clinical trial we can only speculate on the possible clinical implications of these novel atrial selective anti-arrhythmic agents. However, if we assume the best case scenario that also in humans AVE0118 is safe and at least equally effective as currently available drugs, this may have important consequences. First of all, atrial selective drugs will become the first therapy of choice for pharmacological cardioversion of atrial fibrillation. In addition to that, it may be

possible to enhance the cardioversion efficacy of these drugs by additional infusion of a low dosage of I_{K_r} blocker such as *dl*-sotalol, ibutilide or dofetilide. Off course, before using such a strategy, additional studies are required to evaluate safety. However in the preclinical model this combination of AVE0118 and I_{K_r} blockers was such a potent chemical defibrillator, that it is tempting to speculate that it may be an alternative for DC cardioversion of persistent AF. Another important indication would be the prevention of AF recurrences by oral administration of atrial selective drugs. In case this strategy fails it may even be possible to co-administer a small dosage of oral *dl*-sotalol or dofetilide. Using this drug-regime it may be possible to prolong the AERP to such an extent that premature beats will not be able to initiate AF. In addition, if AF occurs, the potent combination of 'early' and 'late' class III agents may prevent the development of persistent AF.

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Samenvatting

Boezemfibrilleren (ook wel atriumfibrilleren genoemd) is een hartritmestoornis die bij veel mensen voorkomt. Met name bij patiënten met een hoge bloeddruk, een lekkende hartklep of een doorgemaakt hartinfarct komt boezemfibrilleren frequent voor. De incidentie van boezemfibrilleren neemt toe met de leeftijd. Bij mensen van 60 jaar heeft 4% van de bevolking de ritmestoornis, bij een leeftijd ouder dan 80 jaar is dit 10%. Door de toenemende vergrijzing van de bevolking zal boezemfibrilleren in de toekomst een steeds belangrijker medisch, maar ook – gezien de kosten van de behandeling - economisch probleem worden.

Tijdens boezemfibrilleren worden de boezems met een zeer hoge frequentie (tot wel 600 slagen per minuut) geactiveerd. Het merendeel van deze elektrische impulsen wordt gelukkig niet voortgeleid naar de kamers, maar wordt grotendeels tegengehouden door de atrioventriculair knoop (de verbinding tussen de boezems en de kamers). Toch zal de hartfrequentie bij een patiënt met boezemfibrilleren veel hoger zijn dan die bij een patiënt met een normaal hartritme (sinus ritme). Atriumfibrilleren veroorzaakt klachten als hartkloppingen, vermoeidheid en kortademigheid. De belangrijkste complicatie van boezemfibrilleren is echter het optreden van beroertes. Dit wordt veroorzaakt doordat er een verhoogde neiging bestaat tot stolselvorming in de boezems. Wanneer deze stolsels stoomafwaarts naar de hersenen doorschieten veroorzaakt dit een CVA.

Het belangrijkste doel van de behandeling van boezemfibrilleren is het verhelpen van de klachten en het voorkomen van CVA's. Dit kan op verschillende wijzen worden bereikt. Ten eerste kunnen de klachten verdwijnen door het normale hartritme (sinus ritme) te herstellen met behulp van medicijnen of een elektrische schok. Deze behandelingsstrategie wordt ook wel 'ritme-controle' genoemd. Een andere behandelingsoptie is de snelle ventrikelfrequentie te vertragen door medicijnen ('frequentie-controle'). In dat geval blijft het atriumfibrilleren echter bestaan. Thrombo-embolische complicaties kunnen grotendeels worden voorkomen door gebruik van bloedverdunders.

Het laatste decennium zijn er talloze niet-medicamenteuze therapieën ontwikkeld die erop gericht zijn om boezemfibrilleren te bestrijden. Op dit moment lijkt echter alleen ablatie van de longvenen een belangrijke plaats te verwerven in het huidige arsenaal aan behandelingsmogelijkheden. Dit is echter een zeer tijdrovende procedure en gezien het grote aantal patiënten met boezemfibrilleren is deze behandeling voorlopig niet voor iedereen weggelegd. De hoeksteen van de behandeling van boezemfibrilleren blijft derhalve het gebruik van medicijnen. Het grote voordeel van medicijnen is dat ze makkelijk te gebruiken zijn en bovendien zijn ze voor veel patiënten toegankelijk. Op dit moment zijn er diverse van deze zogenaamde anti-aritmica beschikbaar. Het werkingsmechanisme waarmee deze middelen sinus

ritme kunnen herstellen is echter zeer verschillend. Zo zijn er zogenaamde klasse 1C anti-aritmica die met name de geleidingsnelheid in het atrium vertragen door blokade van het natriumkanal. Andere medicijnen blokkeren kalium kanalen (I_{K_r}) en kunnen boezemfibrilleren opheffen door een verlenging van de atrial refractaire periode (klasse 3 effect). Helaas kleven er een aantal belangrijke nadelen aan de huidige beschikbare medicijnen. Zo is in verschillende klinische studies gebleken dat anti-aritmica hun effectiviteit verliezen als de ritmestoornis langer dan een aantal dagen bestaat. Bovendien kan het gebruik van anti-aritmica in sommige patiënten leiden tot ernstige ritmestoornissen van de kamers. Het is dus van groot belang voor de behandeling van boezemfibrilleren dat er nieuwe medicijnen worden ontwikkeld die nog effectief zijn als de ritmestoornis lang bestaat en die bovendien veiliger zijn. In dit proefschrift hebben wij in een diersmodel van boezemfibrilleren onderzocht wat de effecten zijn van het langdurig bestaan van boezemfibrilleren op de effecten van diverse anti-aritmica. Wij hebben niet alleen gekeken naar bestaande medicijnen, ook hebben wij een aantal nieuwe experimentele medicijnen getest.

Bij experimenteel onderzoek naar boezemfibrilleren wordt veel gebruik gemaakt van proefdieren waarbij de ritmestoornis kunstmatig wordt opgewekt. In hoofdstuk 7 wordt een overzicht gegeven van de verschillende diersmodellen. Al aan het einde van de 19^e eeuw was bekend dat het mogelijk was om boezemfibrilleren op te wekken door een geringe hoeveelheid elektrische stroom toe te dienen op de boezems. In de loop der tijd zijn er diverse modellen ontwikkeld die hebben bijgedragen aan een beter begrip van de onderliggende mechanismen van boezemfibrilleren. Alle experimenten die in dit proefschrift worden beschreven zijn uitgevoerd in geiten met boezemfibrilleren. Tijdens een open-hartoperatie werden electrodes aangebracht op de linker en rechter boezem en op de inter-atriale spier (Bachmann's bundel). Met gebruik van een versterker en een computergestuurde stimulator was het mogelijk om op elke electrode de elektrische activiteit af te leiden, en elektrische stroompjes toe te dienen. Nadat de borstkas weer was gesloten, en er adequate pijnstilling was toegediend, konden de geiten gedurende 3 tot 4 weken herstellen in de stal. Vervolgens werd boezemfibrilleren opgewekt met een fibrillatie pacemaker. Deze pacemaker kan boezemfibrilleren induceren door de atria gedurende 1 seconde te stimuleren met een frequentie van 3000 slagen per minuut. Op deze manier was het mogelijk om gedurende 24 uur per dag, 7 dagen per week boezemfibrilleren te induceren en te onderhouden. Het gevolg hiervan was dat de ritmestoornis in het begin nog kortdurend was (enkele seconden), maar na enkele dagen duurden de episoden steeds langer en uiteindelijk (na ongeveer 2 weken) stopte de ritmestoornis niet meer vanzelf (persistent boezemfibrilleren). Het mechanisme dat hieraan ten grondslag ligt is een verkorting van de atriale refractaire periode (repolarisatie-tijd). Dit fenomeen wordt wel elektrische remodelering genoemd. De atriale refractaire periode representeert de tijd dat een cel niet meer prikkelbaar is nadat hij kort tevoren is geactiveerd. Des te korter de refractaire periode, des te vaker kan een hartcel worden geactiveerd en des te kleiner is de kans dat de ritmestoornis spontaan stopt.

In hoofdstuk 2 hebben wij onderzocht of het mogelijk is om elektrische remodelering te voorkomen, of teniet te doen, door de geneesmiddelen EMD87505 en EMD125021 toe te dienen. Deze medicijnen zijn remmers van de natrium-waterstof uitwisselaar. Het onderzoek toonde aan dat de medicijnen de verkorting van de atriale refractaire periode niet konden beïnvloeden. Het maakte geen verschil of de medicijnen werden toegediend tijdens, of na het proces van elektrische remodelering. De conclusie van dit onderzoek is dat de natrium-waterstof uitwisselaar waarschijnlijk geen belangrijke rol speelt bij de verkorting van de atriale refractaire periode die optreedt tijdens boezemfibrilleren. In hoofdstuk 3 wordt beschreven wat de effecten zijn van elektrische remodelering op de werking van het klasse 1C medicijn flecaïnide en de klasse 3 anti-aritmica *d*-sotalol en ibutilide. Deze medicijnen worden in de dagelijkse klinische praktijk veel gebruikt om boezemfibrilleren op te heffen. De resultaten van het onderzoek lieten zien dat het werkzame effect van flecaïnide bleef bestaan na 2 dagen boezemfibrilleren. Daarentegen, de effecten van *d*-sotalol en ibutilide waren afgenomen. In normale atria werd -zoals verwacht- een duidelijke verlenging van de refractaire periode waargenomen. Na slechts 2 dagen atriumfibrilleren was dit effect echter nagenoeg verdwenen. De verlenging van de ventriculaire repolarisatie was onveranderd. Dit impliceert dat atriale elektrische remodelering de klasse 3 effecten van I_{Kr} blokkers sterk doet afnemen. Dit is een mogelijke verklaring voor het verlies van effectiviteit van deze medicijnen zoals in de klinische praktijk wordt waargenomen.

Hoofdstuk 4 beschrijft de electrofysiologische effecten van een nieuw experimenteel klasse 3 medicijn (AVE0118). Dit medicijn heeft een ander aangrijpingspunt dan de huidige beschikbare klasse 3 anti-aritmica. Het blokkeert kalium kanalen zoals I_{Kur} , I_{to} en I_{Kach} . Deze kanalen worden vroegtijdig tijdens de repolarisatie geactiveerd en zijn zeer belangrijk in de atria en in mindere mate in de ventrikels. De verwachting is dan ook dat deze middelen een selectieve anti-aritmogene werking kunnen uitoefenen op de atria. De experimenten lieten zien dat het mogelijk was om de atriale refractaire periode te verlengen zonder de ventriculaire repolarisatie (de QT tijd) te beïnvloeden. Bovendien was de verlenging van de atriale refractaire periode toegenomen in elektrisch geremodeleerde atria. Het effect was zo sterk, dat de verkorting van de refractaire periode zoals die werd gezien na 48 uur atriumfibrilleren, volledig ongedaan was gemaakt. Verder bleek het mogelijk om in het merendeel van de geiten met persistent boezemfibrilleren de ritmestoornis te beëindigen. In hoofdstuk 5 hebben wij getest of gelijktijdige toediening van conventionele klasse 3 anti-aritmica (dofetilide en ibutilide) en AVE0118 een sterkere verlenging van de refractaire periode geeft dan de som van de separate effecten. De achtergrond van deze hypothese was de bevinding in hoofdstuk 3 dat de werking van 'late' kalium kanaal blokkers afneemt als de refractaire periode was verkort door elektrische remodelering. Wij postuleerden dat als de refractaire periode verlengt na toediening van AVE0118 (hoofdstuk 4), dan neemt de werkzaamheid van de 'late' klasse 3 anti-aritmica weer toe. Gelijktijdige toediening van dofetilide of ibutilide en AVE0118 zou derhalve een sterkere verlenging van de refractaire periode bewerk-

stelligen in geremodeleerde atria. De resultaten toonden dat de combinatie van 'vroeg' en 'late' klasse 3 anti-aritmica inderdaad een synergetisch effect had in electrisch geremodeleerde boezems. Daarentegen, in normale boezems werd een additieve verlenging gezien. Vervolgens hebben wij gekeken of de combinatie van vroeg en late klasse 3 anti-aritmica ook kan worden gebruikt om langdurig boezemfibrilleren te termineren. Gebruikmakend van de hoogste dosering van AVE0118 en dofetilide of ibutilide was het mogelijk om in alle geiten het boezemfibrilleren op te heffen. Dit ging gepaard met een synergetische verlenging van de fibrillatie cyclustlengte. Concluderend kan worden gesteld dat de combinatie van een vroeg en late klasse 3 medicatie een zeer sterk anti-aritmogeen effect bezit. Dit kan van groot belang zijn voor de medicamenteuze behandeling van boezemfibrilleren.

In hoofdstuk 6 onderzoeken wij of de regionale distributie van de atriale refractaire periode kan worden beïnvloed door de activatie-sequentie te veranderen. Het fenomeen dat de atriale repolarisatie afhankelijk is van de activatie-sequentie wordt ook wel 'atriaal geheugen' genoemd. In zes geiten, waarbij verschillende electrodes op verschillende plaatsen in het atrium waren aangebracht, werd de atriale activatietijd en refractaire periode bepaald na een periode van langdurig atriaal pacen (150bpm) op het rechter en linker atrium. De resultaten lieten zien dat tijdens normaal sinus ritme er een inverse relatie bestond tussen de activatietijd en de refractaire periode. Met andere woorden, des te eerder een plaats geactiveerd werd, des te langer de refractaire periode. Daarentegen, als de activatie-sequentie werd veranderd door het atrium te activeren vanuit de rechter- of linker-boezem, dan leidde dat niet tot een verandering van de distributie van de refractaire periode. Dit suggereert dat het atrium geen 'atriaal geheugen' heeft. In Hoofdstuk 7 onderzoeken wij of de I_{Kr} -blokker dofetilide een preferentiële verlenging van de atriale refractaire periode geeft op de bundel van Bachmann. De achtergrond van deze hypothese was de observatie in hoofdstuk 3 dat I_{Kr} blokkers minder effect hebben als de refractaire periode is verkort door atriale electrische remodelering. Vice versa, op plaatsen met een lange refractaire period (zoals bijvoorbeeld Bachmann's bundel), zou dofetilide dan juist een toegenomen werking moeten hebben. In negen geiten hebben we de effecten gemeten van dofetilide op de atriale refractaire periode op 7 verschillende plaatsen, zowel voor als na een periode van 48 uur boezemfibrilleren. Het klasse 3 effect van dofetilide was inderdaad het grootst op plaatsen met de langste refractaire periode (op de bundel van Bachman). Daarentegen, op plaatsen met een korte refractaire periode was het effect van dofetilide slechts zeer gering. Hierdoor ontstonden er toegenomen regionale verschillen in refractaire periode. Dit bleek ook geassocieerd te zijn met een verhoogde vatbaarheid voor atriumfibrilleren. Mapping toonde longitudinale dissociatie in Bachmann's bundel tijdens initiatie van atriumfibrilleren. Concluderend, in dit hoofdstuk wordt aangetoond dat verlenging van de refractaire periode door klasse 3 anti-aritmica ook een paradoxaal pro-aritmogeen effect kan hebben door een preferentiële verlenging van de atriale refractaire period op de bundel van Bachmann.

IMPLICATIES VAN HET ONDERZOEK

De laatste jaren zijn er verschillende klinische studies gepubliceerd die suggereren dat bij de behandeling van atriumfibrilleren het geen verschil maakt of een patiënt behandeld wordt met een 'ritme-' of 'frequentie-controle' strategie. De mensen zullen evenlang leven en ook de klachten van patiënten zijn gelijk in beide groepen. Echter de meeste huidig beschikbare medicamenten die worden gebruikt voor 'ritme-controle' zijn weinig effectief en bovendien hebben zij soms vervelende bijwerkingen. Het is derhalve van groot belang dat er nieuwe effectieve en veilige medicijnen worden ontwikkeld waarmee boezemfibrilleren kan worden bestreden. In dit proefschrift hebben wij gekeken naar de werking van 'nieuwe' en 'oude' anti-aritmica in een pre-klinisch geitenmodel van boezemfibrilleren. Het onderzoek in dit proefschrift geeft inzicht in de werking van anti-aritmica in geremodeleerde atria. De experimentele bevindingen met nieuwe atrium specifieke medicijnen suggereren dat deze groep medicijnen ook van waarde kan zijn bij de farmacologische behandeling van boezemfibrilleren. Uiteraard zal dit nog in een klinische studie onderzocht moeten worden.

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Curriculum Vitae

Yuri Blaauw werd geboren op 7 november 1972 in Lelystad. In 1992 behaalde hij het eindexamen atheneum aan de Scholengemeenschap Lelystad. Van 1992 tot 1993 studeerde hij geschiedenis aan de Rijksuniversiteit Groningen. In 1993 startte hij de studie geneeskunde aan dezelfde universiteit. Na een keuze-coschap cardiologie in het St. Georges Hospital Medical School te Londen (prof. AJ Camm) werd het artsexamen in 2000 behaald. Tijdens zijn studie was de auteur betrokken bij experimenteel onderzoek naar de mechanismen van boezemfibrilleren. Dit onderzoek werd verricht onder leiding van dr. RG Tieleman en prof. dr. HJGM Crijns. Tevens was hij lid van de eerste redactie van de studenten-editie van het Nederlands Tijdschrift voor Geneeskunde. In 2000 verkreeg de auteur een Zon-MW AGIKO-stipendium. In het kader hiervan werd in juni 2000 gestart met experimenteel onderzoek bij de vakgroep fysiologie aan de Universiteit Maastricht in het laboratorium van prof. dr. MA Allessie. In juni 2004 werd begonnen met het klinische deel van de opleiding cardiologie in het academisch ziekenhuis Maastricht bij opleider prof. dr. HJGM Crijns.

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