Dynamics of propagation patterns and anti-arrhythmic mechanisms during atrial fibrillation

Citation for published version (APA):

van Hunnik, A. (2019). *Dynamics of propagation patterns and anti-arrhythmic mechanisms during atrial fibrillation*. [Doctoral Thesis, Maastricht University]. ProefschriftMaken Maastricht. <https://doi.org/10.26481/dis.20190110ah>

Document status and date:

Published: 01/01/2019

DOI:

[10.26481/dis.20190110ah](https://doi.org/10.26481/dis.20190110ah)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

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Dynamics of Propagation Patterns and Anti-Arrhythmic Mechanisms during Atrial Fibrillation

Arne van Hunnik

Dynamics of propagation patterns and anti-arrhythmic mechanisms during atrial fibrillation

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Dynamics of propagation patterns and anti-arrhythmic mechanisms during atrial fibrillation

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
op gezag van de Rector Magnificus, Prof.dr. Rianne M. Letschert
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op donderdag 10 januari 2019 om 12.00 uur

door

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Dit proefschrift is tot stand gekomen met dank aan subsidies verleend door Nederlandse organisaties en de Europese unie. De subsidiegevers en projecten staan hieronder vermeld.

Cardiovasculair onderzoek Nederland (CVON)

Reappraisal of Atrial Fibrillation: Interaction between hypercoagulability, electrical remodeling, and vascular destabilisation in the progression of AF

De Europese unie:

The international training network AFibTrainNet

The international training network RADOX: RADical reduction of OXidative stress in cardiovascular diseases

The ERACoSysMED H2020

The Center for Translational Molecular Medicine (CTMM)

Project COHFAR

Enkele andere overwegingen

Hoe zal ik dit uitleggen, dit waarom
wat wij vinden niet is
wat wij zoeken?

Laten we de tijd laten gaan
waarheen hij wil,

en zie dan hoe weiden hun vee vinden,
wouden hun wild, luchten hun vogels,
uitzichten onze ogen

en ach, hoe eenvoudig zijn raadsel vindt.

Zo andersom is alles, misschien.
Ik zal dit uitleggen.

Rutger Kopland (1934)

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Chapter 1

General introduction

What is AF?

“The worst is the continuous pounding within my body. It is like I am shook up all over. I feel like I am always close to an attack and this makes me very restless as well.”¹ “Last Monday I was so ill and drowsy and I had to vomit.² I had a bad heart rhythm”. These are just two quotes on a patient forum on the web (www.atriumfibrilleren.nl) illustrating the potential mental and physical burden of atrial fibrillation (AF). In The Netherlands about 200.000 patients have been diagnosed with AF, according to the Dutch Heart Foundation. Another patient: “On hindsight, I think I was affected already for 6 to 8 year”.³ Indeed, AF may come unnoticed as patients might experience very short AF attacks or are just asymptomatic.⁴ Therefore, the Dutch Heart Foundation estimates another 100.000 patients who are not yet aware having AF.

To understand what AF is we need to zoom in on the electrical activity of the heart. During a normal heart beat (sinus rhythm) an electrical wavefront is generated in the cells of the sinus node. This impulse is then propagated to the atrium, where a faster electrical depolarizing wavefront spreads into all directions of the atria initiating atrial contraction. The AV-node is responsible for the transfer of the wavefront towards the ventricles for a similar activation and contraction. After this chain of events a pause occurs during which no electrical depolarizing wavefront is present. During AF, multiple wavefronts propagate over the atrial wall continuously. The atrial electrical activity is not only fragmented in multiple independent wavefronts but activations follow each other up with very short and variable intervals, hence at a high frequency. Fortunately, the AV-node acts as a frequency filter and reduces the number of activations sent to the ventricle but it cannot prevent high and irregular ventricular rates. The fast and irregular rhythm results in a loss of rate adaptation and may reduce exercise capacity, which may disable a patient to a large extent.⁵

Ectopic activity in the pulmonary veins is often responsible for AF initiation. The consequent AF episode may then vary in duration. Most patients will at first experience short AF episodes, but the natural course of AF leads to more frequent AF episodes that last longer.⁶ This progressive stabilization of AF is associated with fibrosis, hypertrophy, and dilatation of the atrial myocardium, which are also commonly seen as a consequence of different comorbidities. AF is rarely manifested as a lone entity but is associated with comorbidities that affect cardiac function (e.g. heart failure, valvular disease), vascular function (e.g. coronary artery disease), and systemic triggers for inflammation and ischemia (e.g. diabetes mellitus, obesity and sleep apnoea).⁷⁻⁹ AF stability is also affected by AF induced adaptations of the atrium, often referred to as remodelling. Structural remodelling, (fibrosis, cellular hypertrophy and dilatation) is increased gradually with prolonged duration of AF in animal models with induced AF.¹⁰⁻¹² These changes disturb the homogeneous propagation of an atrial wavefront and contribute to more individual wavefronts and higher complexity of conduction patterns during AF.^{11,13} More detailed assessment of fibrosis also revealed that fibrosis mainly accumulates close to the epicardial wall.¹¹ It

is hypothesized that epicardial fibrosis is likely to lead to dyssynchrony between the endo- and epicardial layer, increasing AF duration.^{14,15} Endo-epicardial dyssynchrony is an additional factor contributing to the number in wavefronts and complexity of propagation patterns. Electrical remodelling is a type of AF-induced adaptation that facilitates AF stabilization in the first days after AF initiation.^{16,17} The high frequency during AF results in shortening of the refractory period and an increase of AF frequency. Both lead to more complex propagation patterns.¹⁸

Most antiarrhythmic drugs prolong refractory period and thereby terminate AF. Prolongation of the refractory period will slow down fibrillation frequency and destabilize AF. The underlying cause of the shortened action potential after electrical remodelling is due to a shift in the depolarizing and repolarizing currents in the atrial myocyte.¹⁷ Specifically, the reduction of the depolarizing Ca^{2+} current ($I_{\text{Ca,L}}$), responsible for the plateau of the action potential, is reduced. The reduction of a calcium transient does not only affect action potential duration but also leads to a loss of atrial contractility.¹⁹ The loss of atrial contractility thought to, at least in part, attributed to the impaired ventricular function and increased stroke risk for AF patients.²⁰

Stroke is a major risk for AF patients and the strong association between stroke and AF is well recognized for a long time.²¹ About 30% of all strokes can be related to atrial fibrillation.²² The loss of atrial contractility is associated with stasis of blood within the atrial cavity resulting in thrombus formation in the atria found with echocardiography.²⁰ However, the other components of the Virchows triad, endothelial damage and hypercoagulability, are increased in AF patients too.²³ It is known for a long time that AF is a major cause of stroke. However, recent clinical trials demonstrated that stroke and AF are not necessarily associated in time^{24,25} suggesting a more complex causal interaction between AF and stroke.^{8,21} Work from our group suggests that indeed hypercoagulability is not only a consequence of AF but also potentially contributes to AF as it promotes structural remodelling and AF complexity. However, a more detailed discussion of this new perspective on AF is outside of the scope of this thesis.

Management of atrial fibrillation

If AF is left untreated it may lead to stroke²⁶ and even tachycardiomyopathy,²⁷ decreasing life expectancy.²⁸ A cornerstone of AF treatment is stroke prevention by anticoagulation, as this is the main threat of AF. It is claimed that anticoagulation therapy is the only therapy that unequivocally reduces mortality risk.²⁸ Ventricular rate management is the second pillar of treatment because untreated AF may lead to excessive heart rates of 150 beats per minute and more. Rate control, limiting the ventricular rate without the aim to terminate AF, is often an adequate therapy to reduce symptoms and can be achieved by β -blockers and other drugs.²⁹ However, restoring sinus rhythm may be of great relief for some, in general young, patients as expressed by the next patient: “...I got so tired after

exertion, and now Sotalol [a drug with combined β -blockade and antiarrhythmic properties] is exchanged by Tambocor [an antiarrhythmic drug]. Since then a new world opened up to me and I feel energetic again (almost like I used to be)."³⁰ Pharmacological treatment may be an appealing approach as safe administration of antiarrhythmic drugs (AAD) can be widely available. In some patients AADs may be used without the presence of a physician and is a cost-effective therapy.^{31,32} The principle action of most AADs is the inhibition of one or multiple ion currents relevant to the cardiac action potential. Antiarrhythmic drugs are classified into 4 groups depending on the type of current that is inhibited (sodium class I, potassium class III and calcium class IV) or its β -blocking ability (class II). According to the present ESC guidelines for the management of AF, the physician has the possibility to choose between class I and III AADs to terminate an AF episode. The inhibition of sodium current (class I) is effective in the treatment of AF and the mode of action is based on reduced excitability and conduction velocity, and refractory period prolongation. However, the CAST trial revealed that sodium current inhibitors are deleterious in patients with ischemic heart disease.³³ For this reason, the ESC 2016 guidelines advised to only prescribe class I drugs in patients without structural or ischemic heart disease.³⁴ The alternative approach is to target the repolarizing potassium currents (class III). However, ventricular pro-arrhythmias have also been reported for use of class III drugs.³⁵ Class III AADs may prolong the ventricular action potential possibly leading to early after depolarisations, Torsade's des Pointes, and ventricular fibrillation.³⁶ The alternative approach for acute AF termination is a well-timed electrical shock. This is a very effective method that can be used in all stages of AF progression but requires a hospital setting for controlled and safe execution. In other cases, rhythm control can be achieved by ablation of the pulmonary veins (PV).³⁴ However, ablation is an invasive and costly approach and is afflicted with potentially harmful procedural risks. In addition, many patients experience AF recurrences after an ablation procedure, in particular patients with persistent AF.³⁷ Therefore, many efforts are made to optimize ablation strategies.³⁸

Figure 1 Atrial action potential and the normalized inward and outward currents during the atrial action potential. Red current traces point out potential targets for the treatment of AF, details are discussed in the text. The arrow in the bottom highlights the phase where sodium calcium exchanger runs in the reverse mode leading to calcium entry. * currents targeted by vernakalant.

The new antiarrhythmic drug vernakalant

The risk of the initiation of ventricular arrhythmias changed the development of AADs for the treatment of AF. New AADs are now generally designed to target atrial-specific currents. Several currents that are predominantly expressed in the atrium have been proposed as potential targets for the treatment of AF.³⁹ **Figure 1** presents the different currents relevant to the atrial action potential morphology. Red current traces highlight those with an expected atrial specific effect. Probably the most investigated atrial-specific current is the voltage gated ultra-rapid delayed rectifier current (I_{Kur}) facilitated by the $Kv_{1.5}$ channel.⁴⁰ Several drugs have been developed to target I_{Kur} and demonstrated atrial selective antiarrhythmic potential in preclinical models of AF.^{40,41} Inhibition of I_{Kur} is also appealing because it may increase atrial contractility by promoting calcium entry through the sodium calcium exchanger (NCX).⁴² The NCX is a membrane protein that extrudes calcium out of the cell by exchanging 1 calcium ion with 3 sodium ions. The current density of this exchange is dependent on the electrochemical gradient and at sufficiently high membrane potentials it may operate in the reverse mode (calcium entry). Sufficient I_{Kur} inhibition elevates the “early” phase of the action potential, creating a higher driving force for calcium entry into the cell.⁴²

Vernakalant is a drug that targets I_{Kur} and has been approved for clinical use in Europe in 2010.⁴³ However, vernakalant is a multi-channel blocker that also inhibits other repolarizing currents (I_{to} , I_{Kr} and the atrial-selective $I_{K,Ach}$) and the fast sodium current in a use-dependent manner.⁴⁴ A small study in human volunteers demonstrated that vernakalant had atrial-preferential effects despite the multi-channel inhibiting properties.⁴⁵ Larger placebo controlled randomized trials, proved that vernakalant was effective in terminating AF episodes with a maximum duration of 7 days.^{46,47} Vernakalant may be considered for the termination of recent onset AF in the current ESC guidelines.³⁴

Based on the I_{Kur} inhibiting profile we hypothesized that vernakalant may have a positive inotropic effect on the atrium. In **chapter 2** we investigated this hypothesis in an AF model in goats. In this model, electrophysiological and contractile properties of the atrium were measured in conscious animals. We determined atrial contractility before and during vernakalant infusion. It has been reported that I_{Kur} inhibition has a larger effect on atrial refractoriness after electrical remodelling. We, therefore, performed experiments in goats before and after electrical remodelling to assess a possible potentiated effect.

The inhibition of established and repolarizing currents by vernakalant at a cellular level was well determined in previous investigations.⁴⁸ However, how these properties translate into electrophysiological behaviour in whole heart and intact body had not been explored yet. In **chapter 3** we investigated the effect of vernakalant on electrophysiological properties at different stages of AF induced remodelling. We addressed the relative class I and class III effects of vernakalant by studying their properties in comparison to a typical sodium channel blocker and an I_{Kur} blocker.

Mapping of atrial fibrillation

Mapping of the atria has been fundamental for the identification of mechanisms for the maintenance of cardiac arrhythmias. For instance, mapping of the atria has taught us that AF is often initiated by ectopic beats from the PV.⁴⁹ Ablation of the PV is an effective strategy to isolate triggers of AF but about 40% of all patients experience AF recurrence after 1-year follow-up.³⁷ This suggests that extra-PV triggers may in some patients initiate AF. However, different models may explain perpetuation of tachyarrhythmias and have been proposed for AF. These models are based on functional or anatomical re-entry, ectopy or multiple wavelets.^{50,51} Patterns that correspond with all 3 AF mechanisms have been observed by different research groups and with different techniques.⁵²⁻⁵⁶ It is heavily debated how the different patterns should be interpreted and whether specific patterns are truly perpetuating AF.^{57,58} Studying propagation patterns in the presence of an AAD may offer more mechanistic insights in AF perpetuation and the antiarrhythmic mechanisms of the drug. In **chapter 4** we present a study where we performed bi-atrial high-density mapping of propagation patterns before and during vernakalant infusion.

We investigated the dynamical behaviour of AF and investigated parameters that are associated with the different models of AF perpetuation.

It is commonly accepted that in many patients AF is maintained outside the PV. Different signal analysis tools have been developed to identify the AF “source(s)”. These sites may then serve as a valid target for ablation beyond isolation of the PVs. Dominant frequency and complex fractionated electrograms are two electrogram-derived parameters that are well investigated and were proposed to reflect specific mechanisms of AF.^{59,60} Dominant frequency would be associated with high frequency rotor activity leading to fibrillatory activity in the periphery. Fractionation would identify rotors or zones of frequent wave break. Clinical trials, however, did not show ubiquitous superiority of ablating these regions.⁶¹⁻⁶³ Other methods like voltage mapping, ripple mapping and non-invasive body surface mapping need further investigation to address their potential in mapping-guided ablation strategies.³⁸ The underlying assumption of these approaches is that a limited sample (<10 seconds) can accurately reflect the atrial substrate. Atrial sources are often considered to be a stable pattern that is stationary in space or frequently reoccurs.^{54,64} In **chapter 5** we investigated stationarity of AF parameters in space and time. We selected parameters that range from signal derived parameters up to propagation-based parameters. Furthermore, we investigated how stationarity of these properties relate to stable or recurrent propagation patterns. We performed these investigations in AF-recordings of long duration (10 minutes) and investigated stationarity between episodes. To visualize and quantify the dynamical behaviour of propagation patterns we developed a method that identifies recurrent patterns throughout a recording.

Assessment of AF dynamics with Focal Impulse and Rotor Mapping (FIRM) is a different approach to analyse electrograms recorded during AF.⁶⁵ Highly stable rotors in persistent AF patients have been reported with the use of this technique.⁵⁴ The procedure makes use of two catheter with 64 electrodes that are positioned in the cavity of the atria. A high coverage can be obtained because it is shaped as a sphere. Several reports indicate high success rates when FIRM-identified sources were ablated⁶⁶⁻⁶⁸ but later studies failed to reproduce these findings.⁶⁹⁻⁷³ The algorithm is not disclosed but it is reported that the electrograms are converted into a phase signal.⁶⁵⁻⁶⁷ Based on the low density of electrodes, potential poor contact and signal processing steps there are some concerns about the specificity of rotor identification with this method.^{74,75} In **chapter 6** we developed an algorithm to convert raw electrograms into a phase signal. Phase singularities, putative rotor identifiers, can then be detected based on the spatial organization of the phase. In this article, we explored the effect of electrode density and AF complexity on false detections of rotors. Based on these observations we propose a method that limits the degree of false positive rotor detections.

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2

Chapter 2

Vernakalant does not alter early repolarization or contractility in normal and electrically remodelled atria

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Europace. 2018. doi: 10.1093/europace/eux025

Abstract

Aims Besides the inhibition of the sodium inward current, vernakalant also inhibits the ultra rapid rectifier (I_{Kur}) and transient outward current (I_{to}). Inhibition of these currents increases contractility in canine atrial myocytes and goat atria. We investigated the effect of vernakalant on early repolarization and contractility in normal and electrically remodelled atria.

Methods and results Goats were implanted a pressure catheter, piezoelectric crystals, and electrodes to obtain atrial contractility and effective refractory period (ERP). The active component in pressure distance loops was used to compute the atrial work index (AWI). Experiments were performed in normal and electrically remodelled atria at clinically relevant plasma levels of vernakalant. As a positive control, the I_{to}/I_{Kur} blocker AVE0118 was investigated. Monophasic action potentials were recorded in anaesthetized goats and in explanted hearts to determine changes in action potential morphology. Vernakalant did not affect atrial work loops during sinus rhythm. Likewise, vernakalant did not increase atrial fractional shortening or AWI during pacing with fixed heart rate and AV-delay. In contrast, AVE0118 did increase AWI, with a positive force frequency relation. Both in normal and remodelled atria, vernakalant strongly increased ERP but did not prolong early repolarization.

Conclusion In goat atria, vernakalant does not have an atrial positive inotropic effect and does not affect early repolarization. At high rates vernakalant may even have a negative inotropic effect.

Introduction

Patients with atrial fibrillation (AF) have reduced exercise tolerance and a five-fold increase in stroke risk.¹ Electrical or pharmacological cardioversion of AF is often performed to improve symptoms caused by rapid and irregular ventricular activations and restore the active atrial contribution to ventricular filling. However, the atria need to recover from low atrial contractility after termination of AF. This transient loss of contractility contributes to decreased exercise tolerance and might lead to thromboembolic events after termination of AF.¹ The recovery of atrial contractility is dependent on factors such as underlying heart disease, atrial diameter, and the duration of AF.¹ In the goat model a close correlation between AF-induced electrical remodelling and loss of atrial contractility after several days of AF (dAF) has been described in.² Therefore, normalizing electrophysiological properties in the electrically remodelled heart could be a therapeutic target to enhance atrial contractility and thereby increase exercise tolerance and reduce stroke risk in the first days post-cardioversion.

In canine atrial myocytes changes in action potential morphology affect atrial cellular contractility.^{3–5} Specifically, increased plateau amplitude or prolongation of plateau duration facilitates Ca^{2+} entry through the reverse mode of the $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCX) and leads to more pronounced cell shortening.³ In atrial myocytes the transient outward current (I_{to}) and the ultra rapid rectifier (I_{Kur}) largely determine the early repolarization. Inhibition of these currents delays early repolarization and causes a positive inotropic effect in human atrial trabeculae and improved atrial contractility in the goat.^{6,7}

Vernakalant has been available in European countries since 2010 for acute cardioversion of AF.⁸ Vernakalant inhibits both depolarizing sodium current and repolarizing potassium currents including the transient outward current (I_{to}) and the ultra rapid rectifier (I_{Kur}).⁹ Moreover, Torp-Pederson *et al.*¹⁰ showed that a 90-day oral treatment of vernakalant can be safely used in haemodynamically stable AF patients. These properties of vernakalant might allow a positive inotropic treatment strategy after cardioversion. The goal of this study is to explore the possible atrial positive inotropic effect of vernakalant.

Methods

Animal model

The research protocol was approved by the local ethics committee and complied with the European directives concerning the use of animals for scientific purposes. Ten goats with a body weight of 60 ± 5 kg were included into this study. The goats were anaesthetized (i.v. sufentanyl $6 \mu\text{g/kg/h}$ and midazolam 0.8 mg/kg/h) and the thorax was opened using a left-sided approach. To determine local contractility, piezoelectric crystals were placed on the right and left appendage (**Figure 1A**) and left ventricular free wall for segment

length recordings. Additionally, on both atria and the left ventricle a bipolar electrode was fixated, ensuring that the site of electrode implantation would not interfere with the free movement of the segments. After a 2-week recovery period an additional trans-venous 5 Fr Micro-Tip pressure catheter (Millar Inc., Houston, Texas, USA) was chronically implanted in the right atrium. To prevent thrombus formation, the goats were anti-coagulated with a daily dose of nadroparin (2850 IU SC, GlaxoSmithKline, Zeist, The Netherlands).

Study protocol

Three days after pressure catheter implantation, atrial contractility and electrophysiology (EP) were investigated in awake goats. The sequence of experiments was consistent for each goat and followed the study protocol presented in **Figure 1B**. The first experiment (control, CTL) was performed before any episode of AF was induced. The second experiment was performed after 2dAF. AF was initiated and maintained by atrial burst pacing for as described previously in.¹¹ Subsequently, after ≥ 5 days of recovery from AF, AF was reinitiated for another 2 days to perform a positive control experiment with AVE0118. Finally, after full recovery AF was reinduced again for 2 days for a final open chest experiment under anaesthesia (i.v. sufentanyl 6 $\mu\text{g/kg/h}$ and midazolam 0.8 mg/kg/h). Based on the work of Garratt *et al.* no residual effect of the previous AF episodes on AF stability is expected on the atria.^{2,12}

Drug administration protocols

We investigated two different stable plasma levels of vernakalant, about 3 and 5 $\mu\text{g/ml}$ comparable to clinical relevant concentrations. The first and lower dose (LD) was reached by a 15-min loading infusion at 0.25 mg/kg/min followed by a maintenance infusion of 0.13 mg/kg/min . The higher dose (HD) was reached after 15 min loading at 0.30 mg/kg/min followed by a maintenance dose of 0.22 mg/kg/min . All contractility and EP measurements were performed 15 min after start of the maintenance infusion for both dose levels.

The atrial positive inotropic compound AVE0118 was infused at a rate of 1.0 mg/kg/h . Time between experiments allowed full washout (> 5 half lives) of both vernakalant and AVE0118.

Haemodynamic and electrophysiological measurements

Measurements were performed at baseline (saline infusion, BL) and the two different dosages of vernakalant (LD, HD) or in the presence AVE0118. At each of these conditions the atrial effective refractory period (ERP) was measured and contractility recorded during sinus rhythm and during right atrial (RA) pacing at different basic cycle lengths (BCLs).

ERP was measured with a S1S2 protocol, using a pacing output at four times above threshold.

Contractility recordings were performed during atrial and ventricular pacing with a fixed delay from atrium to ventricle (150 ms), about 30 ms shorter than the intrinsic AV-conduction. In previous goat studies¹¹ we have observed a shift in the Wenckebach cycle length after vernakalant infusion. As the occurrence of AV block dramatically alters pre- and after- load conditions of the atrium, we performed Segment lengths, RA pressures (P_{ra}) and electrograms were recorded for 20 s (eight-bit AD conversion, 250 Hz sampling frequency, Sonometrics corporation, London, Ontario, Canada) (**Figure 1C**).

During the final open chest experiment the effect of vernakalant on the morphology of the atrial action potential duration (APD) was determined using a Franz catheter (7F-110 cm–2 mm, Sachs Elektronik, Germany). Monophasic action potentials (MAPs) were recorded on the epicardial RA wall at a 1kHz sampling frequency (Mpaq, Maastricht instruments, The Netherlands).

Haemodynamic analysis

The atrial haemodynamic cycle consists of a passive and an active component. The active contraction is delineated by atrial shortening and an atrial pressure rise (**Figure 1C**). The active phase ends when the volume has returned to its initial value (**Figure 1D**). This point is followed by ventricular contraction, leading to passive pressure, and distance changes. Haemodynamic parameters of the active contraction, such as diastolic distension, fractional shortening (FS), and developed RA pressure (ΔP), were manually determined offline in 20 s recordings (SonoSOFT 3.3.52, Sonometrics corp, London, Ontario, Canada) as described previously in.² In the same recordings the atrial work index (AWI) was derived from the pressure-distance loops (**Figure 1D**). Custom-made analysis software (MATLAB 8.1, The Mathworks, Inc., Natick, Massachusetts, USA) was used to calculate AWI as the area under the curve in the active component of the loop (**Figure 1C**). Only signals recorded during the resting phase of the respiratory cycle were analysed.

Figure 1 Outline of the experimental protocol and signals acquired during the experiments. **A** Diagram of implanted sensors in and on the goat atria for the measurements in awake goats. Abbreviations: SVC, superior caval vein; IVC, inferior caval vein; and PV, pulmonary vein. **B** Outline of the study protocol depicts sequence of experiments. Within the first three studies atrial contractility (contrac) and EP were investigated in the awake goat, at baseline and after vernakalant or AVE0118 infusion. Vernakalant was investigated in atria without any previous AF episode (control) or after 2dAF. AVE0118 was only investigated after 2dAF at a single dose. The effect of vernakalant on MAPs was recorded under anaesthesia. The intervals between experiments were designed to be >5 half lives of the respective compound to ensure drug washout. Additionally, the intervals allowed full recovery from the previous AF episode before AF was reinduced. **C** Example traces of 1.5 s during RA pacing, in order from top to bottom: left ventricular segment length, RA segment length, RA pressure, left atrial (LA) electrogram, and left ventricular electrogram. Note that the LA electrogram is depicted because the RA electrodes are used for pacing. Dotted arrows indicate the start of atrial or ventricular contraction. The pacing artifact was used to identify initiation of atrial activation. **D** The atrial work loop comprises the RA pressure and the distance of the segment. The AWI was calculated as the surface of the loop during active contraction indicated by the grey area. The active part of the loop begins at the start of atrial contraction and ends when the distance returned to the length at the start of contraction.

Optical mapping

In seven goats (57 ± 9 kg) electrodes were implanted on the pericardium at the site of the left atrium that allowed trans-pericardial stimulation. Optical mapping was performed after 3 weeks of AF induction. Hearts were excised under anaesthesia (sufentanyl $6 \mu\text{g/kg/h}$ and midazolam 0.8 mg/kg/h , i.v.) and perfused with Krebs-Henseleit solution and prepared for optical mapping as previously described in detail.¹³ In brief, the hearts were loaded with the voltage sensitive dye di-4-ANEPPS. Fluorescence movies of a 2×2 cm area at the site of the crista terminalis were recorded with a 100×100 pixel CCD camera

(Micam Ultima, Brainvision inc., Tokyo, Japan). The excitation-contraction uncoupler blebbistatin (10 μ M) was used to prevent movement artifacts in the recorded optical potentials. Optical action potentials were recorded at a cycle length of 300 ms pacing in the presence of 0, 10, and 30 μ M of vernakalant.

Statistics

Data were analysed using IBM SPSS statistics version 19.0.0. Parameters were tested with linear mixed effects model using a diagonal covariance structure with drug, dose, pacing cycle length, and/or stage of remodelling taken as fixed variables and animal identification taken as random variable. Results are reported as estimated mean $\pm$ SEM. Statistical significance was taken as $P < 0.05$.

Results

Haemodynamics effects of vernakalant during sinus rhythm and pacing

2dAF strongly reduced RA contractility. During sinus rhythm the AWI declined from 4.7 ± 1.8 at CTL to 1.1 ± 0.7 mm-mmHg at 2dAF. Infusion of vernakalant shifted the atrial work loop to the right in both control and 2dAF experiments (**Figure 2**). Despite this increase in preload during sinus rhythm, vernakalant restored or increased AWI neither in control nor in 2dAF experiments.

To rule out possible AV-delay dependent effects, we performed pacing with fixed atrium to ventricle delay. Haemodynamic parameters, such as end-diastolic segment lengths, FS and ΔP are summarized in **Table 1**. FS in CTL experiments was comparable between both atria. Vernakalant did not significantly change FS and ΔP . In correspondence with the AWI, FS decreased in both atria after 2dAF. Also, under these conditions, vernakalant did not increase or restore FS in either atrium. Left ventricular FS was not affected by vernakalant (data not shown).

To explore possible rate dependent effects, we also investigated the AWI at 450, 350, and 250ms BCL in CTL and in 2dAF experiments (**Figure 3A**). As during sinus rhythm, AWI was strongly reduced in 2dAF experiments. Vernakalant did not significantly affect AWI at 450 and 350 ms, but at the shortest cycle length, AWI significantly decreased at the highest dose. Revealing a use-dependent effect on the atrial contractility.

Monophasic APD and excitability

We hypothesized that delaying 'early' repolarization will lead to an increase in both ERP and AWI. As described earlier, no positive inotropic effect of vernakalant was found in the awake goats in different conditions. Yet, vernakalant strongly increased ERP in a dose-dependent manner. The degree of increase was comparable in both CTL and 2dAF

experiments as can be appreciated in **Figure 3B**. Therefore, the lack of positive inotropic effect could not be explained by an inadequate dose that did not affect atrial electrophysiological properties.

Figure 2 A Representative recordings of pressure distance loops during sinus rhythm in control and 2dAF experiments. Despite a rightward shift apparent for both experiments, no increase in AWI can be appreciated. **B** All data AWI during sinus rhythm in control and 2dAF experiments. Control experiments are depicted in green and 2dAF experiments are depicted in red. 2dAF significantly reduced AWI but vernakalant did not affect AWI in either control or in 2dAF goats.

Table 1 Atrial haemodynamics

	Control			2dAF		
	Baseline	Low dose	High dose	Baseline	Low dose	High dose
Diastolic length (mm)						
Left atrium	27.9 ± 2.3	30.5 ± 2.3**	30.4 ± 2.3*	29.0 ± 2.3	30.8 ± 2.3*	30.8 ± 2.3*
Right atrium	19.1 ± 1.7	20.1 ± 1.7*	20.1 ± 1.7*	22.0 ± 1.5	23.2 ± 1.5*	23.0 ± 1.5*
FS						
Left atrium	0.21 ± 0.04	0.22 ± 0.04*	0.21 ± 0.03	0.16 ± 0.03	0.12 ± 0.02*	0.12 ± 0.02*
Right atrium	0.22 ± 0.02	0.17 ± 0.2*	0.23 ± 0.02	0.12 ± 0.03#	0.09 ± 0.03	0.10 ± 0.03
Developed Pressure (Δ mmHg)						
Right atrium	1.3 ± 0.4	1.2 ± 0.4	1.7 ± 0.4	0.87 ± 0.32	0.26 ± 0.32	0.47 ± 0.32

Effect of vernakalant on average atrial haemodynamic characteristics determined in 20 s recording. Cardiac rhythm was controlled by RA pacing at 450 ms with a fixed AV-delay of 150 ms. Vernakalant vs. control.

* $P < 0.05$; ** $P < 0.01$; 2dAF vs. control; # $P < 0.05$

A

B

Figure 3 **A** AWI measured during pacing with a fixed AV-delay (150 ms) at three different cycle lengths (450, 350, and 250 ms BCL). No significant effects occurred at 450 and 350 ms but a drop in AWI occurred at 250 ms cycle length pacing. **B** ERPs measured in the right atrium. At all cycle lengths the ERP increased in the presence of vernakalant. Vernakalant vs. BL; ** $P < 0.01$; *** $P < 0.001$. HD vs. BL; # $P < 0.05$,

To further explore the effects of vernakalant on early repolarization, we performed ERP and MAP measurements in a small subset ($n = 6$) in the above described goats. These experiments were performed after 2dAF. APD₃₀, APD₇₅, and the plateau potential were not affected by vernakalant while ERP was significantly increased (**Figure 4**). The prolongation of ERP without APD prolongation implies a reduction in excitability. This class I action should affect electrophysiological parameters describing excitability. Therefore, we also explored the effect on pacing threshold and interatrial conduction time. Pacing thresholds were increased from 0.95 ± 0.12 mA at baseline to 1.09 ± 0.17 mA and 1.21 ± 0.19 mA ($P = 0.041$), LD and HD respectively. Furthermore, the interatrial conduction time prolonged from 73 ± 7 ms at baseline to 97 ± 17 and 105 ± 15 ms ($P < 0.001$), LD and HD, respectively.

We also evaluated action potential morphologies with high-resolution optical mapping ($n = 7$) of the RA wall. In agreement with the earlier findings, vernakalant did not change the action potential morphology (**Figure 5**). Only a minor prolongation ($+4.3 \pm 5.7$ ms) of APD₂₀ was observed at a concentration of 30 μ M.

Figure 4 **A** MAPs recorded (BCL of 350 ms) on the RA epicardial wall in the anaesthetized goat. MAPs were normalized to the maximal amplitude. Prior to the experiments AF was maintained for 2 days to induce electrical remodelling. **B** ERP in the right atrium at 350 ms BCL. **C** Average level of the action potential from 10 to 50 ms after the upstroke and the duration to 30 and 75% recovery of the action potential. All measurements were performed at 350 ms BCL. Vernakalant vs. BL; ** $P < 0.01$; *** $P < 0.001$.

Figure 5 Atrial action potentials recordings after 3 weeks of AF using optical mapping. Isolated atria were perfused with Krebs-Henseleit solution only and with two concentrations of vernakalant. **A** Illustration of the isolated goat atria with the recorded location indicated on the right atria by a black rectangle. **B** The three panels show isochronal maps with different concentrations of vernakalant during pacing at 300 ms BCL. The lower panel illustrates three representative normalized (to maximal amplitude) and superimposed optical action potentials, illustrating a lack of effect on APD and morphology. **C** Effect of vernakalant on the APD at APD_{20} , APD_{30} and APD_{80} in the seven individual atrial preparations. Vernakalant vs. baseline; * $P < 0.05$

Positive inotropic effect of AVE0118

In six awake goats, we also investigated AVE0118 after 2dAF. Dosage was chosen based on historical data to achieve comparable ERP prolongations to vernakalant. **Figure 6** shows that both drugs produced comparable and significant prolongations of ERP. AVE0118 displayed a moderate frequency-dependent increase in AWI. At a BCL of 450ms AVE0118 did not increase AWI but it did increase AWI from 4.6 ± 1.9 to 6.6 ± 4.4 mm-mmHg and from 8.7 ± 4.4 to 13.5 ± 6 mm-mmHg at pacing intervals of 350 and 250 ms, respectively. In contrast, vernakalant exhibited a negative frequency-dependent relationship that became significant at 250 ms BCL.

Figure 6 **A** Effect of vernakalant and AVE0118 on atrial refractory period in awake goats. The effect was measured at three different cycle lengths. Dosage of AVE0118 was targeted to result in a comparable increase of ERP. **B** The effect of vernakalant and AVE0118 on AWI. AVE0118 showed a moderate increase in AWI with the largest effect at 250 ms. In contrast, vernakalant did not increase AWI and revealed a negative force frequency relation.

Discussion

This study investigates the effect of vernakalant on atrial contractility in normal and electrically remodelled atria. We hypothesized that the I_{Kur} inhibiting properties of vernakalant would cause an increase in calcium influx by slowing early repolarization and would therefore increase atrial contractility. However, vernakalant did not increase atrial contractility or the work index and at shorter cycle lengths even reduced atrial contractility. These effects of vernakalant were found in both normal and electrically remodelled atria despite significant prolongation in atrial refractoriness. In addition, vernakalant did not affect early repolarization of the action potential, in keeping with its lack of effect on atrial contractility.

Restoration of atrial contractility after cardioversion of AF

Reduced atrial contractility is commonly observed after spontaneous, electrical and pharmacological cardioversion of AF.¹ This reduction in atrial contractility is associated with decreased left ventricular function and reduced exercise tolerances.^{1,14} After cardioversion, improvement of left ventricular function and oxygen extraction may take up to 1 month after cardioversion, illustrating a delayed improvement of exercise tolerance.¹⁵ Therefore, acutely restoring atrial contractility might be an attractive strategy to improve recovery from AF. However, many positive inotropic strategies (e.g. β -adrenergic stimulation and phosphodiesterase-III inhibitors) have proarrhythmic effects on the ventricles.¹⁶

In the goat model of AF, we have previously demonstrated that the initial decrease in atrial contractility went along with atrial electrical remodelling.² Electrical remodelling is characterized by shortening of the APD due to downregulation of $I_{Ca,L}$ and the upregulation of the inward rectifying current I_{K1} .¹⁷ The $I_{Ca,L}$ is—besides release of Ca from the sarcoplasmic reticulum—the most important source of intracellular free Ca^{2+} and an important determinant of atrial contraction. Indeed, Wakili *et al.*⁴ demonstrated that atrial tachypacing induced action potential shortening and was associated with a reduction in peak $I_{Ca,L}$, reduced Ca^{2+} transients and an attenuation of cell shortening. Interestingly they demonstrated that in these cells a restoration of control-like action potentials, by action potential clamp experiments, increased Ca^{2+} transients and improved cell shortening. Furthermore pharmacological changes in action potential, by modulating acetylcholine-mediated potassium current, reveals a strong relation between APD and contractility.⁵ These observations are in agreement with our previous work where we demonstrated that action potential morphology in non-remodelled atrial myocytes, more specifically the plateau phase, is crucial for cellular contractility.^{3,18} Elevation of the action potential plateau in atrial myocytes by blocking the atrial specific I_{Kur} current in combination with the transient outward current (I_{to}) by AVE0118 revealed a linear relationship between plateau duration and cell shortening.³ de Haan *et al.*⁶ translated this observation to a whole animal model, demonstrating that AVE0118 prolonged plateau duration and fully restored atrial work in remodelled goat atria. Given that vernakalant is a known I_{to} and I_{Kur} blocker in HEK cell expression systems⁹, a positive inotropic effect could be expected for vernakalant. However, despite a marked increase of ERP, we did not detect any effect on AWI or FS during sinus rhythm. In fact, pacing at higher frequencies revealed a negative inotropic effect.

Why does vernakalant not increase atrial contractility?

Loss of atrial contractility and disappearance of action potential plateau after cardioversion of AF is caused by attenuated calcium transients and reduced cytosolic $[Ca^{2+}]$. Specifically, the down-regulation of L-type calcium channels and up-regulation of repolarizing K^+ -currents are responsible for the loss of contractility and action potential plateau.¹⁷ Hence, increasing calcium influx through L-type calcium channels, by e.g. beta-adrenergic stimulation or an L-type channel agonist, will only have a limited effect in electrically remodelled atria. Indeed, in the goat model of AF the sympathomimetic drug dobutamine or the L-type Ca^{2+} agonist BayY5959 had no or a limited effect on atrial work.⁶ Alternatively, calcium entry that occurs in the early phase of repolarization through the reverse mode of the NCX may offer a potential target. Indeed, patch clamp experiments have provided evidence that the positive inotropic effect of AVE0118 is mediated by Ca^{2+} entry through this reverse mode of the NCX during early repolarization.³ Based on the data found in the HEK cells one might expect a similar effect in of vernakalant on early repolarization.⁹ However, in this series of experiments, vernakalant only led to a small increase

in APD₂₀ as measured in the optical action potentials with a negligible effect size of only 4 ms. Moreover, vernakalant did not alter the APD₃₀ or the plateau phase. This absence of an effect on the action potential plateau may in part explain why vernakalant did not possess any positive inotropic effect. Of note, Wettwer et al.¹⁹ observed a similar lack of effect on the plateau phase in isolated human trabeculae. Despite a small but significant prolongation of the APD₂₀ in trabeculae of chronic AF patients, vernakalant did not increase the membrane potential during the plateau phase.

Various processes could have prevented an elevation of action potential plateau and an increase of contractility. It might be that the dosage used was not sufficient to substantially inhibit I_{Kur} . In the optical mapping experiments, we saw a small APD₂₀ increase at a high concentration of vernakalant. This minor prolongation of APD₂₀ was found at a concentration that is about three-fold higher than the therapeutic plasma concentration. Such a condition was not investigated in vivo because we aimed to explore vernakalant's positive inotropic potential at clinically relevant plasma concentrations. Further, the degree of remodelling may have affected Ca²⁺ handling to such an extent that a positive inotropic effect through the NCX pathway could no longer be possible. However, in this case a positive inotropic effect would have occurred in healthy atria, which was not the case in our experiments. Moreover, in the same animals AVE0118 did increase atrial contractility at similar baseline conditions. Taken together, these findings indicate that the I_{Kur} inhibition that has been shown by others⁹ has a limited effect on early repolarization and thus does not increase atrial contractility.

Class I effects of vernakalant

In a previous study, we have reported that vernakalant's main antiarrhythmic effects in the awake goat are a slowing of conduction and a refractory period prolongation due to post-repolarization refractoriness.¹¹ This study shows an use-dependent effect on atrial contractility and a decrease in excitability described by increased pacing thresholds and slowing of conduction. The use-dependent effect of vernakalant^{19–21} on the sodium current may explain the negative inotropic effect at faster pacing rates. These haemodynamic and electrophysiologic observations *in vivo*, and *ex vivo* measurements,¹⁹ underscores the potent and dominant class I effects of vernakalant. This inhibition of sodium current presumably will affect NCX function. Specifically, the reduced voltage dependent sodium current will lead to a decreased cytosolic sodium concentration, lowering the driving force for Ca²⁺ influx through the NCX. Thereby limiting the potential contribution of the NCX reverse mode on atrial contractility.

Limitations

This animal model requires chronic implantation of electrodes and crystals. It is likely that this has caused a local inflammation and in turn affected local electrophysiological and contractile properties. In control conditions and after the infusion of AVE0118 significant

atrial work could clearly be measured but this does not exclude a negative effect on atrial contractility of the model itself.

Second, in patients with longstanding persistent AF, LA contractility is affected to a greater extent than the right atrium.²² Our study only evaluated RA work loops because of the risk of clot formation caused by chronic implantation of a pressure catheter in the left atrium. We assume that RA work is in agreement with LA work, comparable to the lack of effect on FS in both atria. Furthermore, differential effects on action potential morphology are not expected as we did not detect differences in effect of vernakalant on ERP or conduction velocity between both atria a previous study.¹¹

Conclusions

Vernakalant does not affect atrial contractility, arguably because it does not affect early atrial repolarization. Most likely vernakalant does not significantly inhibit I_{Kur} at clinically relevant plasma levels. Our data confirm recent observations that vernakalant mainly acts by its effects on the sodium current.

Acknowledgements

Sanofi Aventis (Frankfurt, Germany) kindly provided us AVE0118.

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3

Chapter 3

Antiarrhythmic effect of vernakalant in electrically remodeled goat atria is caused by slowing of conduction and prolongation of postrepolarization refractoriness

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Heart Rhythm. 2016 doi: 10.1016/j.hrthm.2015.12.009.

Abstract

Background Vernakalant inhibits several potassium currents and causes a rate- and voltage-dependent inhibition of the sodium current.

Objective The aim of this study was to evaluate the antiarrhythmic mechanism of vernakalant in normal and electrically remodeled atria.

Methods Fourteen goats were instrumented with electrodes on both atria. Drug effects on refractory period (ERP), conduction velocity (CV), and atrial fibrillation cycle length (AFCL) were determined in normal goats (control) and after 2 (2dAF) or 11 (11dAF) days of pacing-induced atrial fibrillation (AF) in awake goats. To evaluate the contribution of changes in conduction and ERP, the same experiments were performed with flecainide and AVE0118. In a subset of goats, monophasic action potentials were recorded during anesthesia.

Results Vernakalant dose-dependently prolonged ERP and decreased CV in CTL experiments. Both effects were maintained after 2dAF and 11dAF. After 11dAF, conduction slowed down by 8.2 ± 1.5 cm/s and AFCL increased by 55 ± 3 ms, leading to AF termination in 5 out of 9 goats. Monophasic action potential measurements revealed that ERP prolongation was due to enhanced postrepolarization refractoriness. During pacing, vernakalant had comparable effects on CV as flecainide, while effect on ERP was comparable to AVE0118. During AF, all compounds had comparable effects on median AFCL and ERP despite differences in their effects on CV during pacing.

Conclusion The antiarrhythmic effect of vernakalant in the goat, at clinically relevant plasma concentrations, is based on both conduction slowing and ERP prolongation due to postrepolarization refractoriness. These electrophysiological effects were not affected by long-term electrical remodeling of the atria.

Introduction

Atrial fibrillation (AF) is the most common sustained tachyarrhythmia in adults, with a prevalence of up to 2% in Europe.¹ It is desirable to treat AF early after its onset, as AF patients have an increased risk for thromboembolic events (eg, stroke) and reduced left ventricular systolic function, leading to increased morbidity and mortality. Pharmacologic cardioversion of AF can be considered in hemodynamically stable patients with recent-onset AF. The efficacy of vernakalant to convert AF for episodes lasting shorter than 7 days was about 50% in clinical trials.² However, when vernakalant was used in patients with AF of longer duration, the cardioversion rate was not significantly different from that of the placebo group. This illustrates that, like other antiarrhythmic drugs (AADs), vernakalant loses its antiarrhythmic effect after prolonged atrial remodeling.

Fedida et al³ have demonstrated that vernakalant blocks several potassium channels (I_{Kur} , I_{to} , I_{Kr} , and $I_{K_{ACh}}$) as well as voltage- and rate-dependent sodium channels in patch clamp experiments. In vivo animal and human studies have so far focused on its effect on effective refractory period (ERP), with little data available regarding its effect on conduction velocity. In healthy human volunteers, an atrial-specific ERP increase has been shown, suggesting a dominant role of I_{Kur} blockade.⁴ However, Burashnikov et al demonstrated an increase in ERP without an increase of the action potential duration at 75% repolarization (APD₇₅), suggesting that sodium channel blockade resulting in postrepolarization refractoriness underlies the observed ERP prolongation.¹⁰

In this study, we have evaluated the antiarrhythmic properties of vernakalant by comparing its atrial electrophysiological effects to the I_{Na} blocker flecainide and the I_{to}/I_{Kur} blocker AVE0118 in an established model of rapid atrial pacing–induced AF in conscious goats. To investigate the effectiveness of vernakalant during early progression of AF, we compared its electrophysiological effects at different stages of electrical remodeling: control, and 1–2 (2dAF) and 11 days of maintained AF (11dAF).

Methods

Animal model

Fourteen goats weighing 61 ± 12 kg were included in the study. Under general anesthesia (intravenous sufentanyl 6 μ g/kg/h and midazolam 0.8 mg/kg/h), thoracotomy was performed to implant custom-made plaques of 4 x 4 electrodes (5 mm interelectrode distance) on both atria and a 3-electrode plaque on the ventricles. Instrumentation and AF induction was performed as previously described.⁵ All experiments complied with local institutional and national guidelines. The local animal ethics committee approved the study protocol.

Drug administration and study protocol

Three different drugs were investigated: vernakalant (Merck, Darmstadt, Germany), flecainide (Meda Pharma bv, Amstelveen, The Netherlands), and AVE0118 (Sanofi Aventis, Frankfurt, Germany). The infusion regimen of vernakalant was designed to reach stable plasma levels during electrophysiological measurements. Target plasma concentrations were 3 $\mu\text{g/mL}$ for low-dose (LD) and 5 $\mu\text{g/mL}$ for high-dose (HD) administrations, plasma levels known to be effective in patients.⁶ Dosages for flecainide (3 and 6 mg/kg/h) and AVE0118 (1 and 3 mg/kg/h) were targeted to reach an equivalent increase in AF cycle length (AFCL).

In each goat, all drugs were studied at 3 stages of AF-induced remodeling: control (CTL), after 1–2 days of AF-induced remodeling (2dAF), and after 11 days of AF-induced remodeling (11dAF). Measurements were performed at baseline (BL, saline infusion) and at the LD and HD of the drug. The order within CTL and 2dAF experiments met the following rules: (1) random order of drugs and experiments; (2) >2 days apart to allow full recovery from 2dAF; (3) >5 half-lives of the drug. When these experiments were completed, AF was maintained for 8–15 days. The final 11dAF experiments were performed consecutively in random order with, on average, 11 days of AF for each drug. Within each experiment, a 1-hour window with constant infusion rate was chosen for BL, LD, and HD.

Six goats in a separate group were anesthetized (sufentanyl 6 $\mu\text{g/kg/h}$ and propofol 10 mg/kg/h) after 24–48 hours of AF. Using a Franz catheter (7 F–110 cm–2 mm, Sachs Elektronik, March-Hugstetten, Germany), monophasic action potentials (MAPs) and ERPs were measured in the right atrium. This experiment was only performed with vernakalant.

Measurement of electrophysiological parameters

After 2 weeks following instrumentation, electrophysiological measurements were started after the animals had acclimatized to a dedicated quiet room. Both unipolar atrial electrogram and electrocardiogram were recorded during all measurements. Offline analysis was performed using custom-made analysis software (MATLAB 8.1; The Mathworks, Inc, Natick, MA). Conduction velocity (CV) was measured at different cycle lengths (range 200–500 ms) and during AF. The CV was calculated as the reciprocal of the steepness of a plane fitted through the activation times of at least 3 electrodes, as described previously.⁷ CVs between 20 and 120 cm/s were included for analysis. ERP was determined by an S1-S2 protocol. For each S1-S2 protocol, it was documented when AF was induced and the duration of the AF episode was recorded. AF was defined as rapid irregular activations lasting more than 2 seconds. AF recordings, with durations of 10–60 s, were used for analysis. Local deflections of the electrograms were identified using a probabilistic annotation algorithm,⁸ allowing the determination of AFCL and CV during AF. The fifth percentile (p5) of the AFCL histogram was used as a surrogate parameter for ERP during AF.⁹

From the MAPs, the duration to reach 20% (APD₂₀) and 70% (APD₇₀) repolarization was determined. APD₇₀ was in good agreement with ERP at baseline and therefore was used to calculate postrepolarization refractoriness (PRR). The relative plateau potential (phase 2/phase 0) was determined by calculating the average level between 10 ms and 50 ms after the upstroke, divided by the MAP amplitude.

Statistics

Data were analyzed using IBM SPSS statistics version 19.0.0. Electrophysiological parameters were tested with a linear mixed-effects model using a diagonal covariance structure with drug, dose, basic cycle length (BCL), and/or stage of remodeling (CTL, 2dAF, or 11dAF) taken as fixed variables and animal taken as random variable. Results are reported as estimated mean $\pm$ standard error of the mean, unless mentioned otherwise. AF duration was analyzed using a signed rank test. A *P* value $< .05$ was considered to be statistically significant.

Results

Goat model of atrial fibrillation

Eleven goats were included for electrophysiological measurements in the baseline CTL state. In 8 2dAF goats (*n* = 6 24-h maintenance, *n* = 2 48-h maintenance) the median AF episode duration increased from 15 ± 4 s to 177 ± 58 s after 1–2 days of remodeling. Left atrial ERP and AFCL shortened by 39 ± 5 ms and 24 ± 10 ms to 84 ± 2.9 ms and 111 ± 3.2 ms, respectively. After 11dAF (*n* = 9 goats), AFCL decreased further to 96 ± 4 ms and AF became persistent in 8 out of 9 goats.

Effect of vernakalant on electrocardiographic parameters

Plasma samples showed similar concentrations of vernakalant during the CTL and 2dAF experiments and significantly higher levels during the 11dAF experiments (**Table 1**). The effect of vernakalant on electrocardiographic parameters during sinus rhythm or slow atrial pacing is presented in **Table 2**. Vernakalant caused an initial negative chronotropic effect, increasing the RR interval with LD from 621 ± 52 ms to 739 ± 37 ms, although HD vernakalant did not increase the RR interval further. The PQ interval was increased by 54 ± 6 ms and 48 ± 3 ms, respectively, during baseline CTL and 2dAF experiments with LD vernakalant. Again, no further changes were observed with HD vernakalant. Both CTL and 2dAF goats showed a 10% increase in QRS width during LD vernakalant administration in CTL atria and HD vernakalant administration in 2dAF goats. During fixed-rate pacing (450 ms), no changes in QT interval were observed at any dose of vernakalant in any group (CTL or 2dAF).

Table 1 Plasma levels of vernakalant obtained during the 3 different experiments; target plasma concentration was 3.0 µg/mL and 5.0 µg/mL for low and high dose, respectively

	Low Dose (µg/mL)	High Dose (µg/mL)
Control	3.4 ± 1.2	5.8 ± 1.4
2 days of AF	3.8 ± 1.0	6.2 ± 2.3
11 days of AF	5.0 ± 1.7	8.3 ± 2.9

AF=atrial fibrillation.

Table 2 Effects of vernakalant on the electrocardiogram; data obtained in control and 2dAF experiments

		Baseline	Low Dose	High Dose
Control	RR (ms)	621 ± 152	739 ± 37*	726 ± 30*
	QRS (ms)	64 ± 3.6	69 ± 4.6	70 ± 3.6
	PQ ₄₅₀ (ms)	181 ± 12	234 ± 15***	237 ± 9.2***
	QT ₄₅₀ (ms)	263 ± 6.8	255 ± 6.6**	255 ± 5.1**
2 days of AF	RR (ms)	530 ± 18	850 ± 57***	773 ± 37***
	QRS (ms)	60 ± 3.3	64 ± 3.1	70 ± 4.2**
	PQ ₄₅₀ (ms)	159 ± 15	198 ± 21***	205 ± 14***
	QT ₄₅₀ (ms)	255 ± 4.9	242 ± 4.5	263 ± 10

AF=atrial fibrillation. Versus baseline : * $P < 0.05$, ** $P < .01$, *** $P < .01$.

Effect of vernakalant on atrialelectrophysiological parameters

Although differences in CV and ERP were present between the right and the left atrium (LA), there were no differences in drug effects between atria. Here, we will only discuss data obtained in the LA.

Vernakalant led to a dose-dependent increase in atrial ERP in CTL and 2dAF goats (**Figure 1A**). This increase was consistent at all pacing cycle lengths without rate-dependent effects on ERP. Vernakalant attenuated the abbreviation of atrial refractoriness seen with 2dAF. ERP approximately reached CTL baseline values (117 ± 6 ms vs 125 ± 5 ms) at 250 ms BCL. This effect was less pronounced at 400 ms BCL (108 ± 4 ms vs 128 ± 4 ms). Overall, when all cycle lengths and dosages are considered, the absolute increase in ERP in both the CTL and 2dAF groups was comparable. Furthermore, vernakalant induced a dose-dependent slowing of conduction at all cycle lengths in both baseline CTL and 2dAF groups (**Figure 1B**). The degree of conduction slowing was comparable at different cycle lengths in both groups with no rate-dependent differences.

Figure 1 Effects on basic electrophysiological parameters for control and 2dAF (1–2 days of maintained atrial fibrillation) experiments at regular pacing intervals. **A:** Effective refractory period (ERP) was increased at all cycle lengths. **B:** Conduction velocity (CV) was decreased at all cycle lengths. Versus baseline: *** $P < .001$. BCL = basic cycle length.

Effect of vernakalant on monophasic action potentials

MAPs were recorded in 6 goats (represented in **Figure 2A**). The relative plateau potential (reflecting early repolarization) was not altered by vernakalant at any BCL (**Figure 2B**). Indeed, APD_{20} was unaltered by vernakalant at both cycle lengths. At 400 ms pacing a significant increase of ERP occurred at HD. At this cycle length vernakalant did not yet lead to PRR. However, ERP was significantly longer than APD_{70} at 250 ms BCL (**Figure 2C**), indicating that vernakalant caused PRR.

Effect of vernakalant on atrial fibrillation

Median AF duration was significantly shortened in 2dAF goats from 177 ± 58 s to 34 ± 38 s with HD vernakalant (**Figure 3A**). AF was induced in 46% of S1-S2 applications at CTL. This vulnerability to AF was increased to 93% in 2dAF. AF vulnerability was not affected by vernakalant, either in CTL or in 2dAF atria (**Figure 3B**). In longer-lasting AF (11dAF), cardioversion was seen in 5 out of 9 goats with HD vernakalant, whereas only 1 goat cardioverted to sinus rhythm with saline infusion (**Figure 3C**). Furthermore, LD and HD vernakalant increased AFCL in CTL atria by 43 ± 4 ms and 62 ± 6 ms, respectively. Similar increases were observed in 2dAF (34 ± 2 ms and 57 ± 4 ms) and 11dAF (33 ± 3 ms and 55 ± 4 ms) at LD and HD, respectively (**Figure 3D,E**).

Figure 2 Effects of rate on monophasic action potential (MAP) morphology and effective refractory period (ERP) in the presence of vernakalant. **A:** Representative recordings of MAPs (normalized to the maximum amplitude) at 250 ms burst cycle length (BCL) in the right atrium. **B:** The “average plateau potential” from 10ms to 50 ms after the upstroke, corrected for MAP amplitude. **C:** APD₂₀, APD₇₀, and ERP at 250 ms BCL. **D:** APD₂₀, APD₇₀, and ERP at 400 ms BCL. Versus baseline: * $P < .05$, $n = 6$. BL = baseline; HD = high dose; LD = low dose; APD₂₀, APD₇₀ = action potential durations at, respectively, 20% and 70% repolarizations.

Because of the technical limitations in directly obtaining ERPs during AF, the p5 of the AFCL histogram was used as surrogate parameter for ERP. Vernakalant caused a dose-dependent prolongation of AFCL and p5 resulting in a rightward shift of the AFCL histogram. The p5 of the AFCL histogram increased to the same extent in the 2dAF (21 ± 3 ms and 51 ± 6 ms) and 11dAF (26 ± 3 ms and 46 ± 3 ms) groups, for LD and HD, respectively (**Figure 3F**). In addition, atrial CV during AF (CV_{AF}) was comparable in 2dAF and 11dAF atria (62 ± 3 cm/s and 61 ± 3 cm/s). Again, a similar effect was seen for 2dAF and 11dAF animals (**Figure 3G**). Taken together, during AF vernakalant maintained its electrophysiological effects on ERP, CV, and AFCL at all AF durations.

Figure 3 Effect of vernakalant during atrial fibrillation (AF). Data from the left atrium are depicted. **A:** AF inducibility. **B:** Duration of AF paroxysms. **C:** Efficacy of vernakalant in 11 days of pacing-induced atrial fibrillation (11dAF). **D:** Example atrial fibrillation cycle length (AFCL) histogram illustrating a rightward shift due to high-dose vernakalant. **E:** Median AFCL. **F:** Fifth percentile of AFCL distribution. **G:** Conduction velocity during AF: * $P < .05$, ** $P < .01$, *** $P < .001$. BL = baseline; HD = high dose; LD = low dose; p5 = fifth percentile.

Vernakalant in comparison to AVE0118 and flecainide

The results described above indicate both slowing of conduction and ERP prolongation as main antiarrhythmic mechanisms of vernakalant. To better understand the relative contributions of I_{Na} and I_{to}/I_{Kur} inhibition, vernakalant was compared to flecainide (I_{Na}) and AVE0118 (I_{to}/I_{Kur}).

At 250 and 400 ms BCL, flecainide showed a moderate increase in ERP but marked slowing of conduction (**Figure 4A, B**). In contrast, AVE0118 showed a considerable ERP prolongation, without an effect on CV. In comparison, vernakalant demonstrated a similar effect on atrial ERP as compared to AVE0118, while its effect on atrial CV was comparable to flecainide. Electrical remodeling augmented the ERP-prolonging effect of AVE0118 (at short cycle lengths), but not of vernakalant (**Figure 5**).

A

Refractory period

B

Conduction velocity

Figure 4 Vernakalant in comparison to flecainide and AVE0118 in the remodeled left atrium during pacing. Both absolute values and the normalized change is depicted. **A:** Effective refractory period. **B:** Conduction velocity.

Figure 5 Effect of remodeling on effective refractory period (ERP) prolongation in left atrium at the highest dose for vernakalant, flecainide, and AVE0118. ERP increase from baseline obtained in control (CTL) and 2dAF (1–2 days of maintained atrial fibrillation) experiments. Statistical analyses were performed on level of increase and interaction with burst cycle length (BCL).

During AF, all 3 drugs led to a similar increase in AFCL with increasing drug dosing despite the differences in mode of action between compounds described above (Figure 6A, B). However, at HD, the effect of AVE0118 on AFCL was smaller than that of flecainide and vernakalant, in both the 2dAF and 11dAF groups. Likewise, all compounds led to a comparable increase in p5, with a smaller effect seen with AVE0118 at HD in both 2dAF and 11dAF atria (Figure 6C, D). Little or no CV_{AF} was detected for AVE0118, as opposed to the clear reduction in CV_{AF} with vernakalant and flecainide (Figure 6E, F). However, when only lower drug dosing is considered, no differences in effect on AFCL, CV_{AF}, or p5 of AFCL could be detected between compounds.

Figure 6 Vernakalant in comparison with flecainide and AVE0118 during atrial fibrillation (AF), obtained in 2dAF and 11dAF (respectively, 1–2 days and 11 days of maintained atrial fibrillation) experiments. AF cycle length (AFCL) (A, B), fifth percentile (p5) of the AFCL distribution (C, D), and CV_{AF} (E, F) in the left atrium are depicted. CV = conduction velocity.

Discussion

To our knowledge this is the first in vivo study to address the atrial electrophysiological effects of vernakalant at multiple stages of atrial remodeling. This study was designed to investigate vernakalant at clinically relevant dosages. We conclude that the major electrophysiological determinants of vernakalant's antiarrhythmic effect are slowing of conduction together with ERP prolongation, leading to AFCL prolongation and decreased AF stability. Moreover, we confirm that ERP prolongation is due to enhanced PRR.¹⁰ At different stages of AF-induced remodeling, comparable effects on different electrophysiological parameters were observed. In line with previous observations, the loss of cardioversion efficacy is likely not due to a reduction in the effect of vernakalant on ionic currents.¹¹ We propose that loss of efficacy is rather due to a progression of other processes that stabilize AF.

This study also investigated vernakalant in comparison to the actions of flecainide and the I_{Kur}/I_{to} blocker AVE0118. The strong similarity between the effects of vernakalant and flecainide indicates that vernakalant-induced attenuation of excitability leads to conduction slowing and PRR-dependent ERP prolongation at high rates.

Electrophysiological effect of vernakalant

ERP prolongation is a well-described effect of vernakalant in multiple models and species.^{4,10,12} However, the knowledge of the effect of vernakalant on conduction velocity is more limited. Dorian et al⁴ showed no effect on PH interval, suggesting a limited effect on atrial conduction velocity, while ex vivo studies did show a significant reduction of sodium current, leading to PRR and a reduced slope of the upstroke velocity of the action potential. However, these studies could not clarify to which extent atrial conduction slowing contributes to vernakalant's antiarrhythmic effect. Most studies so far were performed in perfused preparations or cardiomyocytes and evaluated vernakalant only during fixed-rate pacing but not during AF itself. We investigated vernakalant in awake goats with or without AF-induced remodeling. We demonstrate that vernakalant markedly reduces atrial CV and prolongs atrial refractory period during both pacing and AF. We confirm that PRR, occurring especially at shorter cycle lengths, is the primary mechanism for ERP prolongation. However, we did not observe the previously described rate-dependent prolongation of ERP¹⁰ or slowing of conduction. Several experimental factors might contribute to this discrepancy. Our experiments were performed at lower and clinically relevant drug concentrations, with higher stimulation rates and in the presence of autonomic regulation.

Vernakalant's atrial selectivity was described in healthy volunteers during sinus rhythm.⁴ Rate and voltage dependency of sodium current inhibition by vernakalant are relevant to its atrial selectivity.¹³ The less negative resting membrane potential in atrial compared to ventricular myocytes and differences in I_{Na} properties between atrium and

ventricle may both contribute to this selectivity.¹³ Moreover, during AF, there is a large difference in activation frequency between the atria and ventricles, which will enhance use-dependent block of I_{Na} in the atrium. However, other studies did show effects on the ventricle. A moderate QRS prolongation (approximately 10%) was observed in human subjects, in canine hearts with left bundle branch block, and in our present study.^{14,15}

Influence of electrical remodeling on the effect of vernakalant

AF-induced remodeling gradually increases AF stability.⁵ The efficacy of AADs shows a corresponding decrease over time. This loss of efficacy is observed for both class I and III AADs.^{16,17} In patients with comorbidities such as heart failure, hypertension, and diabetes, Roy et al¹⁴ showed that AF cardioverted in 52% of all cases if episode duration was <7 days, while only 7.9% cardioverted at a median duration of 25 days. After maintenance of AF for 11 days in our “lone” AF model, the success rate of cardioversion was 55%. The relatively high success rate at 11dAF is likely due to a relatively short duration in AF and absence of comorbidities. In addition, we infused vernakalant, attaining a stable plasma concentration for an extended period (60 min), as opposed to a bolus injection applied in patients. In theory, electrical remodeling might account for the loss of efficacy of vernakalant. Nevertheless, electrical remodeling in terms of ERP abbreviation occurs within the first 1 or 2 days of AF, whereas loss of efficacy of AADs appears to occur with a slower time course.¹⁸ The presence of electrical remodeling after 2 days of AF did not affect the magnitude of ERP prolongation. Neither did 11 days of AF affect the increase of the p5 of the AFCL distribution, suggesting that the ERP during AF was affected to a comparable degree. The consistent conduction-slowing effect of vernakalant is in agreement with a comparable level of I_{Na} inhibition in cells obtained from sinus rhythm and chronic AF patients.¹¹ The preserved effect of vernakalant on electrophysiological parameters such as ERP, CV, and AFCL supports the view that loss of efficacy to terminate AF is due to the progression of other remodeling processes that increase the stability of AF, rather than to a loss of efficacy of vernakalant to change ionic currents.

Comparison of electrophysiological effects of vernakalant, AVE0118, and flecainide

The profile of ion current inhibition by vernakalant includes several potassium currents as well as a rate-dependent I_{Na} current inhibition, with a modest I_{Na} inhibition at slow rates but a stronger inhibition at faster rates.³ In this study, we investigated the relative contributions of conduction slowing and ERP prolongation caused by vernakalant by comparing vernakalant to the I_{Na} blocker flecainide and the I_{Kur}/I_{to} blocker AVE0118. The dosages of the compounds were chosen to cause a similar prolongation of AFCL. At this equieffective dose, flecainide and vernakalant caused a comparable degree of conduction slowing, both during pacing and during AF.

AVE0118 and vernakalant increased ERP to almost the same extent, but the mechanism by which they increase ERP may be different. The increase in ERP caused by AVE0118 depends on the prolongation of the APD.¹⁹ In contrast, for vernakalant we confirm observations in human myocytes and canine hearts that PRR significantly contributes to ERP prolongation at short cycle lengths.^{10,11} Typically, ERP prolongation by potassium channel blockers (eg, AVE0118, ibutilide, and sotalol)^{16,19,20} is affected by alterations in action potential morphology during electrical remodeling,²¹ while the ERP prolongation by flecainide is not.²⁰ Electrical remodeling also did not affect the ERP prolongation by vernakalant and is thus more comparable to flecainide. Despite this similarity, the degree of ERP prolongation was similar for vernakalant and AVE0118 compared to the smaller effect of flecainide. However, based on our present data we cannot determine why vernakalant causes a much larger prolongation in ERP than flecainide. A possible explanation is block of inward rectifying currents, such as I_{KACH} , I_{K1} , and $I_{K2p2.1}$ by vernakalant,^{3,23} which might depolarize the resting membrane potential and thus reduce excitability. This indirect inhibition of sodium channels may contribute to the observed effect on PRR, comparable to ERP prolongation caused by blockade of SK channels.²²

We propose that vernakalant and flecainide have a similar mechanism of action. Therefore, we postulate that sodium channel inhibition is the major determinant of antiarrhythmic properties of vernakalant based on the effects on CV, AFCL, the lack of sensitivity to electrical remodeling, and increase in ERP resulting from PRR.

Vernakalant, AVE0118, and flecainide have comparable fundamental electrophysiological effects during atrial fibrillation

It is commonly accepted that potassium current inhibition can terminate reentry by ERP prolongation. However, the mode of action of sodium channel inhibition is less straightforward. In theory, conduction slowing is expected to facilitate reentry. However, reduced excitability also limits wave turning, reduces rotor stability, and inhibits wavelet generation.^{24,25} In addition, flecainide also reduces the size of calcium sparks that potentially lead to proarrhythmic calcium waves and triggered activity.²⁶ The resulting suppression of ectopy has been proposed to contribute to its antiarrhythmic action. An interesting observation in this study is that different compounds exhibit distinctly different effects during regular pacing, but their differences during AF are far less distinct. All compounds led to a comparable increase in AFCL and the p5 of the AFCL distribution. At LD, there were also no clear differences between the compounds in their effect on CV. These comparisons show that despite clear differences in ionic mechanisms between the compounds, their effects on elementary determinants of AF dynamics are actually comparable.

Limitations

Our model utilized chronic implantation of electrodes on the heart. This in itself could have caused a local inflammatory reaction. However, we expect this effect to be small and stable because repeated baseline measurements during the course of weeks did not deviate from each other. Furthermore, we aimed to characterize the effect of vernakalant during AF. The p5 of the AFCL distribution was used as a surrogate parameter for the atrial ERP during AF. However, this method does not rule out the possibility that the AFCL increased owing to a prolongation of only the excitable period without an ERP prolongation.

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4

Chapter 4

Bi-atrial high-density mapping reveals inhibition of wavefront turning and reduction of complex propagation patterns as pivotal properties for the class I antiarrhythmic drug vernakalant

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Abstract

Background: Complex propagation patterns are observed in patients and models with stable atrial fibrillation (AF). The degree of this complexity is associated with AF stability. Little is known about the effect of sodium channel inhibition on patterns during AF. The aim of this study was to investigate how sodium channel inhibition by vernakalant changes propagation patterns during AF.

Methods: Two groups of 8 goats were instrumented with stimulation electrodes on the left atrium, and AF was maintained by burst pacing for 3 or 22 weeks. Measurements were performed at baseline and two clinically relevant dosages of vernakalant. Unipolar electrograms were mapped (249 electrodes/array) on the left and right atrial epicardium in an open-chest experiment. Local activation times and conduction vectors, vorticity, the number of waves, and local re-entries were determined.

Results: At baseline, patterns exhibited numerous individual waves conducting in random directions. Vernakalant induced conduction slowing and cycle length prolongation and terminated AF in 15/16 goats. Local re-entries were strongly reduced. Local conduction vectors showed increased preferential directions, less beat-to-beat variability, and reduced vorticity. Breakthroughs and waves were significantly reduced in number. Waves conducted more homogeneously in one direction. Overall, complex propagation patterns were strongly reduced. No substantial differences in drug effects between right and left atria or between goats with different AF durations were observed.

Conclusions: Destabilization of AF by vernakalant is associated with a reduction of complex propagation patterns characterized by reduced AF complexity, slowing of fibrillation frequency, and inhibition of turning, local re-entries, and breakthroughs.

Introduction

Atrial fibrillation (AF) is characterized by fast and irregular atrial activity. On the longer term, AF leads to electrophysiological and structural changes that contribute to prolongation of AF episodes and loss of efficacy of antiarrhythmic drugs.¹⁻⁴ Moreover, AF stabilization is associated with increased fibrillation frequency and a higher number of waves on the atrial surface.^{3,4} AF perpetuation is also suggested to be dependent on stable and/or transient local rotors that act as sources of high frequencies and wave break.^{5,6} Conversely, mapping of fibrillation patterns during drug-induced termination of AF showed that class III antiarrhythmic drugs reduced the complexity of propagation patterns.⁷⁻⁹

The relatively novel antiarrhythmic drug vernakalant inhibits both the fast sodium current and several potassium currents.¹⁰ We previously demonstrated that the antiarrhythmic effect of vernakalant is mainly explained by sodium current inhibition.¹¹ This class I effect was exhibited by a reduction in excitability, slowing of conduction velocity (CV), and effective refractory period (ERP) prolongation dependent on post repolarization refractoriness.¹²⁻¹⁴ Theoretically, the ERP prolongation could lead to a prolongation of the wavelength and thereby reduce the number of wavefronts. However, with class I antiarrhythmic drugs (e.g. vernakalant) ERP prolongation is combined with conduction slowing that might counteract wavelength prolongation.

Alternatively, it has been proposed that the antiarrhythmic effect of class I drugs is due to the decrease of the safety factor for conduction, thereby inhibiting wavefront turning.¹⁵ Computational studies on cholinergic AF showed that rotors were the source of multiple independent wavefronts. With sodium channel inhibition, rotors were more likely to be interrupted by enhanced meandering of rotors, their collision with the boundaries of the anatomical substrate and thus reduction of the number of active wavefronts.¹⁶

We hypothesized that vernakalant administration reduces complex propagation patterns during AF leading to less spatial variability of conduction vectors, reduction of wavefront turning, fewer breakthrough patterns, and less re-entrant activity. To address this hypothesis, we epicardially mapped AF on both atria in goats with either 3 or 22 weeks of maintained AF in the absence and presence of vernakalant.

Methods

Animal model

The research protocol was approved by the local ethics committee and complied with the Dutch and European directives concerning the use of animals for scientific purposes. Dutch milk goats with a weight ranging of 46-86kg were used. Goats were anesthetized (sufentanyl 6µg/kg/h and propofol 5-10mg/kg/h, i.v.) and the thorax was opened using a left sided approach. Electrodes were fixated on the pericardium above the left atrium (LA) and connected to an implanted neurostimulator (Itrel 3/4, Medtronic, Minneapolis,

Minnesota, USA). After recovery, AF was induced by repetitive pacing (1s, 50Hz bursts) every other second in the first week, followed by one every 10s in the weeks thereafter. Output was set to 2 times the threshold for AF induction with a maximum of 10V. AF was maintained for either 3 weeks (3wAF, n=8) or 22 weeks (22wAF, n=8).

Sacrifice experiment

Goats were anesthetized with intravenous sufentanyl 6 μ g/kg/h, propofol 10mg/kg/h and rocuronium 0.3mg/kg/h for an open chest experiment. The femoral artery was cannulated for pressure monitoring in the ascending aorta. Octagonal mapping arrays containing 249 electrodes (2.4mm inter-electrode distance, 14.3cm²) were placed on the right atrial (RA) and LA epicardial free wall and kept in position throughout the experiment. Electrograms were recorded with a 1.039 kHz sampling rate, filtered at a bandwidth of 0.1-408 Hz and digitized with 16bit AD-converter.

To measure ERP and CV, the heart was cardioverted with a synchronized DC shock ($\leq$ 20 Joules, Physio-control Lifepak 9B, Medtronic, Minneapolis, Minnesota, USA). ERPs were measured using an S1S2 protocol (4x threshold), with of 8 S1 pulses followed by an S2. S2 stimulation started well within the ERP and the S2 interval was increased with increments of 2ms. Electrograms were recorded at different cycle lengths (200ms-500ms, 2X threshold) for the offline assessment of CV. All measurements were performed at baseline and during vernakalant infusion. Stable, clinically relevant plasma levels (low dose $\sim$ 3 μ g/ml (LD), high dose $\sim$ 5mg/ml (HD)) of vernakalant were targeted using loading and maintenance infusions, as described previously.¹¹ At least 10 electrogram files of 60 seconds were recorded, starting fifteen minutes after the start of the maintenance infusion. The first file of these 10 recordings was used for analysis. If the AF episode was $<$ 60seconds, we selected the recording with the longest AF duration.

Analysis of conduction direction

Unipolar atrial electrograms were analysed offline using custom-made analysis software (MATLAB 8.1, The Mathworks, Inc., Natick, Massachusetts, USA). Local activation times in the electrograms were identified using a probabilistic annotation algorithm, as previously described.¹⁷ Based on the activation times the AF cycle length (AFCL), unipolar fractionation index (FI) and conduction vectors were determined. To determine conduction vectors, a plane was fitted through the central activation time and its direct neighbours (min. 3 max 8) in space. If the conduction time of a neighbouring electrode implied a CV of $<$ 20cm/s, the activation time of that neighbour was excluded from the plane fit. Using the fitted plane, the direction of conduction (V_θ) and CV were assessed. For each electrode, the degree of preferential direction of conduction (Pref), calculated as $\text{Pref} = 1 - (\text{circular variance of all } V_\theta)$, and the beat-to-beat difference in V_θ (ΔV_θ) were determined. Local temporal organization of conduction was described as 'linking' (≥ 5 consecutive activations with $\Delta V_\theta < 30^\circ$).¹⁸

Next, we investigated the coherence of local conduction vectors in a 2x2 cross (centred around a central electrode, 0.51cm²). Vorticity, the rotation of vectors within a vector field,

was determined at all spatial and temporal samples. This parameter is derived from continuum mechanics, quantifying the degree of spinning motion around a certain point. Vorticity is dependent on both the vector length (CV) and the field curvature of the 2x2 cross. Since vernakalant affects CV, CV was normalized to 1 for each condition to only detect changes in local spinning. Vorticity was calculated as the vector field curvature of the CV vector field at a given time point. Mathematically, the vector field curvature is calculated as the subtraction between derivative of the y-component of the CV over the x-axis of the grid and the derivative of the x-component of the CV over the y-axis of the grid.

Wave and re-entry analysis

Next, we analysed the fibrillation waves propagating on the epicardial surface as previously described by Zeemering et al.¹⁷ In short, clusters of activation times that are connected in space and time by an apparent CV of ≥ 20 cm/s were defined as waves. A starting point of a wave was an activation time that cannot be explained by conduction of another wave at physiological CV from its environment. If the starting point was present on the edge of the electrode the wave was classified as a peripheral wave. A wave was identified to be a breakthrough wave if it occurred elsewhere in the electrode array. The intra-wave preferentiality to conduct in one direction ($1 - (\text{circular variance of all } V_\theta \text{ within the wave})$) was determined in waves covering an area ≥ 10 electrodes.

Re-entrant activity was identified based on conduction paths. In short, conduction paths were determined as the shortest contiguous trajectory between a starting and end point of a wave, considering a CV ≥ 20 cm/s. If the trajectory had ≥ 1 self-intersection it was considered a local re-entry.

Statistics

Data were analysed using IBM SPSS statistics version 19.0.0. Parameters were first tested for normality using a Shapiro-Wilk test. Data were tested with linear mixed-effects model using a diagonal covariance structure with dose and duration of AF as fixed variables and animal identity as random variable. In case of the analysis of local re-entries, all re-entries were treated as independent phenomena. A Friedman test was used to explore the association between local re-entries and AF dynamics. Conditions with < 4 observations were not included for analysis. Results are reported as mean $\pm$ SD. Statistical significance was taken as $p < 0.05$.

Results

Basic electrophysiological effects of vernakalant

Vernakalant induced both slowing of conduction and ERP prolongation in both atria at a large variety of pacing cycle lengths (**table 1 & 2**). The median AF inducibility by S1S2 pacing was not affected. The wavelength, computed as the product of CV and ERP, was not altered by vernakalant.

Table 1 The effect of vernakalant on general electrophysiological parameters during pacing and AF in the left atrium. Data presented (mean±SD and n) were obtained in goats with either 3 weeks or 22 weeks of AF. In case of AF inducibility, the median and the range are presented. NA= not available <4 data points. * p<0.05, **p<0.01, and ***p<0.001.

Left Atrium	3 Weeks AF			22 Weeks AF		
	Baseline	Low Dose	High Dose	Baseline	Low Dose	High Dose
CV ₄₀₀ (cm/s)	75±12 (7)	57±11*** (8)	56±11* (4)	75±5.3 (6)	56±4.7*** (7)	44±5.5** (4)
CV ₂₅₀ (cm/s)	70±15 (7)	50±11** (7)	46±13* (4)	72±5.1 (6)	45±5.5*** (7)	NA
CV _{AF} (cm/s)	68±9.4 (8)	52±9.6*** (8)	47±7.4*** (8)	59±5.7 (8)	50±6.1*** (8)	47±4.7*** (7)
ERP ₄₀₀ (ms)	65±14 (8)	100±24** (8)	142±52 (4)	83±5.7 (8)	109±27* (7)	143±20* (5)
ERP ₂₅₀ (ms)	73±17 (8)	114±22* (8)	NA	94±26 (7)	119±24*** (5)	NA
Wavelength ₄₀₀ (cm)	4.7±1.3 (7)	5.3±1.8* (8)	NA	6.3±2.2 (6)	6.3±1.3 (6)	NA
Wavelength ₂₅₀ (cm)	4.9±1.8 (7)	5.4±1.2* (8)	NA	6.8±2.2 (6)	5.0±0.8 (4)	NA
AF inducibility (%)	100% (8)	100% (8)	75% 50-100% (4)	100% (7)	100% (7)	NA
AFCL	115±13 (8)	161±20*** (8)	205±20*** (8)	132±12 (8)	175±20*** (8)	202±23*** (7)
AFCL _{p5}	86±15 (8)	130±15*** (8)	160±25*** (8)	94±12 (8)	133±17*** (8)	163±27*** (7)
AFCL irregularity (p50-p5/p50)	0.26±0.06 (8)	0.19±0.05* (8)	0.22±0.08 (8)	0.28±0.08 (8)	0.23±0.06* (8)	0.19±0.09 (5)
AFCL beat to beat irregularity (ms)	18±2.8 (8)	20±5.0 (8)	26±9.4* (8)			
Fractionation index	1.6±0.3 (8)	1.6±0.3 (8)	1.5±0.4 (8)	2.3±0.7 (8)	2.0±0.8 (8)	1.7±0.6 (7)

Table 2 The effect of vernakalant on general electrophysiological parameters during pacing and AF in the right atrium. Data presented, mean±SD and n, were obtained in goats with either 3 weeks or 22 weeks of AF. In case of AF inducibility the median and the range are presented. NA= not available <4 data points. * p<0.05, **p<0.01, and ***p<0.001.

Right Atrium	3 Weeks AF			22 Weeks AF		
	Baseline	Low Dose	High Dose	Baseline	Low Dose	High Dose
CV ₄₀₀ (cm/s)	81±10 (6)	67±8.7** (7)	57±6.4** (6)	76±9.9 (7)	60±6.1*** (6)	55±8.9*** (4)
CV ₂₅₀ (cm/s)	80±9.9 (6)	58±9.4** (6)	NA	73±8.1 (6)	51±9.5** (5)	NA
CV _{AF} (cm/s)	58±5.0 (8)	52±8.6 (8)	49±5.4* (8)	57±2.5 (8)	51±18* (8)	50±20* (6)
ERP ₄₀₀ (ms)	81±19 (8)	113±29** (8)	134±43* (5)	111±38 (7)	126±37 (7)	162±41 (4)
ERP ₂₅₀ (ms)	90±16 (8)	129±26** (8)	156±35* (4)	118±32 (7)	138±39 (7)	NA
Wavelength ₄₀₀ (cm)	6.0±1.6 (6)	7.6±2.5 (8)	7.7±2.5 (5)	8.8±3.8 (7)	7.3±2.6 (6)	8.6±1.3 (4)
Wavelength ₂₅₀ (cm)	6.9±1.3 (6)	7.3±1.6 (6)	NA	8.5±3.0 (6)	6.6±2.1 (5)	NA
AF inducibility (%)	100%	100%	100%	100%	50%	NA
AFCL	50-100% (8)	0-100% (8)	0-100% (4)	0-100% (7)	0-100% (7)	
AFCL _{p5}	84±10 (8)	141±25*** (8)	195±28*** (8)	93±14 (8)	141±19*** (8)	197±14*** (6)
	56±11 (8)	115±20*** (8)	158±36*** (8)	66±13 (8)	107±23** (7)	160±34*** (6)
AFCL Irregularity (p50-p50)	0.32±0.09 (8)	0.20±0.06* (8)	0.20±0.09* (8)	0.30±0.04 (8)	0.26±0.10 (7)	0.19±0.14 (6)
Fractionation index	1.3±0.3 (8)	1.2±0.3 (8)	1.3±0.4 (8)	1.7±0.3 (8)	1.5±0.3 (8)	1.4±0.5 (7)

AF termination and wave complexity

Goats were in stable AF (>30minutes) before vernakalant infusion, except for one 3wAF goat. AF terminated earlier in 3wAF goats compared to 22wAF goats (**figure 1A**). In **figure 1** panel B the changes in AFCL, FI, and waves/cycle of a representative 3wAF goat are presented. Vernakalant infusion induced a progressive prolongation of AFCL, a reduction of FI and a decline in AFCL irregularity in both the left and right atrium (**table 1** and **2**). Despite large fluctuations in the number of waves/cycle a strong decrease in number of waves was observed just before cardioversion. The wave maps in **figure 2** illustrate the complexity of propagation patterns in a 3wAF and a 22wAF goat before and after vernakalant administration. In the 3wAF goat 6-7 simultaneous waves were present at baseline. The 22wAF goat had clearly more complex patterns with about 10-11 waves. High dose vernakalant reduced the number of waves in both goats. Overall, 3wAF goats had a lower total number (RA + LA) of waves/cycle compared to 22wAF, 15 ± 2.1 vs. 20 ± 6.0 respectively. In **figure 3**, the effect of vernakalant on the number of waves and breakthroughs is depicted for all goats. Vernakalant reduced both waves and breakthroughs to a comparable extent in the RA and LA irrespective of the animal group.

Figure 1 Termination of AF by vernakalant. **A** AF survival plots at baseline and two dosages of vernakalant for 3wAF (n=8) and 22wAF (n=8) goats. Time to cardioversion was longer in 22wAF goats. The electrogram recordings below illustrates that vernakalant increased AFCL in a dose-dependent manner. **B** Progressive changes in AFCL, fractionation index and number of waves prior to AF termination in a 3wAF goat. The number of waves showed large fluctuations but decreased considerably prior to cardioversion.

Figure 2 Effect of vernakalant on fibrillation patterns. Wave maps depict the territory of individual fibrillation waves within the mapped area during 1 AF cycle. Two cycles at baseline and after high dose administration of vernakalant are presented for a 3wAF and a 22wAF goat. The waves are colour-coded according to sequence of appearance. 3wAF goats displayed fewer waves compared to 22wAF. Both groups of goats had highly variable beat-to-beat changes in conduction directions. After vernakalant administration, the number of waves decreased and conduction directions were more organized and repetitive.

Figure 3 The effect of vernakalant on the number of waves and breakthroughs per AF cycle recorded on the epicardial surface the right (blue) and left atrium (red). Data for both 3wAF and 22wAF goats is presented. Vernakalant decreased the number of waves and breakthroughs. LD or HD vs baseline * $p < 0.05$, ** $p < 0.01$.

Local re-entries

A total of 1667 local re-entries, compared to 66,318 waves, were identified in 429 seconds recording time in the 3wAF goats at baseline conditions. In 22wAF goats a total of 1483 local re-entries, compared to 84,033 waves, in 480 seconds were identified (**table 3**). Only transient re-entries appeared within the field of view of the mapping arrays. The maximum number of consecutive rotations per local re-entry at baseline was 3.5 ± 1.5 and 3.1 ± 1.1 for 3wAF and 22wAF respectively. Vernakalant did not change maximum number of rotations, absolute re-entry lifespan and relative re-entry lifespan (**table 3**). Vernakalant strongly and dose-dependently reduced the occurrence of local re-entries (**figure 4A**).

It has been suggested that during AF, a functional re-entry may be the source of the shortest cycle length (i.e. highest frequency). Furthermore, local re-entry may lead to wave split due to spatial heterogeneities of repolarization.¹⁹ We therefore investigated the impact of local re-entries on AFCL and complexity of propagation patterns (waves/cycle). The AFCL and waves/cycle as a function of time is presented in **figure 4** for a goat with 22 weeks of AF. In these graphs no association between local re-entries and changes in AFCL or waves/cycle are apparent. To quantify whether changes in AFCL or waves/cycle occurred when a local re-entry was present we determined the Δ AFCL and Δ waves/cycle

for the re-entry window compared to a 500ms window directly before initiation of the local re-entry. Similarly, we investigated the 500ms window directly after the end of the local re-entry to detect changes in AF dynamics after the termination of the local re-entry. The results are listed in **Table 3**. The average for Δ AFCL or Δ waves/cycle, during and after the rotor, were centred around zero and vernakalant did not affect this behaviour, indicating that the presence of a local re-entry had no detectable effect on AF dynamics. Despite the lack of differences in the mean, some rotors could have had a large impact on AF dynamics acting as occasional sources of high frequency and wave split. Such phenomena would have led to changes in the Δ AFCL and Δ wave/cycle histograms. To investigate whether this occurred, we compared the entire population of local re-entries to a population that was randomly sampled outside the rotor occurrences. A two-way Kolmogorov-Smirnov test indicated that both populations (random sampling vs. local re-entries) showed similar distributions. This suggests that the detected local re-entries did not affect AFCL and waves/cycle.

Table 3 Local re-entries during AF and their impact on AF dynamics in goats with 3 or 22 weeks of AF. In the first column, the total number local re-entries and the mean $\pm$ SD of the absolute and relative lifespan is listed. In the second column the effect of local re-entries on AFCL, once a local re-entry initiated or when it terminated, is presented as mean $\pm$ SD. In the third column the effect of local re-entries on the number of waves is presented as mean $\pm$ SD. A Friedman test was performed for statistical analysis. * $P < 0.05$ vs BL

	Local re-entry			Δ AFCL		Δ Waves	
	Total observed (range across files)	Lifespan (ms)	Relative Lifespan (Lifespan:AFCL)	Re-entry initiation (ms)	Re-entry termination (ms)	Re-entry initiation (ms)	Re-entry termination (ms)
RA 3wAF							
Baseline	1277 (20-252)	175 $\pm$ 25	2.24 $\pm$ 0.23	-0.03 $\pm$ 0.85	0 $\pm$ 0.68	0.13 $\pm$ 0.13	0.02 $\pm$ 0.07
Low dose	277 (0-80)	217 $\pm$ 57	1.73 $\pm$ 0.57*	1.76 $\pm$ 5.11	-0.92 $\pm$ 4.25	0.25 $\pm$ 0.26	-0.08 $\pm$ 0.3
High dose	47 (0-20)	263 $\pm$ 47	1.49 $\pm$ 0.21	-9.95 $\pm$ 22.12	-6.71 $\pm$ 10.63	0.12 $\pm$ 0.58	-0.2 $\pm$ 0.46
LA 3wAF							
Baseline	390 (10-141)	162 $\pm$ 32	1.49 $\pm$ 0.29	0.23 $\pm$ 1.61	-1.99 $\pm$ 3.75	0.27 $\pm$ 0.23	-0.16 $\pm$ 0.2
Low dose	124 (3-40)	247 $\pm$ 110	1.67 $\pm$ 0.87	1.63 $\pm$ 3.01	1.16 $\pm$ 5.7	0.14 $\pm$ 0.38	-0.04 $\pm$ 0.69
High dose	26 (0-9)	298 $\pm$ 69	1.69 $\pm$ 0.57	9.25 $\pm$ 16.09	-7.34 $\pm$ 13.63	0.27 $\pm$ 1.1	-1.33 $\pm$ 0.42
RA 22wAF							
Baseline	1163 (56-247)	158 $\pm$ 11	1.78 $\pm$ 0.33	-0.4 $\pm$ 0.96	-0.49 $\pm$ 0.73	0.2 $\pm$ 0.11	-0.12 $\pm$ 0.07
Low dose	282 (0-52)	189 $\pm$ 20	1.47 $\pm$ 0.21	0.52 $\pm$ 1.64	-0.94 $\pm$ 1.36	0.22 $\pm$ 0.15	-0.05 $\pm$ 0.38
High dose	77 (0-49)	219 $\pm$ 47	1.33 $\pm$ 0.31	-2.66 $\pm$ 2.2	-1 $\pm$ 3.56	-0.08 $\pm$ 0.48	-0.28 $\pm$ 0.41
LA 22wAF							
Baseline	275 (7-73)	149 $\pm$ 21	1.18 $\pm$ 0.23	-0.84 $\pm$ 1.25	-0.61 $\pm$ 1.12	0.45 $\pm$ 0.55	-0.49 $\pm$ 0.37
Low dose	139 (2-40)	195 $\pm$ 26	1.15 $\pm$ 0.16	1.23 $\pm$ 4.14	0.23 $\pm$ 2.78	0.51 $\pm$ 0.74	-0.65 $\pm$ 0.6
High dose	62 (0-32)	215 $\pm$ 62	1.13 $\pm$ 0.30	1.41 $\pm$ 5.48	-1.18 $\pm$ 4.19	-0.25 $\pm$ 0.66	-0.89 $\pm$ 1.43

Figure 4 The effect of vernakalant on the presence of local re-entries. **A** Vernakalant strongly decreased the prevalence of local re-entries in both right (blue) and left (red) atrium and in both groups. LD or HD vs baseline * $p < 0.05$. **B** The dynamical changes in AFCL and number of waves in a 10s window in a goat with 22 weeks AF. A white column indicates the period when a local re-entry was present. Local re-entries did not lead to consistent changes in AFCL or number of waves, neither at baseline nor in the presence of vernakalant.

Conduction directions and wavefront turning

When wave maps in **figure 2** are inspected in more detail, it can be appreciated that wave trajectories were undergoing sharp pivots and conducted in all directions and neighbouring waves often propagated in opposite directions. After administration of vernakalant much more coherent patterns occurred, in which wave trajectories became more uniformly aligned in the same direction.

To explore the variation in conduction directions we first analysed the temporal variation of local conduction vectors for each electrode, therefore reflecting local turning. In panel A of **figure 5** a circular histogram of conduction directions is presented. The black line (baseline) shows that at this electrode, conduction may go in every direction. This circular distribution remained highly stable when ten consecutive 60-second recordings were considered (data not shown). The red line clearly illustrates a preferential direction was established after vernakalant infusion. Indeed, preferentiality to conduct in one direction significantly increased after vernakalant administration in all animals (**figure 5B**). Sudden changes in direction, beat-to-beat (ΔV_θ), were also reduced (**figure 5C**) and linking became more prevalent (**figure 5D**). These effects were similar for both atria and in both

groups. These parameters demonstrated that in the local temporal domain, the manifestation of conduction properties became more regular. Next, we analyzed turning in the spatiotemporal domain. We determined vorticity within an area of 0.51cm^2 to describe instantaneous local rotation. Vorticity was strongly reduced for both atria and experimental groups (**figure 6A**). This suggests less turning of wavefronts and fewer wavefront/wavetail interactions. Likewise, intra-wave preferentiality increased significantly within the individual waves. This increase of was present in both atria and groups.

Figure 5 Analysis of local conduction vectors in the temporal domain. **A** During a 60-second AF recording about 300-500 conduction vectors per electrode are obtained. A circular histogram for an electrode is depicted from a 22wAF goat. At baseline, variability in direction of propagation was high. However, after administration of vernakalant, a clear preferential in direction was established. **B, C & D** Left atrial data for the degree of preferentiality, beat-to-beat changes in direction and the degree of linking. These parameters indicate that after vernakalant administration changes in direction reduced in the both groups of goats. LD or HD vs baseline * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 6 Analysis of conduction direction in the spatial domain. Vorticity describes the local spinning of conduction vectors. The vorticity maps on the left depict a snapshot of instantaneous vorticity recorded in a 22wAF goat. Dark red zones indicate clockwise wavefront turning, dark blue zones indicate counter clockwise turning, and green zones indicate zones with parallel oriented conduction vectors. White arrows denote the direction of the local conduction vector. Average atrial vorticity in 3wAF and 22wAF goats is presented (panel A). During vernakalant administration, a clear reduction of vorticity was observed in all goats. The coherence of conduction vectors within waves (intra-wave preferentiality) of a minimal waves size of 10 electrodes is presented in the lower panel. The intra-wave preferentiality was increased in all goats. Data presented are the individual averages of waves in the left atrium. LD or HD vs baseline * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Discussion

In the present study, we found that vernakalant reduces complex propagation patterns during AF. Overall, patterns became more organized and unidirectional just before AF termination. In addition, vernakalant strongly reduced AF stability, even after 3 or 22 weeks of AF.

The reduction in propagation pattern complexity was reflected by fewer waves, breakthroughs, and local re-entries and went along with AFCL prolongation and reduction of wavefront turning. We conclude that AF termination induced by vernakalant is associated with: 1 AFCL prolongation, 2 inhibition of wave turning, 3 reduction of the number of waves, 4 inhibition of local re-entry formation, and 5 inhibition of breakthrough waves.

Complexity of propagation patterns

In patients with longstanding persistent AF, more complex propagation patterns are observed than in patients with acutely induced AF.²⁰⁻²² Similarly, goat atria display more complex propagation patterns with the progression of AF-induced remodelling.⁴ Also in this study we found an increase of AF complexity with increasing duration of AF. Complex patterns have been associated with an increase of fibrous tissue depositions contributing to conduction delay and block and dyssynchronous activity.^{2,23} Moreover, shortening of the action potential due to electrical remodelling increases the atrial activation rate.^{1,3} Experimental work by Moe et al. showed that high activation frequencies lead to 1:1 propagation failure in canine atria, potentially leading to conduction block and wave splitting.²⁴ It was later shown that indeed high frequency stimulation of pectinate bundles leads to fibrillatory conduction with generation of new wavefronts.²⁵ In the multiple wavelet model of sustaining AF, based on heterogeneous substrate of ERPs restitution of CV, it was predicted that ERP prolongation results in a reduction of the number of simultaneous waves.²⁶ This prediction was later supported by observations that shortening of the ERP leads to more wavefronts and small re-entrant circuits²⁷ and, conversely, AFCL prolongation by class III antiarrhythmic drugs leads fewer wavefronts.⁷⁻⁹ Therefore, AFCL as such can be considered to be an important determinant for the number of wavefronts and overall complexity. Findings within this study support this important interdependence of AFCL and complexity of propagation patterns.

Wavefront turning

Action potential propagation within cardiac tissue is dependent on a sufficient source current generated by the wavefront to depolarize the unexcited tissue downstream (sink) up to the threshold for sodium channel activation.²⁸ This relation between source and sink is depending on the tissue architecture, degree of coupling between cells, and depolarizing current strength.²⁹ The potent inhibition of sodium current by vernakalant¹¹⁻¹⁴, as also confirmed in this study, affects the source/sink balance and decreases the safety factor for wavefront propagation. At the site of a pivot point, the CV is reduced most strongly when the safety factor for conduction is lowered, leading to wider turns.¹⁵ Atrial tissue oriented perpendicular to a muscle bundle can only be activated by this wavefront when it makes a turn. The local cycle length will be short when the turn is sharp but is longer when a turn is wider. Turning within waves and between waves was indeed potentially inhibited offering a potential mechanism for the observed AFCL prolongation.

Moreover, inhibition of turning may have contributed to the observed reduction of breakthrough patterns. In previous work we found that transmural conduction was the predominant mechanism for breakthroughs in the goat.³⁰ A sharp turn is likely to occur when the epicardial surface is activated by a wavefront that conducted along a small transmural bundle. Breakthroughs may therefore be prone to conduction block as a consequence of the required sharp turn from the bundle to the epicardial surface. Preferential block of breakthroughs in the presence of Na⁺-channel block may also be due to the relatively large mass of the epicardial surface as compared to the small source of the transmural bundle.

In summary inhibition of turning was associated with a reduction in complexity in propagation patterns leading to prolonging of AFCL and prevention of breakthroughs.

Local re-entries

Local re-entries are obviously dependent on turning and the maximal number of local re-entries was lower in the presence of vernakalant. Comparable to our observation, “numerous vortices [local re-entries] that shift in position and direction” were observed in the computer model of sustained AF that was dependent on multiple wavelets.²⁶ On the other hand, rotors have been proposed to be the perpetuators of AF, being a high frequency source that results in wave break and complex conduction.¹⁹ Antiarrhythmic action of (state dependent) sodium channel inhibition in the context of rotor driven AF has been investigated in an *in silico* model.¹⁶ The model was composed of a sheet of nodes that resembles human atrial electrophysiological properties and was delimited by a rectangular border. Within this rectangle structure, islands of very short action potential duration were created. The heterogeneous distribution of action potential duration led to multiple waves that could form new rotors. The researchers reported that sodium current inhibition led to AF termination by an increase in the size of the core of the rotor (leading to AFCL prolongation), less daughter wavelet formation and increased rotor drift leading to rotor termination once it hit a boundary. A number of our observations are in agreement with this study, such as AFCL prolongation, fewer waves, and reduced presence of local re-entries. We did not determine the drift of the local re-entries because they were of very short lifespan, which was not changed after vernakalant administration. The absence of any effect on lifespan suggests that local re-entry formation, rather than stability was inhibited. We also investigated whether local re-entries affected AFCL or the number of waves but we could not identify a relation between the occurrence of local re-entries and AFCL or number of waves. The action of local re-entries as perpetuators of AF is not supported by our observations, but can also not be excluded.

Conclusions

During untreated AF we observed complex propagation patterns with wavefronts that could conduct in any direction. Administration of vernakalant prolonged ERP and AFCL

strongly and reduced the number of waves including breakthroughs and local re-entries. A more periodic process got established as randomness of conduction direction and irregularity of cycle length was reduced. As vernakalant did not increase wavelength it is likely that the inhibition of turning prolonged AFCL which in turn resulted in lowering of the complexity of AF propagation patterns and finally in termination of AF.

Limitations

In this study we used a large (14.3cm²) high-density electrode arrays, one the RA and one on the LA free wall. As with any currently available *in vivo* mapping technique, not the entire atria were mapped. We cannot exclude, but also do not have clear indications, that outside the field of view a stationary re-entrant or focal source were driving AF.

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5

Chapter 5

Stationary atrial fibrillation properties in the
goat do not entail stable or recurrent
conduction patterns

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Frontiers in Physiology 2018. doi: 10.3389/fphys.2018.00947

Abstract

Introduction: Electro-anatomical mapping of the atria is used to identify the substrate of atrial fibrillation (AF). Targeting this substrate by ablation in addition to pulmonary vein ablation did not consistently improve outcome in clinical trials. Generally, the assessment of the substrate is based on short recordings (≤ 10 s, often even shorter). Thus, targeting the AF substrate assumes spatiotemporal stationarity but little is known about the variability of electrophysiological properties of AF over time.

Methods: Atrial fibrillation (AF) was maintained for 3–4 weeks after pericardial electrode implantation in 12 goats. Within a single AF episode 10 consecutive minutes were mapped on the left atrial free wall using a 249-electrode array (2.25 mm inter-electrode spacing). AF cycle length, fractionation index (FI), lateral dissociation, conduction velocity, breakthroughs, and preferentiality of conduction (Pref) were assessed per electrode and AF property maps were constructed. The Pearson correlation coefficient (PCC) between the 10 AF-property maps was calculated to quantify the degree spatiotemporal stationarity of AF properties. Furthermore, the number of waves and presence of re-entrant circuits were analyzed in the first 60-s file. Comparing conduction patterns over time identified recurrent patterns of AF with the use of recurrence plots.

Results: The averages of AF property maps were highly stable throughout the ten 60-s-recordings. Spatiotemporal stationarity was high for all 6 property maps, PCC ranged from 0.66 ± 0.11 for Pref to 0.98 ± 0.01 for FI. High stationarity was lost when AF was interrupted for about 1 h. However, the time delay between the recorded files within one episode did not affect PCC. Yet, multiple waves (7.7 ± 2.3) were present simultaneously within the recording area and during $9.2 \pm 11\%$ of the analyzed period a re-entrant circuit was observed. Recurrent patterns occurred rarely and were observed in only 3 out of 12 goats.

Conclusions: During non-self-terminating AF in the goat, AF properties were stationary. Since this could not be attributed to stable recurrent conduction patterns during AF, it is suggested that AF properties are determined by anatomical and structural properties of the atria even when the conduction patterns are very variable.

Keywords: atrial fibrillation, mapping, conduction patterns, stationary patterns, recurrence quantification analysis, AF dynamics

Introduction

Mapping of conduction patterns has been of fundamental importance to understand mechanisms that maintain cardiac arrhythmias (Nattel et al., 2005). The importance of cardiac mapping in clinical practice was demonstrated by Haïssaguerre's et al. (1998) pivotal finding that atrial fibrillation (AF) was often initiated from the pulmonary veins (PV) and that ablation of ectopic sites in the PVs terminated AF. However, many patients experience recurrences of AF in the months following a PV isolation (Verma et al., 2015), suggesting that other regions in the atria may contribute to AF perpetuation as well.

Detailed mapping studies of AF, in both animal models (Berenfeld et al., 2000; Verheule et al., 2010) and humans (Konings et al., 1994; Allesie et al., 2010; Lee et al., 2014, 2015), have described fast, irregular and seemingly random conduction patterns. AF maintenance can be explained by different conceptual models such as multiple wavelets (Allesie et al., 1996, 2010), rotor activity (Jalife, 2011) and repetitive focal activity (Lee et al., 2015). Notably, these mechanisms are not mutually exclusive and different mechanisms may occur in an individual patient. Several electrophysiological parameters have been used to detect local sources of AF (Nedios et al. (2016)). Complex fractionated atrial electrograms (CFAE) and high frequency zones are thought to reflect driver sites of rotational or focal activity or to correlate with areas demonstrating high complexity of AF. Unlike such electrogram parameters, with focal impulse and rotor mapping (FIRM) conduction patterns are identified that putatively describe focal and rotational activity (Narayan et al., 2012; Swarup et al., 2014). Unfortunately, targeting these substrate parameters has variable outcomes in clinical trials and need further validation (Gadenz et al., 2017; Krummen et al., 2017; van der Does and de Groot, 2017).

A potential factor contributing to the limited success rates are the limitations of the mapping techniques used. The atrial surface can only be mapped with a limited time and spatial resolution. A limited spatial resolution may lead to misinterpretation of conduction patterns (Kuklik et al., 2017; Roney et al., 2017). If higher spatial resolution is obtained, for example by point-by-point mapping typically relatively short recordings are acquired. It is unknown whether these recordings are representative for longer episodes of AF. Also, it is largely unexplored whether AF driver sites are stable across different AF episodes.

In this study we analyzed left atrial epicardial high-density recording in goats with 3 to 4 weeks of maintained AF. We studied the degree of stationarity of local AF properties derived from ten 60-s-long recordings within a single AF episode and between different AF episodes. Moreover, we analyzed complexity and stability of conduction patterns, and rotational activity. For the assessment of stability of conduction patterns, we made use of a new recurrence quantification analysis.

Methods

Animal model

This study was carried out in accordance with the principles of the Basel Declaration and regulations of European directive 2010/63/EU. The local ethical board for animal experimentation of the Maastricht University approved the protocol. In total 12 goats, 6 per study, weighing 60 ± 9.8 kg were included. Goats were anesthetized (sufentanyl $6 \mu\text{g/kg/h}$ and propofol $5\text{--}10$ mg/kg/h, i.v.) and electrodes were implanted on the pericardium above the left atrium (LA). After 2 weeks of recovery from surgery, AF was induced by repetitive burst of stimuli (1 s, 50 Hz, 2 times threshold with a maximum of 10 V) using subcutaneously implanted neurostimulator (Itrel 3 or 4, Medtronic, Minneapolis, Minnesota, USA). AF was subsequently maintained for 3–4 weeks. For open chest sacrifice experiments, goats were anesthetized with parenteral sufentanyl $6 \mu\text{g/kg/h}$, propofol 10 mg/kg/h, and rocuronium 0.3 mg/kg/h.

Data acquisition

A circular mapping array containing 249 electrodes (2.4 mm inter-electrode distance, 14.3 cm² surface area) was placed on the left atrial (LA) free wall and kept in stable position throughout the experiment. Unipolar electrograms were recorded with 1.039 kHz sampling rate, bandwidth of $0.1\text{--}408$ Hz and AD resolution of 16bit. In a 10-min window ten 60-s files were recorded during non-selfterminating AF. In a subset of 6 animals 2 additional 10-min windows were recorded (**Figure 1**). Electrical cardioversion of AF was performed using a ≤ 20 Joules synchronized DC shock (Physio-control lifepak 9 B, Medtronic, Minneapolis, Minnesota, USA). The DC shock was delivered on endocardial catheters of which one was placed in the coronary sinus and the other in the right atrial cavity.

Analysis of local activation time and direction

The recorded signals were analyzed offline using custommade analysis software (MATLAB 8.1, The Mathworks, Inc., Natick, Massachusetts, USA). Local deflections, activation times, and unipolar fractionation index (FI) were identified using a probabilistic annotation algorithm, as previously described (Zeemering et al., 2012; Lau et al., 2015). Based on the activation times, the AF cycle length (AFCL) and conduction direction and velocity (CV) were determined. For calculation of conduction vectors, a plane was fitted through the central activation time and its direct neighbors in space and time (min. 3 max 8). If the conduction time of a neighboring electrode implied a CV of < 20 cm/s, the occurrence of conduction block was assumed and the activation time of that neighbor was excluded from plane fitting. Per electrode the degree for preferential direction of conduction (Pref) was calculated as $\text{Pref} = 1 - (\text{circular variance of all conduction vectors})$. The maximal time

difference of activation times with its neighbors was calculated as a measure of epicardial lateral dissociation (LD).

Goats 7-12

Goats 1-6

Figure 1 Schematic presentation of the experimental protocol. Grey boxes represent periods of AF. Ten consecutive minutes per AF episode were recorded. Only a single AF episode was recorded in goats with ID numbers 7–12, see also **Table 2**. Three different AF episodes were recorded in goats with ID number 1–6. Two episodes were close in time and 1 episode was separated for about 1 h.

AF property maps analysis

Based on the results of the above-mentioned analysis, 6 different parameters, i.e., FI, AFCL, LD, CV, breakthroughs (BT), and Pref were obtained for each individual electrode and used to construct AF property maps. Per parameter the spatial Pearson correlation coefficient (PCC) between property maps, of all 45 possible comparisons within a 10-min window, was determined and averaged per animal, producing an intra-episode correlation of property maps at various time points. To address potential correlations due to chance, we randomly reassigned electrodes in space and recalculated the average PCC. In a subset of 6 animals three 10-min windows were recorded from 3 different episodes of AF, in order to determine the inter-episode correlation.

Analysis of epicardial waves and re-entrant circuits

Next, we analyzed the fibrillation waves propagating on the epicardial surface as previously described by Zeemering et al. (2012). In short, waves were defined as clusters of activation times that are connected in space and time by an apparent CV of > 20 cm/s. The earliest activation time that cannot be explained by its surrounding was identified as the starting point of the wave. This starting point was specified as peripheral, on the border of the electrode, or breakthrough, separated > 1 electrode from the border, (BT) origin. Furthermore, we analyzed re-entrant activity based on conduction paths that can be identified based on the activation times. Conduction paths were determined as the shortest contiguous trajectory between a starting and end point of a wave, considering $CV \geq 20$ cm/s. If the trajectory had ≥ 1 intersection(s) it was considered as a re-entrant circuit (RC).

Recurrence analysis

For a recording with N time samples, a recurrence plot (an $N \times N$ scatter plot with time on the x- and y-axis) can be constructed. If an event occurs at two time points with sufficient similarity, it is considered to be recurrent and can be identified by a dot in the scatter plot. Here, we analyzed the recurrence of conduction patterns in 60-s-recordings. We considered a pattern to be recurrent if the wave front(s); (1) reached the same point in space, (2) propagated in the same direction, and (3) had a similar shape. To achieve this, we developed a method that is based on local activation times and corresponding activation intervals. The linear phase ($-\pi$ to π) was interpolated for every individual interval (**Figure 2A**). This approach does not require Hilbert transformation or time embedding of the raw electrogram as frequently used for the identification of phase singularities.

Phase signals were down-sampled to 10 timesteps per AFCL because the recurrence analysis leads to a large number of comparisons $[(60s \times 1.039kHz)^2 = 3.9e9]$ and long computation times would be required. Based on the phase signals, phase maps were constructed for all time steps. For individual electrodes, the difference in phase was calculated by comparing 2 phase maps. Based on all 249 phase differences, the average phase difference (aPD) was computed. This was executed for every possible comparison between time steps within the 60-s-recording. Recurrent patterns were identified at an aPD of $< \pi/4$, corresponding to 1/8 of the AFCL. The rate of recurrent patterns was calculated as the ratio of the number of observed recurrences to the number of expected recurrences if the pattern was fully recurrent. The number of unique recurrent patterns was subjectively scored (by AvH) with the help of conduction movies.

To illustrate the impact of conduction patterns on the aPD and consequent recurrence identification, we can consider different hypothetical wavefronts. **Figure 2B** demonstrates the effect of time in the recurrence analysis. One large “recurrent” wave front was present in the middle mapping area in the first map, timepoint n . In the second map, $n + 2$ timesteps, the wave front had propagated somewhat further, resulting in an aPD of 1.46. This level of the aPD is above $\pi/4$ (0.7854) and therefore considered to be non-recurrent. At timestep $n + 4$, the aPD increased to a maximum of 2.96. In the following timesteps ($n + 6$ and $n + 8$), aPD decreased until at $n + 10$ the wave front reoccurred at the same site like timepoint n . Here, the aPD was 0 and consequently the pattern was considered to be recurrent.

Figure 2C demonstrates the effect of direction and wave front shape. Map n is again presented as the first map. The wave front in the second map propagated in the opposite direction compared to map n . The aPD was now calculated to be 1.53, thus non-recurrent. In the third map the wave did propagate in the same direction like map n . However, the wave front shape was now set to be convex instead of planar. The aPD between this map and map n was 1.62, therefore also non-recurrent.

A Transformation from electrogram to phase

B The effect of time

C The effect of conduction direction and wave front shape

Figure 2 **A** Illustrates that based on the unipolar electrogram activation times (red columns) are identified. Based on the length of the interval, the linear phase from activation time to activation time was interpolated. **B** Demonstrates the effect of time on the recurrence analysis. One large “recurrent” wave front was present in the middle mapping area in the first map, timepoint n . In the second map, $n + 2$ timesteps, the wave front propagated somewhat further, resulting in an aPD of 1.46. This level of the aPD is above $\pi/4$ (0.7854) and therefore considered to be non-recurrent. At timestep $n+4$, the aPD increased to a maximum of 2.96. In the following timesteps ($n+6$ and $n+8$), aPD decreased again until at $n+10$ the wave front reoccurred at the same site like timepoint n . Here, the aPD was 0 and consequently considered to be recurrent. **C** Demonstrates the effect of direction and wave front shape. Map n is presented again as the first map. The wave front in the second map propagated in the opposite direction compared to map n . The aPD was now calculated to be 1.53, thus not recurrent. In the third map the wave did conduct in the same direction like map n . However, the wave front shape was now set to be convex instead of planar. The aPD between this map and map n was 1.62, thus also non-recurrent.

Statistics

Data are presented as mean $\pm$ sd. Data were tested for normality using a Kolmogorov-Smirnov test. The effect of time was tested using a repeated measure ANOVA. Intra- and inter- episode correlation between property maps was assessed using Pearson

correlation coefficient (PCC). A Bonferroni's correction was applied to correct for multiple comparisons. Inter-episode correlation differences were tested with a Wilcoxon rank sum test.

Results

AF property maps

Twelve goats a 10-min period within a single AF episode was recorded. This 10-min period was divided into ten 60-s- recordings. In **Figure 3** two property maps, the first and last recording in a period, of goat 2 are presented. Only minor differences in the spatial distribution of the AF properties between the 2 recordings are apparent. This suggests that the AF properties remained very stable over this interval of 9 min. This high stability was found in all 6 parameters and all goats (**Figure 4**). We further addressed the spatial stationarity of the different parameters by exploring the correlation between AF property maps. From **Figure 4B** it can be appreciated that the average PCCs were very high, ranging from 0.66 ± 0.11 for Pref up to 0.98 ± 0.01 for FI and all PCCs were significant (**Table 1**). To underscore that this finding was not due to chance we broke the spatial coherence of the maps by randomly reassigning the electrodes within the map. This test diminished the average PCCs to almost 0 (right panel of **Figure 4B**). These observations demonstrate that both a high temporal and spatial stationarity was present.

We also looked at to what extent AF properties vary in space. High correlations between 60-s-recordings could have occurred due to a limited spatial diversity of the parameter. **Table 1** presents the coefficient of variation and range within the property maps. Within the AFCL-maps the coefficient of variation was very low ($4.3 \pm 2\%$), demonstrating a fairly homogeneous spatial distribution. By contrast, for FI-, preferentiality-, and break-through-maps the coefficient of variation was much larger, ranging from 50 to 78%. This indicates that discrete zones on the atrial epicardial surface exhibited different properties. In **Table 1** the overall characteristics of the 6 different property maps is presented.

All property maps are available in the Supplemental Materials. Raw data are not available online because of the large data size, 240 min for 249 unipolar channels at a sampling frequency of 1039 Hz. The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Figure 3 Spatial distribution of 6 AF parameters from goat 2. The left sided column represents property maps of the first 60-s-recording in a 10-min window. The right column depicts the final minute of the 10-min window. A high agreement for the absolute average of the maps was found between the maps. Interestingly, also a high degree of stationarity of spatial organization was found when the Pearson correlation coefficient (PCC) was considered. This was true for almost all parameters but weakest for preferentiality of conduction direction, suggesting highly variable conduction patterns.

Figure 4 **A** Intra-episode PCC for 6 different AF parameters. On the x-axis the individual animals are given. Panel A represents the absolute average of a map in a 60-s-recording. Per animal 10 values of the 10 consecutive maps are given. **B** In the first panel the average PCC, based on 45 correlations, is given per animal per parameter. The right-hand panel confirms that this high degree of stationarity did not occur due to chance since breaking the spatial coherence completely removed the correlation.

Table 1 Spatial variation (coefficient of variation and range) and stability in time (PCC) of the 6 different AF parameters.

	Coefficient of variation	Range	PCC(r)	PCC <i>p</i> -value
Fractionation index	50 ± 0.8%	4.60 ± 1.5	0.983 ± 0.01	<i>p</i> < 0.001
AFCL (ms)	4.3 ± 2%	21.2 ± 8.2	0.956 ± 0.04	<i>p</i> < 0.001
Lateral dissociation (ms)	28 ± 8%	29.8 ± 5.5	0.968 ± 0.01	<i>p</i> < 0.001
Conduction velocity (cm/s)	23 ± 14%	115 ± 76	0.926 ± 0.06	<i>p</i> < 0.001
BT (n/electrode/60 s)	78 ± 34%	53 ± 22	0.799 ± 0.08	<i>p</i> < 0.001
Preferentiality	53 ± 11%	3.1 ± 0.02	0.655 ± 0.11	<i>p</i> < 0.001

Per animal the average coefficient of variation and average range (max-min) of the 10 maps was calculated. The grand mean ± sd is presented in the table. The grand mean of the Pearson correlation coefficient (PCC) was based on all 45 comparisons per animal.

Short term AF dynamics

Potentially, stable conduction properties can be stable on a beat- to-beat level or at somewhat longer timespans, e.g., seconds, could have contributed to the stationary property maps within single AF episodes. Therefore, we explored dynamic behavior of AF (**Figure 5**). On a beat-to-beat level, the AFCL varied by 20 ± 3.7 ms, $16 \pm 3.6\%$ of the AFCL, and conduction direction varied with 66 ± 10 degrees. This high dynamic behavior was also reflected in the large average number of simultaneously present waves per cycle, 7.7 ± 2.3 . In total 453 re-entrant trajectories were found of which 25 lasted >2 rotations. On average re-entrant activity was present for $9.2 \pm 11\%$. The lifespan of re-entrant circuits was 149 ± 18 ms. Hence, the average lifespan of reentries was short and close to the AFCL with a lifespan to AFCL ratio of 1.2 ± 0.3 . These findings demonstrated a large beat-to-beat variability with high dynamic patterns that cannot explain stationary AF properties.

Figure 5 Dynamic properties of conduction during AF. On a beat-to-beat level spatiotemporal variability was found, as reflected by the beat-to-beat difference AFCL and direction of conduction. Furthermore, on average multiple waves were present at every beat. A limited percentage of time re-entrant circuits were present and showed a high degree of variation.

Recurrence of conduction patterns

On average, AF exhibited a highly dynamic behavior in terms frequency and patterns, nonetheless a high degree of spatiotemporal stationarity of AF properties was present. This could be explained by frequent recurrent patterns that dominate the property maps. We therefore investigated the occurrence of recurrent AF patterns.

In **Figure 6**, a representation of the construction of a representative recurrence plot is presented. A segment of the AF recording with recurrent activity is depicted on the left series of maps in panel A. In the right side a series of maps with a segment with non-recurrent activity is presented. In **Figure 6B**, the recurrence plots over 3 cycles of these two segments are shown. The recurrent phase presented diagonal lines while during the segment with chaotic activity no recurrences were found. In the overall 60s-recurrence plot, we can observe that the non-recurrent phase was present throughout most of the time. The recurrent pattern appeared 3 times, at timepoints 32, 48, and 52s, in the 60s-recordings.

Figure 6 A schematic explanation of recurrence analysis of conduction patterns. Goat 10, **Table 2**, was used for this example. **A** Based on all 249 electrodes a phase angle map for each time instance, sample, was constructed. In the phase maps, clearly conducting wave fronts can be identified. The analysis was performed with time steps of AFCL/10 (13.5 ms). In the depicted phase map, we used AFCL/5 (27 ms) for clarity of the illustration. In the left-hand series of phasemaps a period with recurrent activity is illustrated. Three consecutive waves start at the lower left-hand corner conducting toward the right-hand upper corner. This recurrent phase was present for 13 cycles. In the right-hand series of phase maps, a phase with non-recurrent activity is depicted. Multiple wave fronts were simultaneously present propagating in different directions. As can be appreciated from these maps no recurrent patterns were present. **B** Recurrence plots, a $N \times N$ scatter plot with time on the x and y-axis, were constructed by comparing each time instance with any other time instance. A recurrent pattern is visualized by a blue dot. The recurrence plot always has a central diagonal line, line of identity, because at this timepoint it is compared to itself and is by definition recurrent. Two timepoints were considered to be recurrent if the average phase angle difference (aPD) was less than $\pi/4$. Recurrent phase. Because of the periodic nature of conduction during a recurrent phase diagonal lines are formed in the plot. The maximal line length within this phase reflects the duration of the recurrent phase. The periodic behavior also leads to multiple diagonal lines, one for each cycle. Therefore, a recurrent phase will appear as a square around the line of identity. During the non-recurrent phase all time instances display unique patterns. In the recurrence plot this is reflected by the lack of recurrences or single dots (a random recurrence by chance), as can be seen in the right upper corner. Within the 60-s-recording, represented by the middle recurrence plot, a majority of the time no recurrence was found. However, at about 32, 48, and 52 s large recurrence blocks can be seen. These blocks exhibited the pattern as depicted in the left panel A. All recurrence blocks showed the same pattern.

Table 2 Overview of recurrence, wave and re-entry analysis for all 12 goats.

Goat ID	Recurrence map	Predominant recurrent pattern	Secondary recurrent pattern	Tertiary recurrent pattern	Rate of recurrent patterns	Waves/cycle	AFCL (ms)	RC presence (% in time)	RC lifespan (ms)
1		Wave type: PW Origin: NE→SW Time point: 58	NA	NA	0.021	9.9	150	2.30	159
2		Wave type: rBT Origin: NW Time point: 4 10 21 etc.	Wave type: PW Origin: SW→NE Time point: 26	Wave type: PW Origin: E→W Time point: 55.9	0.665	3.8	164	0.21	137
	Wave type: rBT Origin: 3 NW Time point: 1 21 51 etc.	Wave type: PW Origin: SE→NW Time point: 8.5	NA	0.063	7.8	132	8.21	176

Goat ID	Recurrence map	Predominant recurrent pattern	Secondary recurrent pattern	Tertiary recurrent pattern	Rate of recurrent patterns	Waves/cycle	AFCL (ms)	RC presence (% in time)	RC lifespan (ms)
4		Wavetype: PW Origin: NE→SW Time point: 45	Wavetype: PW Origin: SW→NE Time point: 24	NA	0.014	10.8	144	0.74	115
5		Wavetype: PW Origin: W→E Time point: 0 10 39 etc.	NA	NA	0.172	5.17	135	2.19	136
6		Wavetype: Collision of 2 PW's Origin: N and S Time point: 3	NA	NA	0.008	8.20	128	10.3	147

Goat ID	Recurrence map	Predominant recurrent pattern	Secondary recurrent pattern	Tertiary recurrent pattern	Rate of recurrent patterns	Waves/cycle	AFCL (ms)	RC presence (% in time)	RC lifespan (ms)
7		Wavetype: Collision of 2 PW Origin: S and N Time point: 5 8 10 etc.	NA	NA	0.200	5.87	127	2.32	151
8		Wavetype: rBT Origin: NE Time point: 2 15 25 etc.	Wavetype: RC Origin: Core in the centre Time point: 32	NA	0.038	6.03	121	14.0	178
9		Wavetype: - Origin: - Time point: -	NA	NA	0.001	112	103	32.4	158

Goat ID	Recurrence map	Predominant recurrent pattern	Secondary recurrent pattern	Tertiary recurrent pattern	Rate of recurrent patterns	Waves/cycle	AFCL (ms)	RC presence (% in time)	RC lifespan (ms)
10		Wavetype: rBT Origin: N Time point: 32 48 52	NA	NA	0.039	8.61	139	4.43	144
11		Wavetype: rBT Origin: 11 N Time point: 4	Wavetype: PW Origin: NW→SE Time point: 21	Wavetype: Collision of 2 PW Origin: South Time point: 14	0.049	6.38	122	5.00	129
12		Wavetype: rBT Origin: S Time point: 9 56	Wavetype: rBT Origin: N Time point: 30	NA	0.006	8.44	102	28.0	157

Recurrent patterns were subjectively scored to be most frequent (primary), second (secondary) or third (tertiary) in line. For each pattern the wavetype, origin and time point of appearance (in seconds) is indicated. Acronyms used are: PW, peripheral wave; rBT, repetitive breakthrough; RC, reentrant circuit; N, north; E, east; S, south; W, west; NA, Not available.

Recurrence plots, wave and re-entrant circuit analysis for each individual animal are presented in **Table 2**. In the majority of goats (9 out of 12, goat number; 1, 3, 4, 6, and 8-12) no frequent or long-lasting recurrent patterns were found. Only 3 goats had higher recurrence rates. Overall 1.6 ± 0.7 recurrent patterns per goat were found, with either peripheral or BT origins. Sixty-four percent of the reentrant circuits with >2 rotations were identified as recurrent events. No predominant origin of conduction pattern was found. Frequent disruption occurred, initiating unique states resulting in a recurrent pattern rate of 0.11 ± 0.19 (**Figure 7**). The association of recurrent pattern rate was strong with the number of waves but was weak with the number of reentrant circuits.

Figure 7 Recurrent pattern rates of conduction patterns. **A** Recurrent pattern rates were generally low with only 3 out of 9 animals expressing higher rates. **B** Recurrent pattern rates as such had a strong, logarithmic association with the number of waves, but not with re-entrant circuits.

AF property maps and effect of time and AF episode

Potentially, AF property maps could gradually change over a period of several minutes. Therefore, we investigated the effect of time on these property maps. In the section above, we determined the average PCC for all comparisons within the 10-min period. Here we averaged the PCC for the different possible time intervals (1-9 min) between the recordings within the 10-min period (**Figure 8**). No changes in PCC occurred for all parameters at the various time intervals. Thus, within a single AF episode a high degree of spatiotemporal stationarity was found.

Figure 8 Intra-episode spatiotemporal stationarity of AF parameters. PCC was assessed for all the possible time differences between the 60-s-recordings in a 10-min window. Note, with increasing time difference the number of comparisons decrease, from 9 comparisons at $\Delta t = 1$ to 1 comparison at $\Delta t = 9$. The parameters are ordered corresponding to the legend order. Data is presented as mean $\pm$ sd of the 12 animals.

Figure 9 Inter-episode spatiotemporal stationarity of AF parameters. Three different AF episodes were compared. **A** Intra-episode stationarity. The average PCC based on the 6 goats in the first episode. **B** PCC for inter-episodes stationarity with short time difference. **C** PCC for inter-episodes stationarity with ~ 1 h time difference. Changes in PCC were tested with a Wilcoxon rank sum test without post-hoc analysis. * $p < 0.05$ vs within episode.

High stationarity could reflect the underlying anatomical structure. In that case one would expect that different AF episodes would display comparable AF property distribution. We therefore explored the spatiotemporal stationarity in 2 additional AF episodes

($n = 6$). We chose 2 episodes to be closely apart, 2.1 ± 1 min of AF interruption, and the third episode after ~ 1 h of AF interruption. This resulted into a time difference of 63.4 ± 16.7 min for episode 2 vs. 3 and 75.3 ± 17.9 min for episode 1 vs. 3, (**Figure 1**, goats 1-6). The 2-min AF interruption led hardly to any changes in average PCC (**Figure 9**). However, larger changes for some animals occurred than for others, specifically for CV. Interestingly, after the 60-min interruption of AF the PCC led to a large inter-individual variation. The decrease of PCC was significant for AFCL, CV, and FI.

Discussion

Major findings

In the goat model of persistent AF, we demonstrated that in 60-s-recordings the spatio-temporal distributions of AF parameters are stationary if measured within a single AF episode. A short interruption of an AF episode had little effect on this stationary behavior but interruption of longer duration changed AF parameters significantly. Sixty-second recurrence analysis illustrated that recurrent patterns were scarce and only found in a minority of the animals. AF was also shown to have highly dynamic behavior at a beat-to-beat level. Therefore, stationary spatiotemporal properties could not be attributed to stable recurrent conduction patterns during AF suggesting that the atrial myocardium may determine average AF properties even when the conduction patterns are highly variable.

Stationarity of ablation targets

Complex fractionated electrograms (CFAE) were reported to occur during induced AF at sites of conduction block in Wolf-Parkinson-White patients and therefore reflect areas with higher complexity of AF (Konings et al., 1997). Consequently, they might reflect zones that act as sources for AF. It was hypothesized that CFAE sites might be suitable targets for AF ablation (Nademanee et al., 2004). Initially high success rates were reported but recent randomized controlled trials failed to show superiority of PV isolation + CFAE ablation above PV isolation alone (Verma et al., 2015; Vogler et al., 2015). In other studies, high dominant frequency (DF) was found to associate with rotor sites in cholinergic AF in sheep hearts (Berenfeld et al., 2000; Mandapati et al., 2000). This notion led to hypothesis that ablating DF sites might be a valid target for AF ablation (Mandapati et al., 2000; Sanders et al., 2005). Indeed, the presence of high frequency sites inversely correlated to procedural success rates (Gadenz et al., 2017; Kimata et al., 2018) but targeting DF sites did not consistently lead to improvement of clinical outcome (Atienza et al., 2014; Gadenz et al., 2017). It is unclear whether these negative outcomes entail that CFAE and high DF indicate bystander sites instead of AF driver sites or are due to technical limitations of the methods applied.

Irrespective of the applied methods, the intrinsic assumption entail that driver sites are stable in time and space. In most studies, DF and CFAE was determined on electrograms of 2-6s. Lau et al. (2012), Gadenz et al. (2017) The question is whether these recording lengths are sufficient to capture potential transient appearances of CFAE's or DF's. Salinet et al. reported limited reproducibility of DF zones in non-contact intra-atrial electrograms (Salinet et al., 2014). However, continuous recordings of bipolar contact electrograms showed that about 75% of CFAE's remain stable in time (Lau et al., 2015). Similarly, we found very high spatiotemporal stationarity for both FI and AFCL. Recordings of 60 s within a single episode of AF were more than sufficient to reliably capture patterns. However, the determination of the minimal recording length to obtain representative information was not addressed in this study and would require further investigations, preferably in human recordings.

Stationarity of conduction properties

Both AFCL and FI are parameters that are derived from single electrograms. Therefore, they reflect AF properties of a limited area. Parameters that account for spatial characteristics of conduction, such as CV and LD had comparably high stationary properties too. Stationarities were lower when AF patterns were considered. Preferentiality of conduction direction was subject to largest variability, indicative of dynamic conduction patterns. Breakthroughs were found at almost all electrodes and clear preferential regions of epicardial focal spread of activation were present. Recurrence analysis revealed that half of the goats exhibit periods of repetitive BT's of variable durations. Whether these sites are important for the perpetuation of AF, as proposed by Lee et al. (Lee et al., 2015), warrants further investigation.

In general, AF wave and recurrence analysis revealed multiple propagating wave fronts with a small number of recurrent patterns. Hence, it is unlikely that the high degree of stationary of AF property maps is caused by stable and frequent conduction patterns. Therefore, we conclude that the atrial structure and local electrophysiological properties are likely to determine average AF properties even if the conduction patterns as such are very variable. This might imply that the relatively large variation in AF complexity (3.8–11 waves/cycle), among animals without substantial structural remodeling (Verheule et al., 2010), may—at least partly—be driven by the individual atrial architecture.

Are AF properties independent from the AF episode?

If AF properties would only be determined by the atrial architecture, then the initiation conditions should not affect AF properties. We therefore examined the effect of different episodes of AF. We found that stationarity is conserved if two AF episodes are relatively close in time (~2 min). This observation is in line with Redfearn et al. who found that ~80% of CFAE regions were confirmed when the atria were remapped after a 10 min interval of sinus rhythm (Redfearn et al., 2009). This implies that atrial structure has a

prominent role on conduction properties during AF. However, stationarity of AF properties was lower when AF was interrupted >1 h. Because atrial structure does not change in this time interval, we propose that some form of acute recovery from electrical remodeling affected the atrial substrate. This observation might be of clinical importance since some symptomatic AF patients are cardioverted to sinus rhythm before AF ablation. Re-induction of AF during the procedure may reveal other AF patterns or drivers than originally were present with the risk that the leading mechanism cannot be identified.

The use of recurrence quantification analysis

Recurrence quantification analysis is a technique to identify recurrent states in non-linear dynamical systems. Recurrence quantification analysis has been used in various biomedical disciplines, e.g., postural fluctuation and heart rate variability (Riley et al., 1999; Arcentalles et al., 2011). In AF, recurrence analysis has been used mostly for (fractionated) electrogram morphology (Censi et al., 2000; Ng et al., 2014). We made a first step toward recurrent conduction pattern analysis by applying principal component analysis on all electrograms from a high-density array (Zeemering et al., 2015). This approach showed a good correlation with the number of waves but lacked directional and wave front information. Hence, recurrence of the degree of complexity but no recurrence of patterns could be discriminated. Here, we introduce a method accounting for both directionality and complexity of conduction patterns. An important finding is that recurrent patterns were scarce in the overall data set. The frequency of recurrent patterns was inversely related to the number of waves. Recurrent patterns mainly occurred in animals with a low degree of AF complexity. A recurrent pattern in animals with a higher average number of waves per cycle is more likely to be disrupted by other waves. In addition, if more independent wave fronts are present, than the likelihood that all fronts show a recurrence, i.e., synchronize in time and space, is relatively low.

Future perspectives

The introduced recurrence analysis allows addressing AF on a large time scale and may generate new ideas about AF perpetuation. For instance, the identification of a recurrent period within an episode of AF could indicate maintenance of AF by large circuits, while non-recurrent periods reflect more localized mechanisms. In that case the corresponding dimensions of the recurrence area that matches the local mechanism should be found. Alternatively, recurrent periods could reflect large transient reentrant circuits that act as oscillators of the fibrillation process. These resonators may speed up fibrillation rate with consequent breakdown in distant areas of the atria. Recurrent patterns may also occur before cardioversion of AF when AF complexity becomes low.

Limitations

An obvious limitation of this study is that the recordings were performed in the goat model of persistent AF in the presence of limited structural remodeling. Additional investigations are required to investigate whether similar patterns occur in patients with AF. Also, the effect of the degree of structural remodeling on recurrent patterns should be addressed in future studies.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2018.00947/full#supplementary-material>

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6

Chapter 6

Identification of rotors during human atrial
fibrillation using contact mapping and phase
singularity detection:
technical considerations

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IEEE Transaction on biomedical engineering. 2017 doi: 10.1109/TBME.2016.2554660.

Abstract

Objective: To explore technical challenges of phase singularity (PS) mapping during atrial fibrillation (AF) using direct contact electrograms.

Methods: AF mapping was performed in high-density epicardial recordings of human paroxysmal (PAF) or persistent (PersAF) ($N = 20$ pts) AF with an array of 16×16 electrodes placed on atrial epicardium. PS points were detected using subsets of electrodes forming rings of varying sizes.

Results: PS detection using a 2×2 electrode ring identified 0.88 ± 1.00 PS/s in PAF group and 3.91 ± 2.51 per s in PersAF group ($p < 0.001$) in 2.4×2.4 cm mapping area. All detected PS had a short lifespan with the longest being 1100 ms (6.8 rotations). Exploration of the PS detection in a numerical model demonstrated that at least eight electrodes are required to avoid frequent false positive PS detection due to chance. Application of a detection grid consisting a double ring of electrodes (2×2 and 4×4 rings) decreased the number of false positive detections. The double ring was more resilient to electrode swapping (with just three instances of false positives versus 4380 false positives using 2×2 ring).

Conclusions: The number of detected rotors critically depends upon the parameters of the detection algorithm, especially the number of electrodes used to detect PS. Based on our results, we recommend double ring comprised of 2×2 and 4×4 grid of electrodes for robust rotor detection.

Significance: Great methodological care has to be taken before equating detected PS with rotating waves and using PS detection algorithms to guide catheter ablation of AF.

Introduction

The hypothesis that atrial fibrillation (AF) is driven by stable rotating waves (rotors) is undergoing a renaissance.

This is due to a set of clinical studies using a 64-electrode basket catheter and custom signal processing technique for detection of rotors and focal sources of AF (focal impulse and rotor modulation—FIRM approach)[1]–[4]. This approach identified stable rotating waves and focal sources in both paroxysmal and persistent AF (PersAF) patients [5]. Identification of these sources, as employed in the FIRM approach, is based on the construction of electrogram phase maps and identification of phase singularity (PS) points and point sources of activation [5] [in this manuscript, we equate rotors with phase singularities lasting for more than one local AF cycle length (CL)]. 3-D basket geometry is then unwrapped onto a rectangular map on which an electrogram phase map is presented with identified rotors and point sources of activity [4]. Ablation at the center of such sources resulted in a high rate of acute AF termination and promising long-term results [1]. Impressive success rates reported in studies using FIRM approach raised a hope of finding a truly efficient ablation technique for patients with PersAF. However, positive clinical findings are not uniformly reproduced [6]. Additionally, stability of conduction patterns and size of individual waves revealed by FIRM approach [5], [7] seem to differ from the results found in other studies that used high density mapping and revealed complex, variable conduction patterns, most often made up of small waves [8]–[11].

Due to promising results and potential clinical value of this approach, there is an urgent need for the exploration of technical aspects of rotor detection using contact electrograms, especially in a light of validation studies demonstrating difficulties in confirmation of rotor detection with activation time mapping [12] and technical difficulties of mapping using basket catheter in general [13]. For instance, AF mapping using basket catheter differs in a number of aspects to high-density mapping. Apart from the larger electrode distances, the elastic nature of the basket catheter may cause individual splines to diverge once pressed against the atrial wall. This effect of interelectrode spacing on rotor detection is unclear and requires deeper investigation.

Every method of rotor detection, either based on phase mapping or activation time mapping, is basically a detection of a sequence of consecutive activations along a set of electrodes forming a closed loop: From “early” to “late”. Of course, the smaller the loop of electrodes selected for the PS presence test, the more precise is the localization of the rotor. However, low number of electrodes used for PS detection leads to a high number of false positives as noticed by others [14]. We hypothesize, that the cause of this effect lies in a high probability of artificial phase sequence occurrence leading to false PS detection.

Here, we explore the problem of PS detection using a highdensity contact mapping dataset and considerations based on numerical modeling. We explored: (1) The effect of

the number of electrodes on PS detection, (2) their spacing, and (3) the effect of the correlation between electrical activity of neighboring electrograms.

Methods

Calculation of the electrogram phase

The first step in phase mapping is calculation of the electrogram phase. In case of contact electrograms, a cycle is defined by two consecutive intrinsic deflections. In course of time, phase changes from minimum value (π) after first deflection and reaches maximum value ($+\pi$) reaching consecutive deflection (see **Figure 1**).

Phase mapping of cardiac fibrillation is an established technique in context of cardiac optical mapping in ventricles [15], [16] and atria [17]. However, phase mapping based on contact electrograms has primarily been used in context of ventricular contact mapping [18], [19]. Phase reconstruction of atrial contact electrograms is challenging because of the complex atrial electrogram morphology that results in complex morphology of reconstructed electrogram phase. In order to overcome this obstacle, we previously introduced a concept of sinusoidal recomposition which combined with the Hilbert transform was demonstrated to robustly reconstruct electrogram phase [20] (see example in **Figure 1** and Supplementary Materials for a MATLAB function used in computations).

Figure 1 Phase reconstruction of contact atrial electrogram. **(a)** Example electrograms from PAF (left) and PersAF (right). **(b)** Signal after application of sinusoidal recomposition to electrograms. **(c)** Phase of recomposed signal.

Detection of phase singularities

Re-entrant wave circulating around a small-sized area in a tissue [21] leaves a very specific footprint in phase map: a PS point [see white circle in **Figure 2b**]. PS is a point surrounded by elements of the system in all possible phases of electrical activation. PS detection using the classical approach [22] is based on a closed path integral of the phase gradient:

$$\oint_c \nabla \phi d\vec{l} \quad (1)$$

where c is a closed loop surrounding given point, ϕ is a phase map in space, and ∇ is a spatial derivative. In theory, integral (1) results in $\pm 2\pi$ value at PS point and zero elsewhere. In other words, a PS is detected if, taking the consecutive phase differences between neighboring points moving along the path c , there is only one large phase difference corresponding with transition to a new cycle. An example of the phase map with a clearly defined PS is shown in **Figure 2b**. This example demonstrates a gradual phase transition plus one large phase change (red to blue) along the path encompassing the PS point. Loop c should be infinitesimally small in order to result in the highest precision of pinpointing PS location.

There are several different approaches to identify PS [14], [19], [22]–[24]. The specifics of each method were based on the properties of the dataset used in the study. Optical mapping and computer simulation datasets are characterized by very high spatial density along with signals (optical signal or transmembrane voltage, respectively) reflecting well-localized activity. In our study, we have rather opposite properties of the dataset: comparatively sparse spatial sampling along with unipolar electrograms reflecting combined electrical activity of the electrode neighborhood. Thus, we adapted detection method to the specifics of the dataset we used in this study.

In case of a discrete mesh of electrodes, integral (1) has to be discretized and approximated [14], [24]. Then, loop c becomes a ring of electrodes encircling a point tested for PS presence. Such ring can be for example formed by 2×2 ring of electrodes [see **Figure 2a**]. Such a small ring is sufficient to detect a PS, and due to low number of electrodes may be perceived as a preferable choice in case of low-density mapping approach (e.g., in case of basket catheter recordings with 64 electrodes in total). PS detection on such a ring translates then into a condition of the presence of just one phase difference greater than π (half of the full cycle of 2π), while taking consecutive differences between phases of electrodes of 2×2 ring. If PS is present at given point, there will be three small phase differences and one large at the phase transition point. However, a low number of electrodes may result in high degree of uncertainty, since with few electrodes there is a higher probability of PS detection by chance (even if electrograms are random, as we will demonstrate below).

High density mapping studies of PersAF demonstrated a presence of plurality of small waves [25]. In such scenario, PS detection grid may encompass more than one wave, leading to occurrence of artificial phase transition sequences. In order to investigate the

sensitivity of the PS detection to random sequences of phase progression and transition, we explored properties of the detection rings formed by different number of electrodes. An alternative to a ring comprised of single 2×2 ring, is a grid comprised of two rings: the inner ring and outer ring [referred as “ $2 \times 2 + 4 \times 4$ ” ring further in the manuscript; see **Figure 2c**]. Such double-ring PS detection grid results in positive PS identification if PS is detected by both inner and outer rings simultaneously. We explored properties of such ring in greater detail in comparison with a grid comprised of single 2×2 ring of electrodes.

Figure 2 Principle of phase mapping and PS detection based on 16×16 grid of electrodes. **(a)** PS detection using 2×2 ring of electrodes. **(b)** Example phases map in case of well-defined PS point. **(c)** Phase is colorcoded with blue corresponding with beginning of the cycle and dark red with end of the cycle (a line between dark red and blue corresponds with a front of the wave). A schematic of $2 \times 2 + 4 \times 4$ detection grid. **(d)** Example phase map in case 2 PS points present.

PS was identified as a rotor and included into analysis if the PS lifespan was greater than one instantaneous AF CL. Instantaneous CL calculation was based on intrinsic deflection annotation as described in [26]. CL was defined as interval between consecutive local deflections. Instantaneous CL of given PS, was defined as mean CL of all electrodes forming the trajectory of PS drift throughout the time period of PS presence.

PS in the manuscript denotes a PS point that lasted for more than one instantaneous CL. PS lifespan was expressed as both lifespan in milliseconds and also as the number of rotations it completed during its lifespan. The number of PS per second was calculated as the total number of detected PS of lifespan longer than local CL divided by recording length.

The drift of PS points was taken into account by clustering them into a single trajectory (see Fig. S1 in Supplementary Materials). Given the temporal sampling in our mapping dataset of 1 ms and inter-electrode distance of 1.5 mm, the maximum PS drift velocity that would be identified as a single PS trajectory was 1.5 m/s.

High-density epicardial mapping and reconstruction of individual waves

Direct, contact high-density epicardial mapping was performed in 20 patients with AF undergoing open chest surgery. Eleven pts were in paroxysmal AF (PAF) and nine pts were in PersAF. Clinical characteristics of the patients are presented in **Table 1** in the Supplement Materials. A rectangular plaque (16 x 16 electrodes, 1.5 mm interelectrode spacing) was placed on the epicardial surface of the atria. The plaque was positioned on the left atrial posterior wall between the pulmonary veins and the right atrial free wall. Atria were mapped sequentially for 10 s at each site with a 256-channel mapping system (bandwidth, 0.56 to 408 Hz; sampling rate 1 kHz, resolution 12 bits). Far-field ventricular activity was subtracted from the unipolar fibrillation electrograms by single beat cancellation method [26]. After exclusion of poor contact recordings, we included 21 recordings in PAF group (10 in LA and 11 in RA) and 17 recordings in PersAF group (eight in LA and nine in RA).

To illustrate PS dynamics, we created phase movies demonstrating conduction pattern 100 ms before, during, and 100 ms after the PS occurrence. Each phase movie represents 16 x 16 mapping array with phase color coded (the same color scale as used throughout the manuscript). PS point denoting the center of the rotor is represented as a circle. Example phase movies can be found in Supplement Materials.

Numerical scheme for generation of random electrograms phases

In general, PS detection is based on the presence of a specific sequence of activations at electrodes surrounding suspected PS point. We hypothesized, that in case of a small number of electrodes constituting detection ring and a lack of correlation between electrograms (e.g., due to large spacing between electrodes), such a sequence may arise due to chance. In order to explore this hypothesis, we performed a simulation consisting of

following steps: 1) sets of 10000 activations with random intervals was generated using following equation: $CL_{i+1} = CL_i + \eta$ where CL_i is i th generated CL and η is a random number obtained from normal distribution with standard deviation σ , 2) for each set of generated activations, the corresponding phase signal was constructed, 3) generated electrograms were spatially organized into rings (resembling multielectrode contact measurements), 4) PS detection was performed. Phase signal was reconstructed from set of consecutive CLs assuming linear change of phase within each CL - producing characteristic “saw-tooth” shape of the phase signal [see **Figure 3b**].

This simulation allowed us to assess the percentage of time a PS was present due to randomness, the number of PS per cycle and PS lifespan as a function of standard deviation of the noise σ used in random signal generation. Based on epicardial contact mapping studies, we found that standard deviation of CL during paroxysmal and PersAF in humans varied between 5 and 70 ms. Therefore, we assumed σ ranging between 0 and 100 ms.

Figure 3 Examination of PS detection based on 2 x 2 ring of electrodes when a random sequence of activations is applied. **(a)** Example set of random timings between consecutive activations. **(b)** Phase signal corresponding with random timings. **(c)** Example histogram of PS lifespan detected in case of electrograms length 60 s consisting of 300 activations with mean CL 200 ms and standard deviation of CL 10 ms. Analyses were performed for varying standard deviation of added noise σ . % of time PS was present at different levels of σ . **(e)** PS number per second. **(f)** Mean PS lifespan.

Statistics

Results are expressed as mean $\pm$ SD unless otherwise stated. Statistical analysis of normally distributed variables was performed using one-way ANOVA. $p < 0.05$ was considered statistically significant.

Results

Numerical scheme for generation of random electrograms phases

Results of PS detection using random electrograms and 2 x 2 ring of electrodes are presented in **Figure 3**. An example of a train of randomly generated intervals (assuming mean CL of 200 ms) between consecutive activations is shown in **Figure 3b**. This set of intervals was then used to reconstruct corresponding phase signal as presented in **Fig. 3b**. After random electrogram phases were generated, PS detection was performed using 2 x 2 detection ring. An example histogram of PS lifespan generated for 300 activations and standard deviation of added noise σ equal to 10 ms is shown in **Figure 3c**. Ninety two percent of PS (73 out of 79) in this example were short lived (< 1 CL lifespan) with only few PS with life span longer than 1000 ms (reaching 2500 ms).

In order to explore the effect of the level of noise added to the CLs on PS detection, calculations for a range of the noise levels were conducted. Results of analysis in case of 2 x 2 ring of electrodes and PS lifespan > 1 CL are shown in **Figure 3d–f**. Horizontal axis presents the level of noise added to the electrograms - σ . At low σ , generated PS were rare but had very long life span - low noise level resulted in rare occurrences of PS, but once it occurred, it took longer to disappear (due to low noise level it took longer to disrupt sequence of activations) [see **Figure 3d**]. With increasing σ , the number of PS per second [see **Figure 3e**] increased but lifespan rapidly decreased. At even higher levels of noise the required sequence for PS detection was destroyed more quickly by noise and both number and lifespan of PS decreased. The balance between the number of PS and their lifespan determined % of time PS present, which reached level of 17–20% at a SD level of about 20 ms, levels that are frequently observed in human AF [see **Figure 3f**]. With further increase of σ , PS number and lifespan decreased, since with an increasing level of noise intensity, sequence resulting in PS detection was more easily destroyed by noise.

The effect of the number of electrodes taken for PS a detection ring on % of time PS present is shown in **Fig. 4**. In case of CL of 200 ms and a standard deviation of 20 ms in a ring of four electrodes (2 x 2 ring), PS was present by chance roughly 18% of the time. With increasing number of electrodes, the chance of a random formation of a PS is rapidly decreasing dropping to $< 0.2\%$ for ring consisting eight electrodes, and $< 0.01\%$ for a 2 x 2 + 4 x 4 ring. This low number of false positive detections for 2 x 2 + 4 x 4 ring was the reason for choosing it as an alternative to more noise sensitive 2 x 2 ring. Below, we compared robustness of those two types of PS detection rings in human recordings.

Figure 4 Effect of the number of electrodes taken for PS detection on % of time PS was present. PS detection based on artificial, random sequence of intervals. Horizontal axis denotes standard deviation of the noise added to the signals. With increasing number of electrodes forming the PS detection ring, % of time PS was present strongly decreases.

High-density epicardial mapping

1) Effect of a Disrupted Spatial Organization on PS Detection: In order to test whether in experimental recordings PS can occur as a result of chance, we performed an analysis we randomly swapped positions of all electrodes in mapping plaque prior to PS detection. Hence, all spatial organization of activation patterns is destroyed and replaced by a random pattern of activations without any spatiotemporal relation [see **Figure 5a**].

One would expect that in such case, no PS should be detected since there is no coherent propagation present. However, as it can be appreciated in **Figure 5a**, that randomization of electrode locations led to a larger number of PS detections using 2 x 2 ring (black circles denote PS points). Analysis of the whole dataset demonstrated a significant increase in the number of detected PS [from 851 in total to 4380; **Figure 5b**] and maximum rotation number [from 6.8 rotations to 21 rotations; **Figure 5d**]. In case of 2 x 2 + 4 x 4 grid, the procedure of random swapping of electrode positions resulted in significant drop in the number of detected PS (from 138 to 3 detected PS points) and shorter maximum lifespan (drop from 3.2 to 1.2 rotations); see **Figure 5c, e**.

Figure 5 Effect of lack of spatial organization on PS detection by random swapping of electrodes positions. Human recordings were used. **(a)** Example phase map before and after electrodes swapping. **(b)** Detected PS are denoted as circles. Number of detected PS without (control) and after swapping using 2×2 ring. **(c)** PS with longest lifespan (expressed by number of rotations) found in dataset before and after swapping. **(d)** Number of PS detected using $2 \times 2 + 4 \times 4$ grid and **(e)** the maximum encountered lifespan.

2) PS Incidence and Lifespan: In total, using 2×2 detection grid, 184 PS were detected in PAF group and 665 in PersAF group (0.88 ± 1.00 and 3.91 ± 2.51 PS per s, respectively). The longest surviving PS had lifespan of 1100 ms (6.8 rotations). Application of $2 \times 2 + 4 \times 4$ PS detection grid resulted in 83% drop in the number of detected PS compared to using the 2×2 PS detection ring: 47 PS were detected in PAF group and 91 in PersAF group (0.22 ± 0.026 and 0.54 ± 0.36 PS per s, respectively). The longest surviving PS had lifespan of 584 ms (3.2 rotations). PS incidence in each recording and distributions of lifespan are presented in **Figure 6**.

Figure 6 Characteristics of PS using high-density epicardial mapping recordings in human PAF and PersAF. (a) and (b) PS incidence and life span using 2 x 2 and 2 x 2 + 4 x 4 detection grids for PAF and PersAF patients. PS incidence per second. (c) and (e) Distributions of PS lifespan expressed as a number of rotations.

On average, PS were present $5.5 \pm 6.2\%$ of the time in PAF group and $10.6 \pm 7.0\%$ of the time in PersAF group. With respect to the chamber, PS were present $8.3 \pm 6.6\%$ of the time in LA and $7.3 \pm 7.4\%$ of the time in RA.

Example animations with phase maps with PS detected using 2 x 2 + 4 x 4 grid (starting 100 ms prior PS occurrence and continuing 100 ms after PS disappearance) can be found in Supplement Materials.

3) Effect of Interelectrode Distance: The problem of the role of interelectrode distance is especially important in context of AF mapping using basket catheter, during which the distance between electrodes at neighboring catheter splines may exceed 1 cm. In case of rotors occupying large portion of the atria, interelectrode distance during PS detection should not play an important role. However, if the size of individual activation waves is smaller than interelectrode distance, there may be a possibility of random PS occurrence due to poor correlation of electrical activity (electrograms of neighboring electrodes would belong to different activation waves). In such scenario one would expect, from robust PS detection method, the number of detected PS to drop to zero at large

interelectrode distance (since there would be no rotor in the area between electrodes but rather several independent small waves).

In order to address this issue, we performed analysis with progressive removal of electrodes from the mapping plaque. In the first step, only every second electrode was included in analysis. In the second step, every third electrode, and so on, until in the last step only four corner electrodes remained [see **Figure 7a**].

In order to illustrate the effect of this analysis, we applied this methodology in two individual recordings: One with well-defined rotating wave [see **Figure 7b**] and one with several simultaneous waves present within the mapped field [see **Figure 7d**] - in both cases using a 2 x 2 ring. In case of well-defined rotating wave the number of detected PS points did not change with the progressive removal of the electrodes [as expected, since in case of large rotating wave interelectrode spacing should not influence PS detection; see **Figure 7b,c**]. However, when multiple waves are present, PS number decreased with progressive electrode removal [see **Figure 7e**]. Next, we estimated how many random PS occurrences could be expected based on the numerical model described in Section D of the methods. The model was inputted with the mean and standard deviation of the AF CL of the individual recordings. The relation between electrode distance and random activity on the number of PS detections is depicted as a red line in **Figure 7c, e**. The number of detected PS in the recording with multiple waves was close to the predicted number of PS points assuming random electrical activity (red dotted line).

The same methodology was applied to the whole high-density mapping dataset showing a decrease in PS number with the progressive electrode removal [see **Figure 7f** for case of 2 x 2 ring used, and **Figure 7h** for case of 2 x 2 + 4 x 4 grid used]. However, the number of PS in case of 2 x 2 ring did not drop to zero at large inter-electrode spacing, unlike in a case of 2 x 2 + 4 x 4 detection grid [see **Figure 7h**].

In order to take into account the fact that with progressive electrodes removal the number of measurement locations drops [resulting in only one measurement site in case of only four corner electrodes remaining—see **Figure 7a**], the number of detected PS was divided by the number of available quadruplets of the electrodes. This value was defined as “PS number normalized to number of measurement sites.” When the number of detected PS was normalized with respect to the number of measurement sites, the number of detected PS became linearly proportional to the electrode spacing in case of 2 x 2 ring [see **Figure 7g**]. In contrast, at largest possible inter-electrode distance (6 mm) a 2 x 2 + 4 x 4 grid did not detect any PS [see **Figure 7i**].

Figure 7 Effect of electrode spacing on PS detection. **(a)** Schematic representing progressive removal of the electrodes from the mapping ring. **(b)** and **(c)** PS was performed on the remaining subset of electrodes. Example of the number of detected PS as a function of inter-electrode distance in case of well-defined rotating waves **(d)** and **(e)** in case of complex conduction patterns with several waves present. **(f)** and **(i)** Red line denotes estimated number of expected PS points in case of random electrical activity with the same mean and standard deviation of activations as in experimental recording. Results for the whole dataset analysis. **(f)** PS number as a function of inter-electrode spacing in case of 2×2 ring used for PS detection and. **(h)** in case of $2 \times 2 + 4 \times 4$ grid. **(g)** The same dependency, but with PS number corrected for the number of measurement points available using 2×2 ring and **(i)** $2 \times 2 + 4 \times 4$ grid.

Discussion

Phase mapping based on contact electrograms allowed us to identify rotating waves defined as phase singularities. The main findings of this study can be summarized as follows: 1) PS detection using low number of electrodes (e.g., 2×2 ring of electrodes) is vulnerable to noise and may result in false positive detection, 2) due to the presence of small waves during AF, large inter-electrode distances may result in coverage of different activation waves and subsequent increased chance of false PS detection, and 3) extension of 2×2 PS detection ring by second 4×4 ring results in much greater robustness in epicardial PS detection.

Effect of the number of electrodes used in PS detection

Since PS detection is basically a detection of a sequence of consecutive activations around PS point, there is always non-zero probability of random alignment of activations giving rise to false positive detections. We explored this phenomenon in a numerical simulation, finding that random electrograms of mean CL 200 ms with random sequence can result in PS present even for 20% of the time if only four electrodes are used [see **Figure 3f**]. A similar effect was present in a high-density mapping after randomly swapping electrodes positions, effectively destroying coherent conduction pattern. The number of detected PS dramatically increased along with their lifespan [see **Figure 5b, d**]. Those results led us to conclude that the number of electrodes used to check for PS presence is a critical parameter.

Although in case of large rotors, such as in case presented in **Figure 2b**, a 2 x 2 ring may be sufficient providing there is a coherent pattern of activation, it is not specific enough in case of small fibrillatory waves as present during PersAF. Since accidental PS formation is a matter of chance, we explored the possibility that a ring with a greater number of electrodes will lower the probability of false positive detection. We used the same numerical model but with increasing number of electrodes. Result can be seen in **Figure 4**. The percentage of time a PS is present by chance dramatically decreased with the number of electrodes forming the ring, practically dropping to zero after the threshold of eight electrodes. This prompted us to combine a 2 x 2 ring with a greater 4 x 4 ring encompassing the 2 x 2 ring. This condition indeed led to the desirable effect of almost no PS detected after destroying spatial coherence by swapping electrodes positions. Only three PS were still detected—however, since it is a random phenomenon there is always nonzero probability that specific phase sequence will occur [see **Figure 5c**]. This means that a 2 x 2 + 4 x 4 grid is much more robust with respect to false positive detection due to noisy signals or lack of coherent pattern than a 2 x 2 ring and as such can be used in basket mapping, during which we can expect a substantial lack of coherence between electrograms. Since we do not have a gold standard, we cannot comment on specificity of PS detected by a 2 x 2 + 4 x 4 grid.

Effect of electrode spacing on PS detection

One of the biggest challenges in endocardial panoramic mapping using a basket catheter is spacing between recording electrodes. Although electrode spacing along a basket spline is fixed (depends on catheter model but usually is between 2–7 mm), the spacing between individual splines varies due to deformation of the basket during maneuvers in atrial cavity. Inter-spline distance may reach 1–2 cm in case of basket being pushed laterally against the atrial wall (neighboring splines then slide in opposite direction leaving a large “gap”). The effect of such large spacing on PS detection needs to be explored. This is especially important in case of individual wavelets having size comparable to, or smaller than interelectrode spacing. Then, activity at neighboring electrodes may belong to

different waves while the phase map reconstructed using basket recording gives the impression of single wave propagation. In this study, we approached this problem by performing PS detection on sub-selection of electrodes while progressively increasing inter-electrode distance [using the high-density epicardial mapping dataset, increasing distance from 1.5 mm to 2.4 cm; see **Figure 7a**]. Assuming that rotors are spatially extended entities, the sequence of activations should remain similar irrespective of interelectrode distance. However, our analysis showed that the number of detected PS (normalized with respect to the number of measurement sites) was actually increasing [see **Figure 7g**]. This effect is to be expected after taking into account the presence of wavelets smaller than larger inter-electrode distance (especially present in PersAF, see phase movies in Supplemental Materials) which would result in false positive detections (there would be a proper sequence of activations along the ring, but resulting from accidental timing of activations at electrodes belonging to different waves rather than real rotor). Application of the $2 \times 2 + 4 \times 4$ grid removed this effect, resulting in no PS detected after inter-electrode distance reached 6 mm (so that $2 \times 2 + 4 \times 4$ grid had a size of 1.8 cm). Similar result was obtained in a study [27] applying similar protocol but with bipolar electrograms and rotor detection performed by experienced electrophysiologist.

Determination of the optimum range of inter-electrode distance required for robust PS detection would be a clinically important achievement. Due to small sample size in case of large inter-electrode spacing (only 38 detection grids in total in case of 6 mm interelectrode spacing and $2 \times 2 + 4 \times 4$ detection grid) we cannot conclude what maximal inter-electrode spacing should be used. Larger dataset is required to determine this threshold with greater confidence.

Rotor detection uncertainty

One of the interesting aspects of the rotor detection problem, we found in our study, is the uncertainty of rotor detection. The general picture of wave conduction we found in our recordings was not composed of clearly rotating waves contrasted with remaining, not pivoting, set of conduction wave patterns, but rather revealed that majority of waves demonstrated some degree of turning/pivoting. The histogram of PS lifespan [see **Figure 6e, f**] shows only PS with lifespan greater than one local CL.

However, there were numerous PS detected that were present for shorter time period. Those short-lived PS were rejected in favor of “long lasting” rotors (>1 rotation) which are in focus of studies interested in mechanistic insight into AF. Additionally, the number of detected rotors depends on the number of electrodes used in the test for PS presence, as shown for example in **Figure 4**. Those factors demonstrate that rotor detection in case of complex wave conduction is related with a degree of uncertainty and specific parameters have to be assumed in order to obtain quantitative measurements. A change of the parameters in detection algorithm will result in a different number of detected phase singularities. Therefore, we focused on designing a detection method

resistant to confounding factors such as random activity and presence of small waves (resulting in uncorrelated activity between electrodes). The detection method presented in our study is not a definitive approach but rather a method adapted to specific type of AF recordings and wave conduction pattern.

Clinical implications

Identification and ablation of rotors in clinical setting has become one of the most important subjects in contemporary electrophysiology. Mapping tools which can be used in clinical setting are limited with respect to the number of electrodes, coverage or both. This limitation introduces a degree of uncertainty to rotor detection. Our study highlights this important link between the characteristics of the mapping tool used (mainly electrodes number and interelectrode distance) and rotor detection. Especially the phenomena of false rotor detection due to random effects. Thus, several technical considerations specific to be used for mapping tool have to be taken into account before rotor identification and ablation.

Since $2 \times 2 + 4 \times 4$ grid of electrodes takes up a quarter of the basket catheter mapping surface, such approach may not be practical in clinical context. However, the main message of our study is that more electrodes are needed to confirm PS existence than just 2×2 grid. Therefore, a potential implementation of our findings in practice, may be detection of long-lasting PS using 2×2 grid of electrodes using a basket catheter, and then, confirmation of suspected points using a circular catheter.

Limitations

There are several limitations of presented study: 1) We mapped only a limited portion of the atria (2.4 cm^2) —it can be argued that such limited area is not reflecting global pattern, 2) epicardial surface of the atria was mapped—whereas endocardial surface is mapped when basket catheters are used, 3) we just explored in detail two types of PS detection grids excluding other possible configurations of PS detection rings (such as 3×3 or 4×4 grids). The main reason for this is the effect of increased number of neighboring PS points being detected (see Fig. S2 in Supplement Materials —with larger rings, several points are indicated as possible PS location). Only superposition of 2×2 and $2 \times 2 + 4 \times 4$ rings allowed us to combine beneficial effect of larger number of electrodes in the ring with the absence of this effect.

The limitation of the mapping to only epicardial surface could have an impact on obtained results in terms of rotors incidence and lifespan. The work by Hansen *et al.* demonstrated AF driven by intramural reentry waves that manifested more frequently on endocardial surface [28]. Simultaneous endocardial and epicardial mapping would be required to have better mechanistic insight. However, we believe our methodological insights are valuable even when based only on epicardial dataset, especially expecting epicardial rotors to be less frequent and more unstable.

We tested only one PS detection method. There are several approaches to this problem including sophisticated approaches based on wavefront curvature and kernel convolution methods [14], [23]. However, since those methods were inspired by high-density mathematical modeling or optical mapping studies, we reverted back to original equation for PS detection (1) and adapted it in context of low resolution and often poor quality unipolar electrograms recorded during AF.

Mathematical modeling study would be beneficial in exploring and validating presented method. However, it would involve implementation of various AF models taking into account different hypothesis of wave conduction during AF (e.g., mother rotor with large, stable rotating wave or multiple wavelet hypothesis with small wavelets exhibiting small degree of pivoting/turning). Such detailed modeling study unfortunately exceeds scope of this paper.

Conclusions

The number of detected rotors critically depends on the interelectrode distance and parameters of the detection algorithm, especially on the number of electrodes used to detect rotors. Based on our results, we recommend use of a double ring comprised of 2×2 and 4×4 grid of electrodes for robust rotor detection.

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7

Chapter 7

General discussion

More than 70.000 hits are returned after a search for “atrial fibrillation” on PubMed. This huge number exemplifies that atrial fibrillation (AF) is a complex disease with a high relevance to society and healthcare. So far, limited success is achieved for therapy that is designed to maintain normal sinus rhythm. Higher success rates should be aimed at by increasing our understanding of AF maintaining mechanisms and better designed drugs. Chapters 2-4 were focussed on the new antiarrhythmic drug vernakalant. This drug is targeted at multiple currents that are present within the atrial myocyte. Its characteristics offered the prospect for a safe and effective therapy that can be used to restore normal atrial rhythm and contractility. In chapter 2 and 3 we addressed how the inhibition of different currents translates to atrial electrophysiology and contractility. We concluded that vernakalant mainly acts as a sodium channel blocker and that atrial contractility was not improved by it. In chapter 4 we performed high-density mapping to study the changes in propagation patterns induced by vernakalant. Vernakalant reduced different mechanisms that are associated with AF perpetuation. We concluded that vernakalant’s antiarrhythmic mechanisms led to a reduction of the overall complexity that was associated with a strong inhibition of turning.

In chapter 5 we investigated AF dynamics and stationarity of AF properties when long recordings are considered. This knowledge is relevant for catheter ablation of AF as these procedures may use a short sample of AF that may be affected by temporal and spatial variability. We identified stationary AF properties for a range of parameters but conduction properties were subject to the largest variation. The stationarity of AF parameters could not be explained by stable patterns. Most of the time unique patterns the mapping area.

It has been proposed that rotors are the source of stable AF patterns at certain sites and are the fundamental perpetuators of AF.¹ However, there is a lot of controversy about the stationarity and contribution to AF perpetuation of rotors. The Focal Impulse and Rotor Modulation (FIRM) approach is a method to terminate AF by targeting rotors. It has been reported that FIRM has a high success rate to restore and maintain sinus rhythm but the algorithms used for rotor detection is not fully disclosed.² However, there are concerns about specificity of rotor detection when the fundamental approach of this technique is considered. In chapter 5 we investigated the potential pitfalls of the identification of rotors with the use of phase singularities. We concluded that false positive rotor detections are increased by low electrode density and high complexity of conduction patterns. We believe that phase singularity-based rotor identification requires confirmation by activation mapping. We propose a dual ring model to reject rotors that are most probable to be a false positive detection. The findings of this thesis are discussed in this general discussion below.

Rhythm control

Atrial fibrillation (AF) is a disease that affects a substantial part of the western society. Both the Framingham Heart study and the Rotterdam study recognized that those with an age above 40 have a 20-25% risk to develop AF over their lifetime.^{3,4} Increased mortality risk, stroke and hospitalization are independently associated with AF.⁵ AF induced reduction of left ventricular function and quality of life may have a significant impact on daily life.⁶ Treatment of AF, either by limiting the ventricular frequency (rate control) or by restoring sinus rhythm (rhythm control), lowers mortality risk and improves quality of life.⁶⁻⁸ According to the guidelines for the management of atrial fibrillation, the clinician can choose between rate or rhythm control for the treatment of AF.⁵ Restoring normal sinus rhythm seems to be the obvious first choice of therapy. During sinus rhythm the heart can adapt its rate, dependent on the physiological demands, and atrial contraction may contribute to ventricular contractility. However, no superiority of rhythm control by antiarrhythmic drugs over rate control was reported by several clinical trials (e.g. AF-FIRM⁹, RACE¹⁰ and PiAF¹¹). Patients on rhythm control above 70 had even poorer outcomes in a sub-study of the AFFIRM trial.¹² The lack of superiority may be due to unsuccessful restoration of sinus rhythm. In the RACE trial, only 39% of patients remained in sinus rhythm at the end of the study.¹³ Failure may be even higher since ~40% of AF patients have asymptomatic (unidentified) AF episodes.^{14,15} Moreover, patients in these trials often had a long duration of AF.¹⁶ This may be of critical importance since AF itself initiates different kinds of remodelling (e.g. electrical, structural, contractile and neuro-humoral) that stabilizes the disease.^{17,18} It is therefore not surprising that with progression of AF pharmacological therapy becomes less effective.¹⁹⁻²² Moreover, the use of antiarrhythmic drugs for the treatment of AF is limited due to the risk for ventricular proarrhythmic effects.²³ To overcome the limited success rates of pharmacological rhythm control, it is proposed to start therapy at an earlier stage and more aggressively.¹⁶ The “Early treatment of Atrial fibrillation for Stroke prevention Trial” (EAST), where patients are treated from their first presentation with an AF episode, will give more insights into effectiveness of early rhythm control. On the other hand, patients do not necessarily present themselves at an early stage of the disease, particularly when they have non-symptomatic (‘silent’) AF episodes.

In any case, it is desirable to improve pharmacological rhythm control. The development of agents should aim for a higher potency for AF termination and with a lower risk for ventricular arrhythmias and/or extracardiac toxicity.

Potential targets for new antiarrhythmic drugs for the treatment of AF

Atrial fibrillation leads to a reduced $I_{Ca,L}$ transient and changes expression levels of K^+ channels and their function.²⁴ These adaptations lead to a shorter atrial action potential duration²⁵, refractory period and AF cycle length, known as “electrical remodeling”.²⁶ The challenge of pharmacological cardioversion of AF is to design a drug that prolongs the AF cycle length to a level that AF termination occurs. The required degree of AF cycle length prolongation to terminate AF becomes higher with longer AF durations.²⁰ The atria undergo structural remodelling with prolonged AF duration leading to more complex propagation patterns that are more resistant to antiarrhythmic drugs.²⁷ Current antiarrhythmic drugs increase the proarrhythmic risk on the ventricular level to an intolerable degree when these cycle lengths are targeted.²⁰ It is therefore desirable to develop a more atrial-specific strategy. Several atrial selective/preferential targets have been proposed. In **figure 1** a schematic presentation of an atrial action potential is presented. The corresponding currents that contribute to the atrial action potential are depicted in the lower panel. The currents that may serve as atrial specific targets are depicted in red and discussed below.

Figure 1 Atrial action potential and normalized inward and outward currents during the atrial action potential. Red current traces point out potential targets for the treatment of AF, details are discussed in the text. The arrow at the NCX current highlights the phase where the NCX runs in the reverse mode leading to calcium entry. Vernakalant targets currents that are indicated by an asterisk.

It has been suggested that an increased basal inward rectifier current abbreviates the action potential and lowers the resting membrane potential.^{28,29} The subsequent faster recovery of the fast sodium current channels increases atrial excitability. This would stabilize re-entry and a higher persistence of fibrillation waves.²⁸ The basal inward rectifier current is formed by the I_{K1} current and is complemented with the **G-protein gated $I_{K,ACh}$ current** when acetylcholine is present.³⁰ Kir2.3 channels (I_{K1}) are expressed in both the atrium and the ventricle but Kir3.1 and Kir3.4 channels ($I_{K,ACh}$) are predominantly expressed in the atrium.³¹ Kir3.1 and Kir3.4 channels are activated by vagal stimulation under normal physiological conditions. $I_{K,ACh}$ is constitutively activated, independent from activation by acetylcholine, in chronic AF patients and contributes significantly to the background inward rectifier current. Some class I and III antiarrhythmic drugs are described to have $I_{K,ACh}$ inhibiting properties but with a low potency.^{32,33} Some investigational drugs with better selectivity for $I_{K,ACh}$ have been developed and delay repolarization thus eliciting an antiarrhythmic effect.³¹ The first clinical trial that investigated the potential of $I_{K,ACh}$ block (BMS 914392) was designed to explore the potential to reduce paroxysmal AF burden. A reduction in AF burden could, however, not be demonstrated.³⁴ It is not yet understood whether the lack of effect was due an inadequate efficacy of $I_{K,ACh}$ inhibition as a target or if it was due to technicalities like dosing, patient selection or others.

Another well-recognized atrial specific potassium channel is $Kv_{1.5}$, which is responsible for the **ultra rapid delayed rectifier current** (I_{Kur}).³⁵ The ultra rapid delayed rectifier current is activated in the earliest phase of repolarization and together with I_{to} determines the triangular shape of the atrial action potential.³⁶ Selective I_{Kur} block by 4-Aminopyridine leads to an elevation of the plateau potential.³⁷ I_{Kur} block significantly increased the action potential duration at 90% repolarization (APD_{90}) in atrial bundles from in patients with AF but shortened APD_{90} in bundles of sinus rhythm patients. Indeed, I_{Kur} inhibition promoted AF susceptibility in a dog model with healthy atria.³⁸ Contrarily, I_{Kur} inhibition potentially reduced AF inducibility and stability in electrically remodeled atria.³⁹ A computer model of the atrial action potential gave insight in these contradicting observations.³⁷ In the healthy atrium, $Kv_{1.5}$ channels are inactivated during terminal repolarization but inhibition of I_{Kur} does increase the driving force for I_{Kr} . Hence, the larger driving force for I_{Kr} leads to a larger current density at the terminal repolarization and shortens APD. Electrical remodeling shortens the APD resulting in an activated $Kv_{1.5}$ channels during terminal repolarization. Indeed, a potentiated effect on effective refractory period (ERP) prolongation by I_{Kur} inhibition were demonstrated in different models of AF.^{37,39,40} A whole range of I_{Kur} inhibitors have been developed to exploit the potential of I_{Kur} but did not pass phase I and II trials.⁴¹

Two pore domain channels (K_{2P}) contribute to the resting membrane potential and may affect repolarization.⁴² Eighteen isoforms are currently known of which 6 are expressed in the heart. Different existing antiarrhythmic drugs have I_{K2P} inhibiting properties and may thereby be supported in their mode of action.⁴³⁻⁴⁵ TREK-1 ($I_{K2P2.1}$) and TASK-1 ($I_{K2P3.1}$) channels are expressed in the ventricle and atrium but respond to different

stimuli.⁴⁶ $I_{K2p2.1}$ is sensitive to stretch and $I_{K2p3.1}$ is modulated by extracellular acidosis and α -adrenergic stimulation. Both TREK-1 and TASK-1 are down regulated in patients with left ventricular dysfunction.⁴⁷ In contrast, TASK-1 is up regulated in atria of patients with chronic atrial fibrillation and inhibition prolongs APD₉₀.⁴⁶ This type of differential remodeling may offer an opportunity to affect the atrium with limited effect on the ventricle, particularly in higher risk patients. Yet, it must be noted that two pore domain channels are also widely expressed in other organs.⁴⁸ Multiple isoforms are often expressed within a certain cell type and targeting one might be compensated by the repolarization reserve. In the pulmonary system, however, smooth muscle cells exclusively express $I_{K2p3.1}$. The inhibition of $I_{K2p3.1}$ by endothelin-1 leads to contraction of a pulmonary smooth muscle cell and may cause severe pulmonary hypertension. Targeting TASK-1 clearly needs further *in vivo* investigations.

In contrast to the above describe channels, gating of **small-conductance calcium-activated potassium channels** is not voltage-gated but regulated by binding of calcium to calmodulin that induces conformational changes of the SK channel.⁴⁹ Currently, 3 isoforms of these channels have been identified, SK1 SK2 and SK3. The latter two are expressed in human atria but are somewhat down-regulated in AF patients.⁵⁰ SK current inhibition leads to post-repolarization refractoriness and APD prolongation in human and rat atrial cells.^{50,51} A selective SK channel blocker, AP14145, was recently evaluated in pigs and demonstrated atrial-selective refractory period prolongation and a high potency to terminate vernakalant-refractory AF.⁵² Despite these promising results it remains a concern that SK channels are not only expressed in the heart but also in other tissues like the brain. Another concern is that SK channels may also have an important feedback mechanism to neutralize spontaneous calcium release events. Depolarization of the membrane potential with consequent activation of the fast sodium current may occur when the sarcoplasmic reticulum releases a calcium spark of sufficient size. The SK channels may buffer elevations of membrane potential caused by calcium releases and thereby protect against ectopic activity due to spontaneous Ca^{2+} release events.⁵³ Moreover, SK current inhibition led to APD heterogeneity, alternans and wave break in canine left atria.⁵⁴ Further investigations are warranted to address the potential usefulness of SK inhibition for the treatment of AF.

Biophysical properties of **sodium channels** differ between the atrium and the ventricle.⁵⁵ These differences can be exploited to reach an atrial-preferential effect, as demonstrated for ranolazine and amiodarone during regular pacing.^{56,57} During AF, the excitable period is this short that a new action potential is initiated before full recovery of the sodium channel and resting membrane potential is achieved.⁵⁸ Additionally, the rate in the atrium is far higher than in the ventricle. A sodium channel blocker with use-dependency may, therefore, be functionally more active in the atria during the high rates of AF. However, the CAST trial raised strong concerns about the use of sodium channel blockers particularly in patients with structural and ischemic heart disease.⁵⁹ Conduction slowing

induced by sodium channel block makes these patients more susceptible re-entrant tachyarrhythmias.

In summary, different targets have been proposed as potential strategies. However, some targets may be downregulated during AF or strong inhibition of these targets may increase the propensity for other arrhythmias and extracardiac side effects. Alternatively, **multichannel approaches** may be designed that stay within the safety limits and may be more efficacious because of synergistic actions between channels. For instance, the combination of the “early” I_{Kur} and “late” I_{Kr} has a supra-additive effect on ERP and was very effective in terminating persistent AF in the goat.²¹ Additionally, atrial-preferential sodium current inhibition may be augmented when the atrial action potential is modulated.⁶⁰ The potential of this approach was observed in *in-silico* models, isolated dog hearts, and horses.⁶⁰⁻⁶²

Vernakalant, a multichannel approach with predominant sodium channel effects

Available antiarrhythmic drugs are in many cases not 100% specific for a single ionic current. Amiodarone, dronedarone, and vernakalant are well recognized for their ability to inhibit multiple ion channels at therapeutic concentrations.⁶³⁻⁶⁵ Vernakalant is the last of these to be approved for the European market.⁶⁶ Fedida et al. reported that vernakalant inhibited the atrial specific currents I_{Kur} and $I_{K_{ACh}}$, accompanied with an inhibition of I_{to} , I_{Kr} and a use-dependent inhibition of I_{Na} .⁶⁵ Recently, it was shown that vernakalant also inhibits $I_{K_{2p2.1}}$ at clinical relevant concentrations but not I_{SK} (**figure 1**).⁶⁷ Earlier studies on vernakalant have emphasized its ability to inhibit I_{Kur} and high atrial specificity was assigned to vernakalant because of a high potency for the atrial specific currents.⁶⁸⁻⁷⁰ Vernakalant led, indeed, to an atrial-preferential refractory period prolongation in patients.⁷¹ This notion led us to hypothesize that vernakalant could increase atrial contractility, as investigated in **chapter 2**. The hypothesis was based on previous experimental work from our group that demonstrated that during inhibition of I_{to}/I_{Kur} -dependent early-phase-repolarization the sodium calcium exchanger runs in the reverse mode, facilitating calcium entry (arrow **figure 1**). Higher plateau potentials will cause more calcium entry and a more forceful contraction.⁷² If atrial contractility is increased by vernakalant it could help patients to improve exercise tolerance. Unfortunately, vernakalant did not increase atrial contractility in the goat model of AF. We could explain this by the marginal changes in plateau level of the action potential, similar to effects in human trabeculae.⁷³ Moreover, reduced atrial contractility occurred at higher heart rates. This rate dependent effect indicated that I_{Na} inhibition was more important to vernakalants antiarrhythmic mechanism than previously thought.

In **chapter 3** we evaluated the class I and class III properties of vernakalant. We concluded that vernakalant should indeed be considered as a class I antiarrhythmic drug

because; **1** action potential duration increased only marginally, **2** the substantial ERP prolongation was due to post repolarization refractoriness, **3** conduction velocity was slowed, **4** excitability was reduced. This conclusion is supported by *in vitro* investigations that demonstrate that I_{Na} is strongly inhibited, the upstroke of the action potential is slower and only moderate action potential prolongation occurs.^{73,74} In itself it is surprising that it took almost decade after the first reports on vernakalant (aka RSD1235) before post repolarization refractoriness was investigated as a potential mechanism of ERP prolongation.^{65,74} Today, it is questioned whether I_{Kur} inhibition is indeed relevant to action of vernakalant.⁷⁵ However, potassium current inhibition may affect the sodium current indirectly by preventing the full recovery of the membrane potential. This increased membrane potential could lead to partial inactivation of sodium, as proposed by Aguilar et al.⁶⁰ In our study we compared vernakalant with flecainide (a more preferential I_{Na} blocker) and matched dosage to obtain similar increases in AF cycle length. Interestingly, although conduction velocity was reduced to a similar degree, vernakalant produced longer refractory periods. This suggests that the multi-channel profile of vernakalant potentially contributed to its antiarrhythmic effect. Assessment of the diastolic membrane potential with micro-electrode measurements of a multicellular preparation would be of help address this potential mechanism.

The sodium channel inhibiting profile of vernakalant is supported by 2 additional observations we made in several goats, although these were not systematically addressed. **1** The observation of conversion of AF to a persistent form of atrial flutter, as seen with other sodium channel blockers.⁷⁶ Two case reports describe the same transition from AF to stable flutter in patients. In these reports the flutter led to fast ventricular frequencies that required intervention with electrical cardioversion.^{77,78} **2** The failure of vernakalant to terminate atrial flutter, in goats and humans, is another indication of the predominant class I effect of vernakalant.⁷⁹ The risk of vernakalant-induced atrial flutter should, therefore, be considered when patients are treated with vernakalant.

Potency and safety of vernakalant

Cardioversion with vernakalant is often achieved 10-15 minutes after administration in recent onset AF patients. The time to conversion was shorter than other clinical alternatives such as amiodarone⁸⁰, flecainide and propafenone⁸¹, and ibutilide.⁸² Vernakalant is successful in 50% of patients with ≤ 7 days of AF.⁸³⁻⁸⁵ Likewise, vernakalant cardioverted almost half of the goats after two weeks of AF (**chapter 3**). It is therefore remarkable that almost all goats (15/16) cardioverted under anaesthesia despite that AF was maintained for longer periods, 3 or 22 weeks (**chapter 4**), **figure 2**. The reason for this high efficacy under anaesthesia is unclear but could offer an opportunity for improvement of therapy. The basic electrophysiological effects of vernakalant (e.g. AF cycle length, refractory period, conduction velocity) did not change with AF duration, indicating that potency did

not change. Possibly an “external factor” may be required to achieve cardioversion during advanced AF. For instance, one could speculate that the anaesthetics inhibited the autonomic nervous system and thereby improved efficacy. Inhibition of the vagal tone is expected to prolong action potential and AF cycle length. An inhibition of the sympathetic tone may reduce the number ectopic discharges. Experiments where the autonomic system is fully blocked would then illustrate a major improvement on antiarrhythmic drug efficacy. However, several other procedural related effects (e.g. changes in stretch due to reduced arterial pressure and ion channel block due to anaesthetics) may have contributed in consequence of the open chest procedure.

Figure 2 The upper two panels present the effect of vernakalant on the AF cycle length and conduction velocity. The results for 2 (2dAF) and 11dAF days of AF were obtained in awake animals. Results for 21 (21dAF) and 150 (150dAF) days of AF were obtained in anesthetized animals. The AF duration is approximated in days for readability. The lower panels present 25-minute AF survival plots. At baseline only 1 animal cardioverted spontaneously in the 11days AF group, others remained in AF. Despite a longer AF duration, vernakalant cardioverted more goats under anaesthesia than awake goats.

The cardiac safety of vernakalant was tested in several experimental models sensitive to ventricular proarrhythmic events.⁸⁶⁻⁸⁸ In these models vernakalant did indeed not show entirely atrial-specific effects. Action potential duration, refractory period and QT time increased to some degree. However, proarrhythmic markers such as action potential

dispersion, short-term variability and Torsade de Pointes were not increased. Indeed, in clinical trials vernakalant showed to be safe in hemodynamically stable patients^{84,85}, post-operative AF⁸⁹ and patients with a history of ischemic heart disease.⁹⁰ The safety of vernakalant in patients with ischemic heart disease has been investigated in a relatively small number of patients (n=183). To the best of our knowledge there has been no preclinical investigations on safety in models of ischemia or myocardial infarction. Given the important role of sodium current inhibition the propensity to macro-reentrant arrhythmias may be critically increased in these conditions, as illustrated by the occurrence of fast atrial flutters in goats and man.

In summary, the main mode of action of vernakalant is not targeted at atrial-specific ion currents but is largely dependent on sodium current inhibition but proarrhythmic markers in ventricular action potential morphology seem to be limited. Vernakalant can, therefore, be considered as a drug that acts preferentially on atrial electrophysiology.

Vernakalant induced cycle length prolongation

The hallmark of AF is the fast and irregular activation of the atria that is manifested as complex propagation patterns on the atrial surface. The strong association between rate and complexity of propagation patterns has been demonstrated in several experimental studies that modulated fibrillation cycle length.⁹¹⁻⁹⁵ The AF cycle length is composed of the effective refractory period and the excitable period. The refractory period was consistently prolonged (chapters 2-4) but AF cycle length was prolonged to an even stronger extent. Indeed, both class I and III Antiarrhythmic drugs have been shown to considerably prolong excitable period and thereby contributed to AF cycle length prolongation.^{96,97} Allessie and coworkers proposed the concept that inhibition of turning of wavefronts may contribute to AF cycle length prolongation.⁹⁸ Wavefronts frequently encounter functional (refractory) and anatomical obstacles during AF. At the end of these obstacles, the wavefront may encounter a region that is excitable but only can be activated when the wavefront makes a turn. When the region is activated through a sharp turn the local cycle length will be shorter compared to an activation preceded by a wider turn. Hence, reduced turning of conduction paths leads to longer excitable periods and contributes to an increased cycle length.

Conduction paths of wavefronts are not fully random (isotropic) but tend to follow the fiber and/or bundle directions.⁹⁹ The lateral sides of the wavefront will therefore regularly encounter lateral cell-to-cell connections. Sodium channel inhibition preferentially suppresses transverse conduction and thereby the speed of turning.^{98,100} Furthermore, turning around a line of block becomes blunted when excitability is reduced.¹⁰¹ In the study presented in **chapter 4** we mapped both atria to investigate the degree of turning during AF. Turning was abundant during AF and a strong reduction of turning occurred after vernakalant administration (**figure 3**). These data support the proposed hypothesis that

inhibition of turning prolongs excitable period and AF cycle length. Further investigations using the optical mapping technique could identify the ERP and excitable period during AF. This may be of help to more directly proof that vernakalant blunts turning around a functional line of block which contributes to cycle length prolongation.

Figure 3 A graphical summary of the electrophysiological effects of vernakalant. The reduction in excitability caused by vernakalant led to a slowing of conduction, post repolarization refractoriness and reduced turning. The combination of these effects resulted in a lower fibrillation frequency and less complex propagation patterns.

Vernakalant reduces AF complexity.

A reduction in the complexity of fibrillation patterns has generally been observed during the pharmacological treatment of AF.^{93,94,102} Several conceptual models of AF perpetuation have been proposed which may explain the reduction in complexity. Scherf et al.

demonstrated in one of the first “AF models” that an ectopic focus could initiate and maintain fibrillatory conduction (**figure 4A**).¹⁰³ More than half a century later, Lee et al. suggested that in longstanding persistent AF patients all wavefronts indeed can be traced back to focal sources.^{104,105} However, a dual layer model has been proposed that may explain activation patterns that resemble ectopic sources. These breakthroughs patterns are formed by wavefronts that conduct transmurally from endo to the epicardial surface and vice versa.¹⁰⁶ Endo-epicardial dissociation (EED) is required for transmural conduction to occur. In the AF model of the goat, the degree of EED increases gradually with increasing AF duration.¹⁰⁷ Endo-epicardial mapping in the right atrium of patients with paroxysmal and persistent AF confirmed the existence of electrical dyssynchrony in patients.¹⁰⁸ With a similar procedure we demonstrated that transmural conduction is the predominant source of breakthroughs in the left atrium of goats with persistent AF.¹⁰⁹ The overall incidence of breakthrough waves was limited to 10% of all waves and only 13% of these breakthroughs were identified to resemble ectopic activity. The small contribution suggested that ectopic sources were not continuously present. If ectopic beats appear transiently then another mechanism should contribute to AF maintenance in the periods of ectopic silence.

Figure 4 Overview of proposed AF perpetuating mechanisms.

Others have proposed that rotors, a specific type of functional re-entry may maintain AF (**figure 4B**). The rotor is a wavefront that turns and causes a functional re-entry independent from an anatomical obstacle. When these small re-entrant circuits are situated within the atrial wall they may appear as repetitive breakthroughs.^{110,111} Rotors have been observed in experimental studies in isolated sheep hearts and were originally visualized with optical mapping.¹¹²⁻¹¹⁴ Based on such studies it has been proposed that rotors are sources of high frequency that thereby drive AF.¹¹⁵ FIRM, an endocardial technique with high coverage and low density has been developed with the intention to identify rotors based on electrograms.^{2,116} Low AF recurrence rates were reported when stable rotors were located and ablated.² The termination of AF after targeting putative rotors was considered by some as evidence that a rotor maintains AF.¹¹⁷ However, there is large disagreement on the interpretation of these results. There are some technical aspects of the technique that may lead to identification of pseudo-rotors but that will be discussed in the last paragraph of this discussion. To date, several independent clinical investigators used this technique with variable success rates. A randomized multicenter trial is required to gain more scientific evidence for the efficacy of this technique.¹¹⁸ However, the reported conduction patterns are hard to reconcile with high-density epicardial mapping. One would expect that such stable sources should also be present on the epicardium, at least in some patients. High-density mapping of the epicardium of persistent AF, analyzed with different algorithms and by different research groups, did not identify stable rotors.^{104,119,120} It is argued that the high-density maps prevent the identification of rotors due to a limited field of view. Yet, a consistent finding in animal models and human AF is difficult to reconcile with this argumentation. High-density mapping identified narrow waves that conduct in different directions without re-entrant paths.¹¹⁹ This would mean that if rotors need to be identified with a macroscopic view, that the center of the rotor is occupied with complex non-re-entrant patterns. Yet, according to the rotor theory a functional re-entry occurs in the core of the rotor and is thereby the fundamental perpetuator of AF.¹²¹

FIRM ablation may be an effective therapy independently from the identification and ablation of rotors. Ablation of different sites in the atrium affect two important contributors to multiple wavelet re-entry, i.e. total mass and substrate geometry (**figure 4C**). Gordon Moe, who proposed the multiple wavelet re-entry, wrote that "it is difficult to believe that either of these mechanisms [ectopic focal activity or re-entry] would be endowed with sufficient stability to persist for years as fibrillation often does."⁹¹ He therefore proposed that a substrate with short refractory periods, heterogeneity in refractory period and conduction velocity would lead to multiple waves. Stable AF is then achieved when the probability that all waves die out simultaneously becomes sufficiently low.¹²² In support of the multiple wavelet hypothesis, a higher number of epicardial waves have been found in recordings of animal models and human persistent AF compared to acute AF.^{104,119,120} Jalife et al. criticized that the first observations of multiple waves in a canine model of cholinergic AF exhibited far fewer waves than the critical number of waves

needed in the computer model of Moe et al.¹²¹ Yet, complexity, particularly during persistent AF, is likely to be much higher when EED is considered because only one of the layers of the atria is mapped.

In **Chapter 4** we described breakthrough and re-entry patterns and multiple waves within the same recordings. Neither breakthroughs nor re-entry patterns were found to be stable or located in a specific area within the atria. Complexity was highly dynamic and varied between 1 single wave up to >15 waves in a single AF cycle within the same goat. The intervention with vernakalant reduced complexity of propagation patterns, local re-entries and the number of breakthroughs. Hence, the intervention with vernakalant led to a reduction of all 3 AF perpetuating mechanisms. Thus, all 3 mechanisms may contribute to AF maintenance as the manifestation of the number of waves, rotors, and ectopy are interdependent (**figure 4D**). For example, fewer waves involve fewer opportunities to form a rotor. However, fewer rotors may lead to less rotor-mediated wave break and thereby fewer waves. Less ectopic activity will lead to fewer waves or unidirectional conduction block for rotor formation but fewer waves or rotors do not necessarily mean less ectopic activity. Advocates of the ectopy or rotor theory may object upfront to the conclusion that all mechanism coexists because a stable source could have been outside the field of view. Targeting this stable source would then terminate AF. Indeed, the coexistence of all theories can only be disproven when the entire atria (or substrate) can be mapped which is technically not achievable at this moment in time. It is conceivable that continuous interaction between diverse mechanisms will be the actual perpetuator of AF in many patients. A similar interpretation was drawn for ventricular fibrillation.¹²³

Recurrent pattern analysis and stationarity

In **chapter 5** we have introduced a technique that visualizes and identifies changes in AF dynamics. Recurrent pattern analysis was developed to identify patterns with a similar direction and morphology. In contrast to chapter 4 no waves were constructed but a recurrence was solely based on the spatial distribution of activation times. This approach allowed us to identify patterns that appear intermittently or potential recurrent patterns that affect the fibrillation process continuously. Propagation patterns were often very unique in their appearance. Similar to “conventional” wave mapping, no stable re-entrant sources were identified but occasional recurrent epicardial and breakthrough patterns did occur. Despite the high degree of uniqueness of propagation patterns a high degree of stationarity (reproducibility of a parameter when multiple samples taken) of AF properties was found in time and space. This finding is an important illustration that a stationary distribution of AFC cycle length, conduction velocity, AF complexity and others do not necessarily implicate a stable propagation pattern or source at this site. This observation suggests that AF is in general confined to the determining properties of the underlying tissue. However, there was a loss of stationarity after a 1-hour interruption of AF. This

observation may have an important clinical relevance. If AF sources are pinpointed by signal properties then the ablation target may have shifted in space in a short period of recovery in sinus rhythm. This observation is good demonstration that AF is a dynamically changing process that possibly requires multiple (ablation) strategies dependent on the current state of AF.

Figure 5 Recurrent pattern plots obtained during stable AF and vernakalant-induced AF termination. A) A 60s recurrence plot from goat 10, chapter 5. B) A zoomed in section of 5s that visualizes a block of recurrent patterns. It is interrupted for a short instance twice but at the red arrow a >10s disruption of the organized pattern occurred. C) The last 5s before a vernakalant-induced AF termination. The last 5 cycles a recurrent pattern appeared for before AF terminated. D) A cross-recurrence plot visualizes recurrent patterns between two separate recordings. This cross-recurrence plot compared two 5s sections, stable AF (above, x-axis) and AF termination (left, y-axis). The dashed lines illustrated how states on the cross-recurrence plot are related to the individual recurrence plots. The recurrent pattern prior to AF termination appeared for multiple cycles during stable AF.

The true significance of recurrent propagation patterns for AF maintenance remains to be investigated. Stable periods of recurrent patterns, identified as a block of recurrences centered on the line of identity, repeatedly occurred in several animals (**figure 5A, 5B, and table 2 chapter 5**). It is tempting to interpret the appearance and/or disappearance of these events as a reflection of different mechanisms that switch from one to the other over time. Recurrence plot analysis may therefore disclose mechanistically relevant events contributing to AF. Preliminary data have shown that with transition from stable AF towards AF termination, more recurrent patterns occur. Repeated vernakalant-induced cardioversions also indicated that within one individual animal one particular pattern consistently precedes cardioversion (**figure 5C**). This may suggest a common path for AF termination. In most studied goats these “termination” patterns were also observed during stable AF (**figure 5D**). It would, therefore, be of interest to study under which circumstances a termination-associated propagation pattern switches to unique patterns, as the latter seem to prevent AF termination. High coverage mapping experiments, where both spontaneous and drug-induced AF terminations are recorded in the same goat, could be of great value to investigate the occurrence of termination patterns and how they are disturbed over the atria.

Identification of phase singularities as true rotors

Different mapping strategies have been developed over the years.¹²⁴ All techniques aim to identify proarrhythmic areas that can be targeted for ablation. Characterization of the substrate was often based on the signal properties of unipolar/bipolar signals. Specific properties such as complex fractionation and dominant frequency were then thought to reflect sources of AF. However, these techniques failed to show improved outcome when ablation of these “sources” was performed additionally to pulmonary vein isolation.^{125,126} This brought the field to develop methods that can map propagation patterns during AF in patients. Propagation patterns can be visualized if local activations can be identified. Proper identification of local activation times is not straightforward because of fractionation or double potentials. Fractionation can be caused by dyssynchronous activity due to tissue architecture and functional block or slowing of conduction.^{127,128} We developed, and used in **chapters 3 to 5** an approach that identifies the most likely local activation based on the probability distribution of cycle length, that is obtained based on amplitude and steepness of the deflections.¹²⁹ Phase mapping is another approach to visualize electrical activity in the heart. Phase mapping abandons the attempt to identify local activations but transfers the electrogram into phase space. Phase space loops are formed in periodic systems but if an electrogram is transferred into phase without prior processing it leads to chaotic patterns (non-periodic). Preprocessing of the signal is therefore required to obtain meaningful phase analysis but smoothens the signal and causes a phase shift to some degree. Different algorithms can be used but require assumptions like

stable signal morphology or cycle length.¹³⁰ The analysis of multiple phase signals in space provides information on wavefront curvature and allows the identification of sites that are surrounded by all phases (phase singularities). Phase singularities are supposed to identify rotor activity. Stable phase singularities may then indicate stable rotors that drive AF and be relevant ablation targets.

The FIRM technique is designed to identify rotors and phase analysis is integral part of the algorithm.^{2,116} FIRM is applied on the signals obtained with basket catheters that are positioned in the cavity of both atria. The appealing part of this approach is that global coverage is obtained. However, basket catheters contain a limited number of electrodes, 64 per basket. The basket catheter is composed of 8 splines with 8 electrodes with an inter-electrode distance of 5-9 mm. The distance between splines is dependent on how the basket is unfolded in the atrium.¹³¹ The map obtained from the 3D oriented electrodes is presented as a rectangular map that is opened up at the site of the valves. The maps have fixed distances between the nodes on the spline but the map is not corrected for the variable distance between the splines. This hampers the interpretation of conduction velocity. Zones of conduction block are therefore not recognized. Moreover, the identification of a phase singularity does also not take conduction block into account. Identification of rotors based on phase singularities but without activation time conformation may therefore identify phase singularities caused by lines of block. We demonstrated in recent work that in patients with paroxysmal and persistent AF the majority of all identified phase singularities co-localize with block lines.¹³² This excludes that the identified phase singularity reflects a re-entrant mechanism at this site. Another point of concern is the spatial density of the maps. In persistent AF, wavefronts may be as narrow as 2.5mm or less.¹¹⁹ Some of the splines of the basket are often positioned 10mm apart or more. Two neighboring activations are therefore not necessarily caused by the same wavefront. In **figure 6** an example of a map from a goat with 22 weeks of AF is presented. The figure demonstrates that low electrode density and velocity-independent interpretation of activation sequences may lead to apparent re-entrant trajectories.

Independent assessment of these concerns in relation to basket and phase mapping is not possible because the FIRM algorithm is not fully disclosed. We therefore designed our own algorithm to construct phase and identify phase singularities. This algorithm was then applied on high density maps obtained in patients with paroxysmal and persistent AF. Recently, it was confirmed by the developers of FIRM that our approach to identify phase singularities agrees with FIRM. Specifically, phase singularities that were associated with ablation-sites that induced AF termination corresponded well.¹³³ Despite this encouraging finding it is still an important to address how often false detections will occur as ablation of it will lead to unnecessary damage to the atrial wall. In **chapter 6** we investigated how phase singularity detection is affected by electrode distance and organized electrical activity. We found that the probability of phase singularity detection was strongly increased with increased electrode distance. Moreover, when spatial coherence of the map was broken, a significant amount of phase singularities was found. Yet, these

phase singularities could not be caused by re-entrant conduction. Both exercises illustrate that the transformation to phase and consequent phase singularity detection is sensitive to false positive detections. A confirmation of the activation map is indeed needed to reduce false positive detections. We therefore introduced a double ring detection algorithm which only confirms phase singularities if they are detected in two rings of electrodes around the phase singularity point.

A Basket 2D map with 10mm spline distance projected on a HD-map

B Complexity of waves patterns after 22 weeks of AF in the goat

C Isochrones and propagation trajectories zoomed in to the size of a HD-map

D Down-sampling and disregarding conduction block leads to apparent re-entrant circuits

Electrode on a

Block line

Wave trajectory

① Sequence of appearance

↻ Apparent re-entrant circuit

Figure 6 A 2D basket map in relation to high density (HD) map to illustrate the relevance of electrode density and conduction block. **A)** A 2D basket map superimposed on a HD-map used in this thesis. The basket map is based on the largest basket catheter with an inter-electrode distance of 9 mm and an assumed inter-spline distance of 10 mm. The HD-map is comprised of 249 electrodes with a 2.5 mm electrode distance. 56% of the basket electrodes are covered by the HD-map, illustrating a larger macroscopic view of basket mapping. **B)** The basket splines are superimposed on a wave map. Different greyscales signify individual waves and the

numbered labels indicate the consecutive order of appearance. The superposition of the basket electrodes illustrates that a highly complex pattern is present in a limited region of the basket map. **C)** The wave map from panel B zoomed in to 5 splines of the basket. For readability the assigned greyscale to the waves is omitted but isochrones and wave trajectories are unaltered. **D)** The trajectories in panel D are here visually adjusted to the low density of the basket electrodes. Trajectories became straighter often following the direction of the spline. In some cases, the trajectory shifted in space (e.g. 8 and 9). Several apparent re-entrant circuits appear at this point of pattern analysis when conduction block is disregarded.

As different clinical investigations had variable outcome with FIRM ablation, a randomized multicenter trial should be executed to obtain further evidence on the efficacy of FIRM ablation.^{118,134} With proven beneficial effect of FIRM it should then be further addressed if the identified ablation sites are truly rotors or whether other proarrhythmic mechanisms are targeted. This would be essential in the scientific interpretation of AF perpetuating mechanisms.

Conclusions

In this thesis we performed a thorough evaluation of the new antiarrhythmic drug vernakalant. We concluded that although vernakalant is a multichannel blocker the primary mechanism of action is related to I_{Na} block; reduction of conduction velocity, excitability and post repolarization refractoriness (**figure 3**). Vernakalant prolongs refractory period and AF cycle length, inhibits wavefront turning during AF and lowers complexity of fibrillation patterns. All of these factors are associated with a decrease of AF stability. A summary of the antiarrhythmic effects of vernakalant is shown in **figure 3**. During stable AF continuous changing patterns are present but occasional stable patterns occur as well. The use of recurrent patterns analysis may advance our understanding of AF dynamics and stability. And finally, we addressed pitfalls of phase singularity analysis and concluded that low electrode density and high AF complexity may lead to false positive detection of rotors. Activation time mapping should be used in addition for correct interpretation of phase singularities.

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Valorization

Atrial fibrillation (AF) is a rhythm disorder that is often observed in the elderly population.¹ AF increases the risk for several cardiovascular diseases and in particular stroke.² The impact of AF on daily life, e.g. exercise tolerance and cognitive function, can be significant. The psychological burden of not knowing when and for how long AF occurs is substantial in symptomatic AF patients too. This becomes apparent when one reads the posts of AF patients on fora like www.atriumfibrilleren.nl. The joined goal of basic and clinical scientists is to reduce the incidence of AF and limit the burden of AF in daily life for those who already developed AF.

The prevalence of AF is as high as 3% for adults above 20 years of age. About a quarter of middle-aged people are expected to develop AF at some point in their life.¹ This estimate projects 14-17 million AF patients in the European Union in 2030.¹ Healthcare costs as a consequence of AF are considerable. In a combined evaluation of the Cardiovascular Health Study and the Framingham Study, healthcare costs increased by \$18,000 for a patient in the year after the first diagnosis of AF.³ Moreover, 70-80% of AF patients get hospitalized at some point during their disease.⁴ Once hospitalized, costs are about 3-fold higher than hospital costs for the average patient.⁵ The total financial burden of AF on the US healthcare system is estimated between \$6-26 billion.⁵

AF is also an extremely challenging scientific topic. Although it was initially identified as an electrical disorder, AF is a disease that has a more complex nature. Multiple regulatory responses (e.g. inflammation, electrical remodeling) and different cell types are affected during the progression of AF. These all together interact with other comorbidities.⁶ Hence, a highly challenging topic to investigate. The large social and economic impact of AF and its scientific challenges are valid justifications for worldwide efforts being made to come to a better understanding and treatment of the disease.

The work presented in this thesis is primarily focused on the scientific understanding of AF as a disease, with the eventual aim to improve treatment of patients. The data presented in this thesis are mostly obtained from experimental animal studies to address basic mechanism that cannot be studied in humans at the level of detail presented here. However, the topics discussed do directly link to clinical practice as both vernakalant and mapping, as a tool for AF ablation, are applicable strategies in the treatment of AF. Moreover, new algorithms that are presented in this thesis may contribute to more robust analysis of conduction patterns during AF. The work in this thesis is of interest for basic scientists, engineers and clinicians. The work on both, the antiarrhythmic drug vernakalant and mapping of AF, was presented on several congresses and network meetings in the Netherlands, Europe and US in the years 2014 to 2018. Scientific congresses (e.g. ESC, HRS, Europace, EHRA and CINC) that target a large audience with a broad range of backgrounds (e.g. basic, engineering and clinical sciences) were selected to maximize the impact of our work. Four out of five chapters are published in peer-reviewed journals relevant to our field of research: Heart rhythm, Europace, Frontiers in Physiology and IEEE. The other chapter is in preparation for submission in the near future.

Antiarrhythmic drugs

The first main topic of this thesis is the antiarrhythmic drug vernakalant. Vernakalant was approved for the clinical practice not long before the studies for chapter 2 and 3 were started. At that time, the majority of mechanistic knowledge about vernakalant was obtained in *in vitro* and in small animal models. The studies suggested an atrial-specific effect of vernakalant. The novelty of our work was that the effects were characterized during AF, the condition that is treated. Vernakalant was investigated in a large animal model that closely resembles the human electrophysiology. Therefore, the results in chapter 2-4 are expected to be well transferable to patients. The critical finding of the studies in chapter 2-4 was that the actual antiarrhythmic mechanism of vernakalant is dependent on the inhibition of sodium current that is present in the ventricle as well as the atrium. This insight is supported by investigations from two other independent laboratories.^{7,8} This finding is relevant because sodium current inhibition may increase the risk of lethal arrhythmias. It is therefore likely that our findings will contribute to a cautious use of vernakalant.

Another interesting observation is that the multi-ion-channel approach of vernakalant tended to be more efficacious compared to single ion-channel strategies. This finding is in line with numerical and experimental studies that suggest that the block of different currents may lead to synergistic electrophysiological effects.^{9,10} We have therefore setup a project that addresses the potential synergistic effects of combining different drugs. Our aim in this study is to achieve improved AF cardioversion efficacy without the cost of ventricular arrhythmias. Improved cardioversion efficacy will be achieved by the combination of the inhibition of atrial-specific currents and conventional antiarrhythmic drugs. The first results of this study are expected in spring 2019. In case of promising findings (a) phase I trials will be required before a combination approach can enter phase II and III trials to assess efficacy and safety in AF patients. We estimate that the entire trajectory will be at least ten years before it can be applied in standard clinical care.

Mapping and ablation of AF

The second main topic of this thesis is the analysis of propagation patterns during AF. We believe that insights of propagation patterns are fundamental to understand why, where and when AF requires an intervention (drugs, electrical shock, catheter ablation or surgical intervention) to terminate. This understanding may help the scientific community to design new therapies that affect specific mechanisms by for instance ablation.

An ablation approach is designed to eliminate atrial tissue to exclude the local tissue from the atrial activity. When an ablation procedure is performed successfully the patient is free of AF, at least for a longer period compared to pharmacological or electrical AF termination. However, there still is a large group of patients, about 40%, who does

develop recurrent AF in the first year after the procedure. It is therefore of interest for patients and physicians to develop a technology that identifies critical sites for AF maintenance. Moreover, the large group of AF patients represents a large economic value for biotech companies. This is well illustrated by the \$250 million investment of Abbott® in 2014 to acquire FIRM (Focal Impulse and Rotor Modulation) procedure developed by Topera Inc. FIRM requires extensive processing of the atrial signals. The risk of such processing signals is that the technology may lead to false detections of critical sites that will be irreversibly damaged. The work in chapter 6 focuses on the pitfalls of signal processing steps required for the detections of putative AF maintaining sites. Based on these results the concerns were addressed about the probability of false detections and proposed an additional step for improvement. This work is important to make scientists, physicians and engineers aware of the limitations of the technology when it is applied in an AF patient.

A novel analytical approach for the analysis of conduction patterns is presented in chapter 5. This approach is designed to identify patterns that occur repeatedly. The basic principle of this approach is a tool that can be applied to a chaotic system to describe the dynamical behavior of it. A particular pattern reoccurred in some animals of the study. Interestingly, some recurrent patterns matched to AF termination patterns, as discussed in the general discussion. It is therefore of high interest to study the mechanisms that disrupt such recurrent patterns as they might be critical for AF maintenance. If a particular mechanism can be identified, it might serve as an ablation target. Recurrent pattern analysis can be applied to signals obtained in preclinical models and from patient data sets. Moreover, recurrent pattern analysis may also be applied to noninvasive electrical mapping of the heart. Hence, we foresee that recurrent pattern analysis might have a great potential to identify AF maintaining mechanisms. However, further evaluation and validation needs to be applied before it can be used in the clinic. Whether and how frequently recurrent propagation patterns occur in patients remains to be investigated. It would be of potential interest to focus identification of ablation targets on the mechanisms that disrupt recurrent propagation patterns. The time window that is required that recurrent pattern analysis that will be applied in clinics are dependent on the outcome of our preclinical explorations, manpower for algorithm development and analysis, financial opportunities and cannot be estimated at this moment of time.

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Summary

Atrial fibrillation (AF) is a cardiac arrhythmia that leads to fast and irregular heart rhythms. AF occurs in about 3% of the adult population in the western world. The arrhythmia often appears in episodes in the early phase of the disease. However, episodes may become longer and AF may eventually become a chronic condition. The presence of AF increases the risk of a stroke and prevents the normal regulation of the heart rate during e.g. physical activity. It may therefore be desirable to terminate AF and restore normal sinus rhythm. AF can be terminated with an electrical shock, by ablation or antiarrhythmic drugs.

Vernakalant is a new antiarrhythmic drug available in the European clinical practice. Vernakalant can be distinguished from other antiarrhythmic drugs because it targets multiple ion currents, instead of one, without substantial extracardiac side effects. An interesting property of vernakalant is that, among others, it inhibits ion currents that are only expressed in the atrial wall. This property is thought to contribute to a safer use of the drug because targeting an atrial-specific current reduces the risk of drug-induced lethal ventricular arrhythmias. The chapters 2 to 4 investigate the possible effects and antiarrhythmic mechanisms of vernakalant.

In chapter 2 we evaluated the inotropic potential of vernakalant. We hypothesized that vernakalant could increase the membrane potential during the early phase of the action potential and thereby increase atrial contractility. This hypothesis was based on the observed inhibition of the atrial-specific I_{Kur} current by vernakalant. Previous studies in goats and dogs revealed that I_{Kur} inhibition could increase atrial contractility. We studied the effect of vernakalant on atrial contractility in an awake goat model. The goats were implanted with piezoelectric crystals to measure atrial dimensions, an atrial pressure catheter and electrodes. With these sensors we were able to measure contractile and electrophysiological parameters in healthy hearts and after two days of AF. Vernakalant did not increase atrial contractility. Despite the increase of atrial contractility by the selective I_{Kur} inhibitor AVE0118. Yet, vernakalant did substantially increase atrial refractory periods. More detailed analysis of the action potential morphology revealed that the early phase of the action was not affected by vernakalant. This finding suggests that I_{Kur} inhibition has a limited relevance for the effect of vernakalant.

In chapter 3 we explored the relative class I and III effects of vernakalant. The fast sodium current is responsible for excitability of the myocardium and the conduction velocity of a propagating wavefront. Potassium channels are responsible for the repolarization of the action potential and during normal conditions determines the refractory period. Vernakalant inhibits both the fast sodium current (class I) and several potassium currents (class III). We performed experiments in an awake goat model. We investigated how the relative class I and class III effects contribute to the antiarrhythmic action of vernakalant. Goats were implanted with grids of electrodes on the atria to measure refractory periods and conduction velocity and other AF properties. We concluded that vernakalant indeed reduces conduction velocity and increases refractory period in healthy hearts. Moreover, these effects were unaffected by 2 days or after 11 days of AF.

However, the refractory period prolongation was not dependent on a change in repolarization but on a loss of excitability. Indeed, the comparison to a typical class I drug revealed that vernakalant had a similar electrophysiological effect. We therefore concluded that the antiarrhythmic property of vernakalant is mainly based on a class I effect.

In chapter 4 we zoomed in on how vernakalant affects fibrillation waves. The continuous presence of fibrillation waves is a requirement for AF persistence. It is therefore relevant to understand how propagating fibrillation waves are affected by vernakalant. Based on the class I effect of vernakalant one would expect that turning of wavefronts will be inhibited and fewer (breakthrough) waves and re-entrant circuits will occur. We performed a study in anesthetized goats with 3 or 22 weeks of AF. High density mapping of the atrial electrical activity allowed the identification of waves and the discrimination between type of waves and re-entrant circuits. Moreover, the wavefront turning was assessed at different levels of spatiotemporal domains. Vernakalant increased the AF cycle length strongly and led to the frequent termination of AF on both groups. Termination was associated with a reduction in complexity of propagation patterns which was illustrated by fewer waves, breakthroughs and re-entrant circuits. The strong decrease in turning events may have contributed to the overall reduction in complexity.

Mapping of AF has been a cornerstone in the understanding of AF maintaining mechanisms. Since the early days of cardiac electrophysiological research, it was used to identify phenomena like re-entry, focal activity or just simply to describe a wavefront. Currently different mapping modalities have been developed to aid ablation procedures in clinical practice. Some technical aspects of mapping of AF are considered in chapter 5 and 6.

In chapter 5 we evaluated the spatial distribution of AF properties and their relation to the stability of conduction patterns. It is often thought that a site with a certain signal property may identify areas (or sources) that are of critical importance for AF. More specifically, some parameters are thought to reflect rotor activities, e.g. high dominant frequency. This and other parameters are typically sequentially sampled with a limited duration of the sample. This approach implies the assumption of stationarity of AF properties in time and space. We investigated stationarity of AF properties and dynamic propagation patterns between ten 1-minute recordings of AF in the goats with 3 weeks of maintained and stable AF. Our results show that AF properties are highly stable in the temporal and spatial domain. However, the patterns of AF parameters could strongly change when two different episodes of AF, that interrupted for 1 hour, were considered. Analysis of the dynamics of propagation patterns demonstrated that the overall propagation patterns could range from a dominant recurrent pattern to a continuously changing process. We concluded that indeed AF properties are stationary within a single episode but that this stability was independent of a stable propagation pattern.

In chapter 6 we investigated pitfalls for the identification of phase singularities, putative identifiers of rotor activity. The Focal Impulse and Rotor Mapping technique (FIRM) has been designed to identify these specific propagation patterns, instead of an indirect

measure from a single electrogram. The method is based on a specific catheter that acquires a large coverage but has a low density. The signals from the catheter are transferred into the phase domain to identify rotors and focal impulses. Concerns about this approach were raised because the density of electrodes may not correspond to the scale of fibrillation waves. Furthermore, the use of phase (more specifically phase singularities) abandons the idea that a wave should propagate within the range of physiological conduction velocities. These two concerns may affect the degree of false positive rotor detections. We developed an algorithm based the same principles used by FIRM to address these concerns. The development of a new algorithm was required because the FIRM algorithm is not available to the public domain. Our results demonstrated that low electrode density or chaotic fibrillation process contribute to multiple false positive rotors detections. To reduce false positive detections, we conclude that the detection of a rotor with this method should be confirmed by activation time mapping. The use of a double ring of electrodes to confirm rotation helped to significantly reduce false positive detections.

Samenvatting

Boezemfibrilleren (BF) is een hartritmestoornis. Patiënten met BF hebben een snelle en onregelmatige hartslag. BF begint meestal met korte periodes en in de meeste patiënten ontwikkelt het zich tot een vorm van fibrilleren die niet meer vanzelf stopt. Deze hartritmestoornis leidt niet direct tot een plotse hartdood, maar kan wel grote gevolgen hebben voor de fysieke en mentale gezondheid van een patiënt. Patiënten met BF hebben een grotere kans op hartziekten, een beroerte en veel patiënten kunnen een minder grote fysieke inspanning leveren. Dit laatste wordt veroorzaakt omdat de pompkracht van de boezem afneemt en omdat het hartritme zich niet meer kan aanpassen aan de vraag van het lichaam.

In dit proefschrift wordt het medicijn vernakalant onderzocht. Dit is een nieuw medicijn voor een behandeling van BF. Vernakalant kan het snelle en onregelmatige ritme van BF beëindigen. Er zijn meer medicijnen die BF in een vroeg stadium tegengaan. Echter, de meeste courante medicijnen voor het stoppen van BF zijn zeer gevaarlijk voor bepaalde type patiënten. Deze medicijnen veroorzaken ritmestoornissen in de hartkamers, wat tot de dood kan leiden. Dit gebeurt doordat deze medicijnen een effect hebben op eiwitten die zowel in de kamers als de boezem aanwezig zijn. Uniek aan vernakalant is dat het de functie van een eiwit remt die alleen in de boezem aanwezig is. Dit eiwit is verantwoordelijk voor de elektrische stroom die de *ultra-rapid delayed rectifier current* wordt genoemd. In diersmodellen zorgt de remming van de *ultra-rapid delayed rectifier current* voor een vergroting van de pompkracht van de boezem.

In hoofdstuk twee is in een geitenmodel de hypothese dat vernakalant de pompkracht in de boezems verhoogd onderzocht. Helaas bleek uit verschillende metingen dat vernakalant niet leidt tot een verbetering van de pompkracht. Waarschijnlijk komt dit doordat de remming van de *ultra-rapid delayed rectifier current* te beperkt is voor de effectieve werking van vernakalant.

De werking van vernakalant is namelijk complexer dan dat het enkel op één eiwit inwerkt. Vernakalant werkt zowel in op de activerende natrium als op de herstellende kaliumstroom. Dit komt door een remming van minimaal zes verschillende eiwitten. In hoofdstuk 3 is de vraag gesteld welk mechanisme, de activerende natriumstroom of de herstellende kaliumstroom, het belangrijkste is voor de werking van vernakalant. Het bleek dat vernakalant vooral eigenschappen beïnvloedt die afhankelijk zijn van de activerende natriumstroom en niet van de herstellende kalium stroom, zoals de *ultra-rapid delayed rectifier current*. Dit betekent dat vernakalant inwerkt op een elektrische stroom die ook in de hartkamer aanwezig is en mogelijk een dodelijke hartritmestoornis kan opwekken. Dit resultaat is een belangrijke waarschuwing voor het gebruik van vernakalant bij bepaalde type patiënten.

Dit resultaat roept een interessante wetenschappelijke vraag op, namelijk hoe kan vernakalant BF stoppen zonder dat het de golfenlengte beïnvloedt? Echter, voordat we deze vraag beantwoorden, is het interessant om een eerst paar elektrische principes van BF nader te bekijken. In tegenstelling tot een normaal hartritme, waarbij één golf aanwezig is, wordt tijdens BF een groot aantal golven waargenomen. Volgens de *multiple wavelet*

theorie wordt de stabiliteit van BF bepaald door het aantal golven. Als er meer golven in de boezems aanwezig zijn is BF stabiel. Het maximaal aantal golven dat theoretisch binnen de boezems past, is afhankelijk van de beschikbare oppervlakte van de boezems en de totale oppervlakte die een golf inneemt. Bij een medicijn zoals vernakalant neemt, zoals in hoofdstuk 3 is beschreven, de lengte van de golf dus niet toe. De wetenschappelijke vragen in hoofdstuk 4 zijn: Neemt het aantal golven in de boezems af? En waardoor wordt dit veroorzaakt? Vernakalant leidt inderdaad tot minder golven en hierdoor stopt BF. De afname van het aantal golven hangt samen met een verminderde draaiing van de golven. Een verminderde draaiing zal waarschijnlijk tot minder nieuwe golven leiden en de kans vergroten dat een golf uitdooft.

Voor het bestuderen van golven is gebruik gemaakt van een techniek die *mapping* heet. Bij *mapping* wordt met een groot aantal elektroden de elektrische activiteit van de boezems gemeten, zodat individuele golven onderscheiden kunnen worden. Van de elektrische activiteit kan dan een film gemaakt worden. Als deze films bestudeerd worden, dan wordt een zeer chaotisch proces zichtbaar, vele golven. In hoofdstuk 5 is de stabiliteit van de eigenschappen van BF in relatie tot geleidingspatronen onderzocht. De conclusie is dat de eigenschappen van BF zeer stabiel (*stationarity*) zijn. Echter, dit is onafhankelijk van het geleidingspatroon. Verder blijkt dat de eigenschappen van BF veranderen tussen verschillende episodes van BF. Deze informatie kan belangrijk voor de cardiologische behandeling van BF zijn, omdat dit laat zien dat binnen één patiënt de kritische gebieden voor BF kunnen variëren. Met andere woorden de behandeling van vandaag kan toereikend zijn maar de boezems kunnen morgen in die mate veranderd zijn dat de patiënt morgen een andere behandeling nodig heeft. Het is belangrijk om op te merken dat deze gegevens in geiten zijn gevonden en dat nu eerst bewezen moet worden een dergelijk proces ook in de mens plaatsvindt.

In hoofdstuk 6 wordt een specifiek type *mapping* analyse onderzocht. Deze analyse is gebaseerd op de *rotor* theorie, een andere dan de *multiple wavelet* theorie, die verklaard waarom BF niet vanzelf stopt. De rotor heeft een spiraalvormig golffront en draait om een oneindig klein punt heen. De *rotor* blijft continu om zichzelf draaien. Tenzij het centrum van de *rotor* een obstakel tegenkomt. Voor de klinische praktijk is de FIRM-techniek ontwikkeld. Dit is een techniek die *rotor* fenomenen traceert. Op de plaats in het hart waar de FIRM-techniek *rotoren* herkent, wordt boezemweefsel gedood. Dit dode weefsel vervangt het lichaam door littekenweefsel. Er heerst controverse over deze behandeling met behulp van de *rotor* theorie. Sommige onderzoekers vinden een zeer positief effect in de behandeling van BF, terwijl andere onderzoekers deze resultaten niet kunnen herhalen. Om *rotoren* te vinden worden signalen bewerkt en geanalyseerd. Echter, hoe dit precies wordt toegepast is niet openbaar gemaakt. Alleen een paar basisprincipes zijn openbaar. Aan de hand van die basisprincipes zijn er zorgen over het optreden van onterechte herkenningen van *rotoren*. Daarom hebben wij een vergelijkbare methode ontwikkeld. We hebben de gevoeligheid voor onterechte *rotor* herkenningen onderzocht. De uitkomst van dit onderzoek is dat er inderdaad veelvuldig een onterechte herkenning van

rotoren plaatsvindt. Dit wordt veroorzaakt doordat de afstanden tussen de elektrodes van het meetinstrument te groot zijn voor de kleine BF golven. De golven geleiden als het ware tussen de mazen van de elektrode door. Als de onterechte *rotor* herkenning toch wordt behandeld, dan worden er willekeurige littekens in het hart aangebracht. Het is heel raadzaam om het aantal onterechte herkenningen zoveel mogelijk te beperken. Wij hebben daarom een methode voorgesteld waarbij de *rotor* tweemaal bevestigd moet worden. Deze methode verminderde het aantal onterechte herkenningen aanzienlijk.

Dankwoord

Dit proefschrift is een mijlpaal na een lange periode van uitdagingen en leren. Het pad tot mijn proefschrift is waarschijnlijk al begonnen toen ik in 2001 voor de groepen van Maurits Allesie en Frits Prinzen kwam te werken. Daarom zijn er tientallen mensen die aan mijn wetenschappelijke ontwikkeling hebben bijgedragen. Ik ben iedereen erg dankbaar hiervoor. Er zijn natuurlijk een aantal mensen die ik er uit wil lichten.

Professor Schotten, beste Uli, dank je dat je deze promotie voor mij mogelijk hebt gemaakt. Samen met Sander en Stef vormen wij een goed wetenschappelijk team. De samenwerking tussen ons gaat terug tot aan mijn begin dagen in Maastricht. Wij hebben elkaar daardoor jaar na jaar in ons werk zien groeien. Ik kan mij nog goed herinneren, dat zal 2002 geweest zijn, dat we samen op OK1 voor jouw contractiliteitsstudie een geit hebben geïmplantéerd. Was dat niet een studie voor jouw promotie? Ik heb je toen gevraagd wat je in de toekomst wilde doen. Het antwoord was helder en duidelijk. Eerst zo veel mogelijk van Maurits leren om vervolgens met ons je eigen onderzoeksgroep op te bouwen. Dat is je duidelijk gelukt. En ik? Ik doe het meer stap voor stap. “Laat mij eerst maar een goede analist/experimentator worden, daarna zien we wel verder” was mijn antwoord, geloof ik. Jij bent een man met een (lange termijn) visie en je weet hoe je dit moet aanpakken. Relevantie en kwaliteit van het onderzoek staat bij jou altijd centraal. Een negatief resultaat schuw je niet. Dit waardeer ik heel erg. Uiteindelijk proberen wij via wetenschap tot zuivere antwoorden te komen. Uli, bedankt voor je vertrouwen en waardering.

Doctor Verheule, beste Sander, jij bent vaak mijn toetssteen. Jouw deur staat altijd open voor een korte vraag of een lange discussie. Je hebt me vaak geholpen om mijn meanderende overwegingen richting te geven. Ook ben je essentieel geweest om mijn teksten leesbaar te maken. Het zet je dat ik af en toe nodig had om iets nieuws op te pakken, zoals het onderwijs, heb je ook vaker gegeven. Je brede interesses, kennis en humor zijn uniek en maken je tot een bijzonder persoon. De boeken, muziek, podcasts en films, die je vaak aan mij uitleent, prikkelen elke keer mijn nieuwsgierigheid. Ga zo door. Voor één gemeenschappelijke hobby is dat helemaal niet nodig; fietsen! Ik ben benieuwd hoe goed onze collega's dat spelletje begrijpen. Ze moeten toch wel wat hebben opgestoken van onze vele wieler-lunches, denk je niet? Het is me alleen nog niet gelukt om jou op de fiets te krijgen.

Doctor Zeemering, beste Stef, toen wij samen op één kamer zaten had ik verwacht dat we uren over koken en eten zouden bomen. Het is een gezamenlijke passie en een onderwerp van gesprek dat we niet schuwen. Toch zijn onze gesprekken meestal op de wetenschap gericht. Zo kunnen we met gemak twee uur filosoferen over een idee. Ik ervaar dit als mooie momenten die ons werk interessant maken. Je hebt me vaak geholpen om een bepaalde denkrichting op te gaan of een bepaalde analyse mogelijk te maken. Essentieel was daarin jouw terugkerende vraag: “Wat wil je nu precies weten?”. Dat klinkt heel logisch, maar was niet altijd simpel. Dank je wel.

Ik wil de leden van de beoordelingscommissie (professor Crijns, doctor Coronel, professor De Groot en Professor Prinzen) hartelijk bedanken voor de tijd en moeite die zij in de evaluatie van mijn proefschrift hebben gestoken. Ik kijk met spanning uit naar de verdediging.

Beste Ali, dear Ali, *lieber* Ali or should I say *caro* Ali Gharaviri. You are a great, kind and a positive person who always knows how to enjoy live. You are a true *joie the vivre*. We had great times at work, parties and congresses abroad. You live in Lugano now. This makes it a bit more difficult to meet. Where ever we do meet, we know how to spend our time well. I am happy you are my paranymp.

Lieve Jasper mijn grote broer. Ja mijn grote broer. Je bent er altijd voor mij geweest. Ook om van gedachten te wisselen over mijn werk. Je vragen over mijn onderzoek en je kritisch denkvermogen hebben me geholpen om met meer afstand naar de betekenis van mijn onderzoek te kunnen kijken. Ik zal de komende jaren nog wel vaker op je deur kloppen. Ik ben trots dat je mijn paranymf wilt zijn.

Mijn *sister in crime* op de OK is natuurlijk Marion Kuiper. Marion, jij bent de persoon met wie ik blind kan samenwerken. Wij zijn een perfect team en vullen elkaar aan. Samen zijn wij in de wereld van cardiovasculair onderzoek in grote proefdieren de, of één van de beste OK teams. Als ik met jou een project start weet ik dat het goed komt. Jij hebt een wezenlijke bijdrage geleverd aan dit proefschrift. Bedankt voor alle hulp en je inzet op de lange dagen.

Piotr Podziemski, I really like your physics brain and your large interest in everything around you. We often had synergy in our collaboration which is based on our mutual curiosity. Good luck with your Peekaboo project and the other 100.000 things you yearn to initiate. I am happy you are my friend.

Dear Dennis H Lau, you were my first source of information at the start of my PhD. That is much appreciated. You also taught me how to appreciate good wine. We did some nice projects together and I hope we'll finish it someday. Perhaps 2019? Australia is far away, fortunately we meet regularly on the international meetings we have.

Dear Dominik Linz, you are an example to many scientists and clinicians. Not only because you are a successful high-quality scientist, but also because you are such a great and friendly guy and friend. You are very much appreciated by me and I believe by many others. Enjoy your time in Adelaide!

Another Aussie, Pawel Kuklik. You are a very independent person that questions everything. I enjoyed our lengthy discussions about a whole range of topics.

Dear Dragan Opacic, my Big Friendly Giant. According to some, I've been tough on you. But I think you appreciated it. We really get along well. I hope to see you on a regular

basis in the future too. Good luck reaching your goals in surgery, Serbia and maybe cycling.

Cara Giulia Gatta, sorry for my frequent absences on my journey to finalize this thesis. Good luck in finishing up your own. That is (y)our challenge for 2019!

Dear Hussein, you were one of the kindest colleagues I ever had. Thanks for all your support with my experiments and analysis.

I would like to acknowledge my appreciation to Richard Cornelussen, Ward Vanagt, Maura Greiser, Kelly van Bragt, BG Kerfant, Stephanie Reid, Matthias Zink, Joris Winters, Vladimir Sobota, Patrick Schönleitner, Gudrun Antoons, Martijn Gilbers, Billy Scaf, Elisa D'Alessandro and many others.

Ik wil nog een paar mensen uit een verder verleden bedanken.

Ten eerste professor Allessie (Maurits). Maurits, ik ben blij dat ik met je heb mogen samenwerken. Ik bewonder je grote creativiteit, snelle denkvermogen en gedrevenheid. Wat ik nog het meeste in je bewonder is je zoektocht naar de juiste wetenschappelijke interpretatie. Ongeacht of je eerdere beweringen terug moet nemen, of dat anderen je tegendraadse ideeën wel of niet waarderen. Mijn promotietraject kreeg vorm nadat jij met pension bent gegaan. Ik had graag nog meer van je geleerd.

Theo van der Nagel. Ik zal het verhaal van het aluminium folietje uit het pakje sigaretten (Camel?) in de Braziliaanse jungle nooit vergeten. Hiermee heb je mij laten zien dat er altijd een oplossing te bedenken is. Of het nu om een protocol of een falend systeem gaat. Bedankt voor je inleiding tot de wereld van de universiteit en de meetsystemen.

Jens Eckstein. Jij bent het voorbeeld hoe iemand harmonie en samenwerking binnen een groep tot stand kan brengen. Het voorbeeld voor velen. Je bent nu hoofd digitale vernieuwing in het Universitätsklinikum Basel, als ik het goed heb. Je gaat er ongetwijfeld wat moois van maken.

Een groot deel van de periode dat ik aan mijn proefschrift heb gewerkt ben ik lid geweest van de dierexperimentencommissie. Ik heb veel geleerd van het toetsen van de proefopzet van onderzoeksprojecten. Ik wil iedereen uit de DEC bedanken voor onze samenwerking. Twee personen wil ik in het bijzonder noemen. Reinder, je bent er helaas niet meer, maar ik zal je nooit vergeten. Jij was de persoon die liet zien hoe een voorzitter moet zijn. Je voerde deze functie uit met hart en ziel. Puur met als doel om zinvol proefdieronderzoek te garanderen. Marianne, jij bent het centrum van de dierexperimentelecommissie. Jij bent vol overtuiging dat dingen goed moeten zijn. Daar hou ik van.

Bij het Idee (de technische dienst van de Universiteit Maastricht) was ik vaak kind aan huis. Ik kon daar terecht om wat ik ook maar bedacht mogelijk te maken. Bij het Idee lopen veel mannen rond die ik dank verschuldigd ben. Een paar van hen wil ik in het bijzonder noemen. Leon Essers, Enrico Toffoli, Iwan de Jong, Ben Flenterie, Paul Verjans,

Paul Laeven en niet te vergeten Jan Geilen. Ook de centrale proefdiervoorziening was onmisbaar voor het tot stand brengen van dit boekje. Mijn geiten waren altijd in de juiste handen. Huub, bedankt voor al je hulpvaardigheid. Het maakte niet uit of het om de inrichting van de stal ging, of de locatie van huisvesting. Jij was altijd bereid om een oplossing te vinden. Petra en Joyce, ook jullie bedankt voor jullie werk rondom de proefdieren en de vele weekenddiensten.

Beste Bart, in 2008 heb ik jou en Janneke leren kennen. Daarmee heb ik twee vrienden voor het leven voor gekregen. Ondanks de beperkte tijd om elkaar regelmatig te zien kunnen we alle aspecten van het leven met elkaar delen. Enkel mijn wetenschappelijke waardering voor jou uitspreken zou de rest van deze aspecten onrecht aan doen. Wat ik je wil meegeven is dat ik je waardering en vertrouwen in mijn werk heel erg apprecieer. Dat heeft mijn twijfelaarsgeest zo nu en dan nodig.

Lieve Pa en Ma. Wie had dit gedacht. Jullie hebben me wel vaker een laatbloeier genoemd. Dat bleek ook in dit geval te kloppen. Hoe dan ook, nieuwsgierig en leergierig ben ik altijd geweest.

Lieve Janneke, mijn steun en toeverlaat. Het was al op onze eerste *date* dat ik me beklaagde dat ik wat anders wilde gaan doen. Je hebt geduldig naar me geluisterd. Dat verbaast me nog steeds. Later ben ik gaan kijken wat ik nu precies wilde met mijn "loopbaan". Je hebt me toen gestimuleerd om dat te doen wat ik leuk vind. Dat doe je continu, in ons hele leven. Je vertrouwen en aanmoedigingen om uitdagingen aan te gaan brengt mij op plekken waar ik anders nooit zou zijn gekomen. Dat waardeer ik zeer sterk, dank je. Ik hou van je.

About the Author

Arne van Hunnik was born in Amstelveen in 1977. At the age of four he moved to Ede where he grew up and followed secondary school. In 1996 he graduated for his HAVO degree at the Pallas Athene College in Ede. At that point he didn't have a clue what he would like to study. Eventually, he visited the college for laboratory studies in Utrecht. Once he witnessed an experiment on a living animal he knew he wanted to perform physiological studies. Preferably in the field of cardiology. He graduated in 2001 with a specialization in zoology. For his bachelor thesis, he worked a year at the veterinary research institute ID-Lelystad. Here, he set up a model in chickens to determine the absorption capacity of the jejunum and ileum. This model was meant as a tool to determine the potential of food supplements to prevent intestinal infections.

During the year 2000 a number of patients and cows were diagnosed to have Creutzfeldt-Jakob disease. This disease is caused by a prion, a misfolded protein. The disease is a fatal degenerative condition that affects the brain. The prion was thought to be transmittable from cows to man. The federal government therefore decided to screen all cows for Creutzfeldt-Jakob disease. Directly after his graduation, Arne van Hunnik spent some months at ID-Lelystad to train a group of unskilled people to work in a laboratory with potentially hazardous conditions.

Following this experience, he started at the department of physiology at the Maastricht University in 2001. He joined the research group of professor Prinzen and the research group of professor Allessie. Shortly after he started all experienced personnel left. During some intense years he acquired all skills to perform complex physiological experiments. He mastered himself the surgical techniques, technology that is used for data acquisition and the scientific background of the research that was performed. He quickly got fascinated for the search for the correct answers to research questions. Professor Allessie acknowledged this wish and offered him training to comprehend mapping of atrial fibrillation. At that time a laborious task. Almost all maps had to be analyzed by hand. This was a unique and instructive collaboration. This experience underscored that Arne van Hunnik wanted to perform his own research. Following this period, professor Schotten offered him a PhD position where he started in 2012.

In 2010 Arne van Hunnik joined the Animal Experimental Committee installed by Maastricht University. He performed this work until 2016.

Currently, Arne van Hunnik is still working at the department of physiology. He is involved in the supervision of three PhD students. In the foreseeable future his research will continue to focus on the action of antiarrhythmic drugs and propagation patterns during atrial fibrillation. He believes that more advanced mapping modalities will contribute a better understanding of the maintaining mechanisms of atrial fibrillation.

List of Publications

Original research

1. Rotors Detected by Phase Analysis of Filtered, Epicardial Atrial Fibrillation Electrograms Colocalize With Regions of Conduction Block.
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Case Report

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Dynamics of Propagation Patterns and
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10-01-2019