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Introduction
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Clinical relevance

Atrial fibrillation is the most common cardiac arrhythmia. The incidence of atrial fibrillation is 0.7% in people aged from 55-59 and is increasing with aging of the population.\(^1\)\(^,\)\(^2\) Risk factors for atrial fibrillation include age, hypertension, diabetes, valve disease and heart failure.\(^3\)\(^,\)\(^4\) Atrial fibrillation is classified as (i) paroxysmal atrial fibrillation, which is defined as self-terminating atrial fibrillation, (ii) persistent atrial fibrillation, in which episodes last longer than 7 days or require termination by electrical or pharmacological cardioversion or (iii) permanent atrial fibrillation, which is defined as atrial fibrillation in which the decision has been made to accept atrial fibrillation.\(^5\) Atrial fibrillation also occurs without the presence of underlying disease, which is called ‘lone atrial fibrillation’. However, most patients with atrial fibrillation have a concomitant disease. In the Euro Heart Survey, describing data from 5333 patients in 35 countries, 60% of the patients suffered from hypertension and 20-50% of patients suffered from heart failure as a concomitant disease, depending on the progression of atrial fibrillation (Figure 1).\(^6\) In addition, the presence of more concomitant conditions increased with the prevalence of more advanced forms of atrial fibrillation.\(^7\)\(^,\)\(^8\) Atrial fibrillation tends to become more persistent over time,\(^8\)\(^,\)\(^9\) which is both due to atrial fibrillation itself, as well as progression of the associated diseases.\(^10\)\(^,\)\(^11\) Figure 2 shows that underlying diseases already induce changes, i.e. remodeling, in the atria before onset of atrial fibrillation.\(^12\) This remodeling increases susceptibility for atrial fibrillation.

Although atrial fibrillation is not directly life-threatening, it is not benign.\(^13\)\(^,\)\(^14\) Atrial fibrillation increases the risk of stroke,\(^15\)\(^,\)\(^16\) heart failure,\(^17\) hospitalizations, mortality,\(^18\) and for the patients at major importance, it impairs quality of life.\(^8\)\(^,\)\(^19\)\(^-\)\(^21\)

Atrial fibrillation is a complex disease. The pathophysiology underlying atrial fibrillation is diverse. Changes in the shape and duration of the action potential occur, dispersion of effective refractory period, but also changes in the structure of the atria take place. These changes contribute to the initiation and maintenance of the arrhythmia and are both induced by the underlying heart disease and atrial fibrillation itself. Studying the processes occurring before the actual arrhythmia takes place may give insight in the mechanisms of development of atrial fibrillation and may eventually improve prevention of AF.

Mechanisms of AF

AF can be initiated by increased rapid focal ectopic activity (often originating in the pulmonary veins) and reentry. Focal ectopic activity can be caused by abnormal automaticity (spontaneous depolarizations) or triggered activity (early afterdepolarizations and delayed afterdepolarizations).\(^10\) Ectopic activity usually serves as a trigger to initiate atrial fibrillation. Perpetuation of atrial fibrillation depends on the characteristics of the tissue (vulnerable substrate) and reentry. A vulnerable substrate can be caused by changes in structural properties of the atria. For example, atrial dilation, this creates a larger area for reentry, and fibrosis, which interferes with tissue conduction. Changes in (dispersion of) action potential duration may also contribute to this process.
Figure 1: Concomittant diseases in atrial fibrillation, graph is modified from Nieuwlaat et al.\textsuperscript{6} Depicted is the percentage of patients with the specified concomitant diseases. In the study by Nieuwlaat et al. the category others includes, sick sinus syndrome, chronic obstructive pulmonary disease and thyroid disease. Also other concomittant diseases might be present which have not been registered.

Figure 2: Time course of atrial remodeling (adapted with permission from Cosio et al.).\textsuperscript{12} Hypothetical representation showing that both underlying diseases such as hypertension or heart failure and atrial fibrillation induce atrial remodeling. Underlying diseases already induce atrial remodeling long before the onset of atrial fibrillation. Early treatment of these underlying diseases would therefore be beneficial. AF, atrial fibrillation, ECV, electrical cardioversion, SR, sinus rhythm.
Different mechanisms of reentry exist, including circus movement reentry, the leading circle concept, spiral wave reentry and the multiple wavelet hypothesis.\textsuperscript{10} The leading circle concept states that functional reentry establishes itself in the minimum sized circuit for maintaining reentry. This is determined by the wavelength, which depends on the refractory period and conduction velocity.\textsuperscript{10,21,23} Decreasing effective refractory period, slowing conduction velocity and increasing tissue mass increase atrial fibrillation stabilization.\textsuperscript{10,24} The spiral wave theory suggests that reentry is maintained by a rapidly circulating rotor with a wavefront rotating around a central core.\textsuperscript{10,23} The multiple wavelet hypothesis was initially suggested by Moe, stating that multiple independent wavelets might travel through an excitable medium in a seemingly chaotic pattern.\textsuperscript{25} The existence of multiple wavefronts was first shown by Allessie et al. in dog atria stimulated with acetylcholine.\textsuperscript{26} Also in humans, studies have confirmed the multiple wavelet theory.\textsuperscript{27}

**Remodeling**

Cardiac remodeling refers to changes in the heart upon a demanding situation such as pressure overload, volume overload or ischemia. Causes of remodeling include, but are not limited to, rapid electrical stimulation, activation of the autonomous nervous system, calcium-overload, impaired glucose metabolism, hormonal stimulation via endothelin or the renin-angiotensin-aldosterone system (RAAS), stretch, inflammation and oxidative stress. Because stretch occurs due to underlying heart diseases, already before onset of atrial fibrillation, the first part of this thesis focuses on stretch.

Remodeling processes often seen include, but are not limited to, changes in size (hypertrophy), shape (dilation), structure (fibrosis, connexins) and physiology (switch in energy source). These adaptive changes are initially beneficial, for example reducing wall stress by increasing wall thickness.\textsuperscript{28} Remodeling, however, might result in decompensation and an impaired cardiac function. Remodeling in the atria occurs due to underlying heart diseases,\textsuperscript{29-32} for example by increasing atrial pressure, i.e. increased stretch. But remodeling is also caused by atrial fibrillation itself (Figure 2).\textsuperscript{33,34} Early treatment of associated diseases could possibly halt progression of atrial remodeling and could prevent or delay occurrence of atrial fibrillation.\textsuperscript{35}

**Electrical remodeling**

Electrical remodeling relates to alterations in ionic currents and transporters which change the shape and duration of the action potential. In 1995, Wijffels et al. showed that induction of atrial fibrillation in goats reduced the effective refractory period and caused loss of physiological rate adaptation of the effective refractory period.\textsuperscript{36} It was also shown that the vulnerability for atrial fibrillation increased when atrial fibrillation was induced before. Moreover, atrial fibrillation episodes lasted longer when atrial fibrillation was maintained for a longer period, showing the concept of ‘atrial fibrillation begets atrial fibrillation’.\textsuperscript{36} These electrophysiological changes occurred within 24 hrs\textsuperscript{36,37} and were rapidly reversible.\textsuperscript{36,38}
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At the same time, Morillo et al. demonstrated a reduced effective refractory period and dispersion of refractoriness in dogs subjected to rapid atrial pacing. Atrial fibrillation was easily inducible in this model. Also in the chronic AV block dog model, electrical remodeling occurs. In this model it is characterized by an increased action potential duration and increased spatial dispersion. A reduced effective refractory period has also been observed in humans with atrial fibrillation. In experimental heart failure, and also in atria from patients with structural heart disease, action potential duration in single cells remained unchanged, or was even prolonged, in contrast to the reduced effective refractory period in atrial fibrillation. Another aspect of remodeling in AF is contractile dysfunction. The time course of atrial contractile function recovery is related to the duration of AF.

Structural remodeling

Electrical remodeling occurs rapidly, but atrial fibrillation does not get permanent in this short time frame. Wijffels et al. observed that electrical remodeling is completed within 24 hrs, however, atrial fibrillation became sustained after one week, already suggesting that electrical remodeling is not the only factor involved. Todd et al. showed that 4 week periods of atrial fibrillation in goats, followed by cardioversion and recovery of the effective refractory period, also promoted the vulnerability for atrial fibrillation. This suggested the presence of a so-called ‘second factor’. The second factor relates to factors which increase stability of atrial fibrillation and have a longer time-course than electrical remodeling, such as structural remodeling. Structural remodeling seen in atria from patients with atrial fibrillation and atrial fibrillation animal models include hypertrophy, dilation, dedifferentiation, fibrosis and cell death. Morillo et al. were the first to show that atrial tachypacing induced structural changes. In a dog model they observed atrial enlargement, hypertrophy, changes in the mitochondria and disruption of the sarcoplasmic reticulum, but no fibrosis. Similar findings were observed in Allessie’s goat model of atrial fibrillation. Schoonderwoerd et al. showed that not only a high atrial rate, but also a high ventricular rate, inducing heart failure and hemodynamic overload, is an important causal factor in atrial structural remodeling.

In humans, studies investigating structural remodeling caused by lone atrial fibrillation, without underlying disease, are scarce. Hypertrophy, fibrosis, cell death, myolysis and inflammation were observed in biopsies from patients with lone atrial fibrillation. In biopsies from patients with underlying diseases atrial structural remodeling has been shown, including dilated atria, hypertrophy and fibrosis. In addition, atrial structural remodeling has been described in animal models of hypertension, heart failure, mitral regurgitation, shunt induced volume overload, and pressure overload induced by aortic constriction. A recently discovered mechanism contributing to remodeling, are a class of non-coding RNAs, so called miRNAs, which are involved in regulation of gene expression. These have been implicated in for example fibrosis upon ventricular pressure overload. Structural changes may reverse, but this is a very slow process and the help of pharmacological treatments might be essential.
fibrillation and structural remodeling in relation to stretch, i.e. simulating underlying disease associated with pressure and volume overload, is reviewed in Chapter 2.

Cardiac cells and remodeling
Cardiomyocytes occupy 80% of the volume of the heart, but represent only 30% of the total cell population. The other 70% of the cardiac cells are mainly fibroblasts, but vascular smooth muscle cells and endothelial cells are also present. Cardiomyocytes maintain the primary function of the heart; they contract, in this way the heart pumps blood through the body. Fibroblasts regulate the synthesis of the components of the extracellular matrix, and as such regulate the structure of the extracellular matrix and provide structure to the heart. In addition, fibroblasts are involved in infarct healing. Both cardiomyocytes and fibroblasts are capable of changing their appearance and characteristics in cardiac disease in response to stressors such as stretch.

Cardiomyocytes might increase cell size (hypertrophy), differentiate towards a more fetal phenotype to maintain cell viability, secrete cytokines and hormones, such as atrial natriuretic peptide (ANP), angiotensin-II and transforming growth factor β1 (TGFβ1), and change their electrical properties (Figure 3). As a response to stress, fibroblasts differentiate into a more active phenotype, i.e. into myofibroblasts. A myofibroblast is an activated fibroblast-like cell with characteristics of both a fibroblast and a smooth muscle cell. Fibroblasts show increased proliferation, migration, secretion of cytokines and growth factors including TGFβ and interleukin-6, and increased collagen synthesis (Figure 3).

Figure 3: Overview of changes in cardiomyocytes and cardiac fibroblasts upon stress. Cardiomyocytes respond to environmental stimuli via increasing cell size, i.e. hypertrophy, changing towards a more fetal phenotype, i.e. dedifferentiation, electrical remodeling and via secretion of growth factors and cytokines. Fibroblasts activation includes changes in migration, proliferation, production of extracellular matrix, differentiation and production of signaling molecules. AII, angiotenin II; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; IL6, interleukin-6; TGFβ1, transforming growth factor β1; TNFα, tumor necrosis factor α.
Atrial fibrillation and hypercoagulability

Next to the known factors involved in the pathophysiology of atrial fibrillation and atrial remodeling, also previously unrecognized mechanisms might be involved in the pathophysiology of atrial fibrillation. Atrial fibrillation has been recognized as a hypercoagulable state long ago. Patients with atrial fibrillation have an increased risk of stroke.\textsuperscript{15,16} This is related to the fulfillment of Virchow’s triad for thrombogenesis, consisting of changes in blood flow, vessel wall and blood components.\textsuperscript{78} Due to the rapid atrial activation, contractile dysfunction occurs, causing stasis of blood. The other components of Virchow’s triad are also fulfilled: atrial fibrillation is associated with endothelial dysfunction\textsuperscript{79,80} and a prothrombotic state.\textsuperscript{78,81} Because of the increased risk of stroke many patients with atrial fibrillation are treated with oral anticoagulants.\textsuperscript{82}

Coagulation factors are more and more recognized as active players in the pathophysiology of different diseases.\textsuperscript{83-86} A link with atrial remodeling has not been made. Cellular actions of coagulation factors are mediated via a family of G-protein coupled receptors, the protease-activated receptors (PARs). Four PAR isoforms exists, PAR1-4.\textsuperscript{86,87} PARs are expressed throughout the body and exert effects on multiple organs and celltypes.\textsuperscript{87} In the heart PARs are expressed in cardiomyocytes, fibroblasts, endothelial cells and smooth muscle cells.\textsuperscript{85} The most prevalent and studied isoforms in the heart are PAR1 and PAR2.\textsuperscript{86,88} The activated coagulation factor thrombin is capable of activating PAR1, PAR3 and PAR4. Factor Xa, another activated coagulation factor, is capable of activating PAR1 and PAR2.

In the heart PARs have been linked to remodeling. In mice, PAR1 overexpression induced cardiac hypertrophy, whereas PAR1 deficiency reduced left ventricular dilation and attenuated left ventricular dysfunction in a mouse model of ischemia-reperfusion.\textsuperscript{89} In neonatal rat ventricular cardiomyocytes, thrombin has been shown to induce hypertrophy and increase ANP expression.\textsuperscript{90} In more recent reports, thrombin induced hypertrophy and proliferation in cardiomyocytes and cardiac fibroblasts, respectively.\textsuperscript{88,91} In adult cardiac fibroblasts thrombin induced pro-fibrotic changes, but proliferation was not observed.\textsuperscript{82} Also in fibroblasts from other tissues, such as lung and kidney, thrombin induced activation of fibroblasts, i.e. increased expression of smooth muscle actin and induced proliferation.\textsuperscript{93-95} The relation between activated coagulation factors and cellular changes in atherosclerosis and atrial fibrillation is reviewed in Chapter 6. The first cellular data on the effects of thrombin on cardiac cells and the effect of inhibition of coagulation in the goat model of atrial fibrillation are presented in Chapter 7.

Therapy

Because atrial fibrillation is a complex disease, with many different mechanisms inducing atrial fibrillation, treatment is also difficult. Important targets in atrial fibrillation therapy are reducing symptoms, preventing thrombo-embolic complications and prevention or improvement of impaired ventricular function.\textsuperscript{5} This can be achieved by rhythm control, i.e.
restoring sinus rhythm, or rate control, i.e. reducing ventricular rate. Drugs that target ion channels have been first choice to maintain sinus rhythm for a long time. It might however be more efficient to target the underlying pathophysiological processes, instead of targeting the arrhythmia itself. Therefore, early treatments that have an effect on the remodeling process associated with underlying diseases such as hypertension or heart failure may improve maintenance of sinus rhythm. These treatments are called upstream therapies, and include angiotensin-converting enzyme inhibitors (ACE inhibitors), angiotensin receptor blockers, aldosterone receptor antagonists and statins. Although results of upstream therapies are promising in animal models, in humans the results are disappointing and evidence mainly exists for primary prevention of atrial fibrillation. Because of the increased risk of stroke patients with atrial fibrillation often receive anticoagulants.

**Aims**

The aims of this thesis are to investigate the atrial remodeling processes occurring before and after start of atrial fibrillation, induced by differential factors, i.e. stretch which is induced by the diseases associated with atrial fibrillation, but also by atrial fibrillation itself, and activation of coagulation.

In Chapter 2 we review different aspects of structural remodeling as seen in patients with AF and in animal models. Furthermore, we describe downstream signals of mechanical stretch and their contribution to atrial fibrillation and structural remodeling. Chapter 3 provides a primary cell culture model to investigate atrial structural changes caused by stretch. In Chapter 4 and 5 a mouse model of ventricular pressure overload was used and the effects of ventricular pressure overload on the atria were investigated. First, in Chapter 4, we studied the changes in the atria upon ventricular pressure overload and the effect of the angiotensin II receptor blocker losartan on the atrial remodeling. Thereafter, in Chapter 5 we show development and progression of the atrial substrate after different durations of pressure overload. Moreover, atrial fibrillation inducibility is investigated. Chapter 6 reviews the effects of activated coagulation factors thrombin and Factor Xa on cellular processes in both atherosclerosis and atrial fibrillation. Chapter 7 explores the effects of activated coagulation on cardiac fibroblasts and cardiomyocytes and the effects of inhibition of activated coagulation in the goat model of atrial fibrillation. Finally, in Chapter 8, findings of this thesis are summarized and put in to perspective.
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References


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