Introduction

Atrial Fibrillation, clinical aspects

Atrial fibrillation (AF) is currently the most common sustained clinical arrhythmia and is responsible for a substantial proportion of hospital costs incurred in the treatment of cardiac rhythm disorders.1 AF becomes increasingly common with age, having an incidence averaging <0.5% in patients <40 years of age and reaching >5% in patients >65.2 Thus AF is likely to become increasingly important with the ageing of the population. The arrhythmia is defined by a very rapid atrial rate (generally >400/min in humans) along with irregular atrial activation and lack of repetitive pattern of co-ordinated atrial activity. AF is associated with a variety of complications, including thromboemboli resulting from coagulation in the relatively static atrial blood pool, a loss of the fine adjustment of ventricular rate to the body’s precise metabolic needs, potential impairment of cardiac function and subjective symptoms like palpitations, dizziness, breathlessness and chest pain.

AF can occur in paroxysms of a duration shorter than 24 hours (but longer lasting paroxysms are not unusual) with intermittent sinus rhythm. Paroxysmal AF either converts spontaneously or is terminated with an intravenously administered antiarrhythmic drug.3,4 In contrast, during persistent AF, the arrhythmia is continuously present until the moment of investigation, i.e. at least two consecutive electrocardiograms of AF more than 24 hours apart and without intercurrent sinus rhythm. Persistent AF does not convert spontaneously.3,4 AF has the tendency to become more persistent over time. This is illustrated by the fact that about 30% of patients with paroxysmal AF eventually will develop persistent AF.5 Also pharmacological and electrical cardioversion and maintenance of sinus rhythm thereafter become more difficult the longer the arrhythmia exists.6 The cumulative percentage of patients who maintained sinus rhythm after serial cardioversion treatment was not more than 42% after 1 year and 27% after 4 years.6 This relates to progression of the underlying disease and possibly also to electrical remodeling of the atria.7

Mechanism: multiple-wavelet reentry

In 1959 Moe & Abildskov8 showed that AF could be produced by experimental paradigms of both multiple circuit reentry and rapid activity and they suggested that either type of mechanism might cause clinical AF. Moe put forward the ‘multiple wavelet hypothesis’ of AF in 1962.9 This concept described the propagation of reentry waves as involving multiple independent wavelets circulating around functionally refractory tissue. The maintenance of AF then depends on the probability that electrical activity can be sustained by a sufficient number of active wavelets at any time. Experimental support for Moe’s ideas was obtained subsequently with the use of computerized mapping of AF maintained in the presence of acetylcholine in dog hearts.10 It was demonstrated that during
Chapter 1

Figure 1
Schematic representation of inward and outward ionic currents involved in the ventricular action potential, resting membrane potential and cytoplasmic Ca2+ transients.

The numbers 0 through 4 indicate the different phases of the action potential: 0, upstroke; 1, fast early repolarization phase; 2, plateau phase; 3, repolarization phase; 4, resting membrane potential. Adapted from The Sicilian Gambit.47

INa, sodium current encoded by the gene SNC5A; ICaT, calcium current encoded by T-type Calcium channel; ICaL, calcium current L-type Calcium channel; ICl(Ca), calcium dependent transient outward current encoded by the gene Kv4.2; INaCa, sodium calcium exchanger; ITO, transient outward current encoded by Kv4.3; IK1, inward rectifier K+ current encoded by Kir2.1; IKs, slow delayed rectifier K+ current encoded by minK and KvLQT; IKr, rapid delayed rectifier K+ current encoded by HERG, IKACh, acetylcholine dependent potassium current encoded by Kir3.1 and Kir3.4; INa-K, sodium potassium pump.
AF, multiple independent wavelets activate the atria in a random reentrant way. Individual wavelets could brake-up, fuse or collide with each other and wavelets would extinct when they reached the border of the atria or met refractory tissue. From time to time a varying number of wavelets was present in the atria and the duration of each individual wavelet lasted only several hundreds of a millisecond. Further it was shown that the number of wavelets that fit into the atria determined the perpetuation of AF. Below a critical number of wavelets (between 3 and 6), there was a considerable chance for the wavelets to die out all at the same time. When more than 6 independent wavelets were present, the arrhythmia would not convert spontaneously anymore. The number of wavelets that fit into the atria depends on the atrial refractory period, conduction velocity and atrial mass.\textsuperscript{8,9} During the last decade, mapping studies in humans with AF have further confirmed the multiple wavelet theory.\textsuperscript{11}

**Electrophysiology of the atria**

The processes that signal the heart to contract (excitation-contraction coupling) begin when an action potential depolarizes the plasma membrane surrounding the myocardial cell. This electrical signal is generated by the passage of ions through ion channels in the plasma membrane that changes the electrical potential of the interior of the cardiac cell relative to the extracellular space. These ion fluxes include two major inward currents that depolarize the heart. The phase 0 depolarization is initiated by a rapid inflow of sodium ions through the voltage-gated Na-channels and later by calcium ions via the L-type calcium channel ($I_{Ca,L}$) (Figure 1).\textsuperscript{12,13} A large transient outward current has been held responsible for the phase 1 repolarization. This current is composed of a 4-aminopyridine-sensitive component ($I_{To1}$) and a Ca\textsuperscript{2+} activated and verapamil blocked Cl\textsuperscript{-} current ($I_{To2}$ or $I_{Cl(Ca)}$).\textsuperscript{14-16} During the plateau phase (phase 2), there is a delicate balance of inward and outward currents. Inward currents can be carried through the Na\textsuperscript{+} channel and the Ca\textsuperscript{2+} channels. During reverse-mode, the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger will transport three Na\textsuperscript{+} ions outside and exchanged for one Ca\textsuperscript{2+} resulting in a net movement of charge across the plasma membrane from inside to outside. The influx of calcium through the L-type calcium channels is responsible for the plateau phase during repolarization and initiates calcium release from the sarcoplasmic reticulum that binds to the contractile filaments of the myocardial cell, causing contraction (see calcium homeostasis in normal cardiac cells).\textsuperscript{17} Outward currents during the plateau are carried by a number of K\textsuperscript{+} channels ($I_{Ks}$, $I_{Kf}$, $I_{K(Ca)}$) and the Na\textsuperscript{+}-K\textsuperscript{+} pump. For the initiation of phase 3 the contributions of rapidly and slowly activating delayed rectifier K\textsuperscript{+} currents are of key importance. Here the outward potassium current $I_{K1}$ is activated and the action potential returns to its transmembrane resting potential, which remains at that level during phase 4 of the action potential until the cell becomes re-activated.
The duration of the repolarization phase is different between atrial and ventricular myocardial cells. This phase is shorter in the atrium compared to ventricle. An other difference is the distribution and magnitude of the ionic currents responsible for the resting membrane potential. The inward rectifier current, which is responsible for maintenance of the resting membrane potential, $I_{Ks}$ is smaller in atrial cells compared with ventricular cells. Secondly, the acetylcholine-dependent potassium current $I_{KACh}$ is very important in maintaining and hyperpolarizing the resting membrane potential in atrial cells, while less active in ventricular cells. It is this current which is responsible for hyperpolarization and shortening of the atrial action potential during parasympathetic stimulation. Vagal stimulation is indeed one of the oldest models to induce sustained atrial fibrillation by applying vagal stimulation. The resulted increased parasympathetic tone will shorten the atrial refractory period through opening of these acetylcholine-dependent potassium channels. Furthermore, inhomogeneous distribution of vagal nerve endings will increase the spatial dispersion in refractoriness. In this way, atrial fibrillation will persist after induction with premature stimuli or atrial burst pacing as long as the parasympathetic system is stimulated, either by vagal nerve stimulation or by acetylcholine application.

**Figure 2**
Schematic representation of the calcium triggered calcium release in the myocardial cell. Small amount of calcium enters the cell via the Na+/Ca2+ exchanger, but mainly through voltage gated L-type calcium channels, which open in response to the action potential. This causes a much larger amount of calcium to be released by the ryanodine receptors (RyR) in the sarcoplasmic reticulum (SR). Calcium will bind to the troponin C resulting in contraction. The calcium pump of the SR (SR Ca2+ ATPase) has a high affinity for calcium and reduces cytosolic Ca2+ concentrations to levels low enough to dissociate this cation from its binding sites on troponin C. The SR Ca2+ ATPase is inhibited by phospholamban (PLB). When PLB is phosphorylated this inhibition is reversed.
Calcium homeostasis in normal cardiac cells

When an action potential depolarizes the plasma membrane surrounding the myocardial cell, the processes that signal the heart to contract begins. Calcium transients underlying this excitation-contraction coupling in cardiac cells result mainly from calcium release from the sarcoplasmic reticulum triggered by calcium entry during the action potential. This process is called calcium-induced calcium release (Figure 2). Under normal circumstances, calcium entry into cardiac myocytes is carried primarily via $I_{\text{CaL}}$, whereas additional fractions can enter via reverse-mode Na$^+$/Ca$^{2+}$ exchange (one calcium ion is transported inside the cell against three sodium ions outside) and $I_{\text{Cat}}$. All three pathways are capable of triggering sarcoplasmic reticulum (SR) calcium release and contraction, but the relative contribution and efficiency is largest for $I_{\text{Cat}}$. Upon depolarization, influx of calcium through discrete clusters of L-type calcium channels in the plasma membrane triggers the opening of ryanodine receptors in the sarcoplasmic reticulum membrane, resulting in the major release of calcium into the cytoplasm which in turn triggers myofibril contraction by binding of troponin C. Following contraction, the SR calcium ATPase enzyme in the network of sarcoplasmic reticulum surrounding the myofibrils, rapidly pumps the calcium back into the SR lumen, causing the myofibrils to relax. The SR calcium ATPase is regulated by phospholamban, a small protein and when phosphorylated it will activate SR calcium ATPase and the calcium content of the SR will increase.

Modern concepts of excitation-contraction coupling rely on a ‘local-control theory’ a close association between L-type calcium channels and ryanodine receptors and subsequently received experimental support documenting local functional interaction between these channels. Changes in the close association between the L-type calcium channels and ryanodine receptors result in a reduction of calcium release from the sarcoplasmic reticulum and reduced contractility of the cardiac myocyte.

The membrane machinery allows each myocyte to function as an autonomous contractile unit. To produce a heart beat, the contractile capabilities of myocytes that make up the heart have to work in a highly synchronous fashion. This requires both an orderly spread of the wave of electrical activation and effective transmission of contractile force from one cell to the next, throughout the heart.

Electrical remodeling of the atria

Over the past several years, AF-induced remodeling and its underlying mechanisms have been studied in substantial detail. Wijffels and coworkers published the first study in 1995 which part of the underlying electrophysiological changes explaining the progressive nature of AF was demonstrated. In healthy goats they showed that repetitive induction of AF increased the duration of successive episodes of AF, until AF finally did not convert
spontaneously any more. They discovered that the increased tendency of the atria to fibrillate was paralleled by a progressive shortening of the atrial effective refractory period (AERP) and loss of the physiological rate adaptation of the refractory period which they termed atrial electrical remodeling. After cardioversion of atrial fibrillation that had been present for two to four weeks, this so-called atrial electrical remodeling appeared to be completely reversible within one week after restoration of sinus rhythm.

In 1995 Morillo et al. also showed that rapid atrial pacing (400 bpm) strongly promotes the ability to maintain AF in dogs, with changes quite similar to those observed by Wijffels et al. Later observations by Wijffels et al. suggested that acute volume loading, opening of the ATP-dependent potassium channels, neurohumoral activation or an increase in ANP were not responsible for the altered electrophysiological characteristics in this experimental model. This supports the idea that AF-induced remodeling is primarily due to the rapid atrial activation rates caused by AF. Consequentially, many investigators have used rapid atrial pacing in experimental models to study the electrophysiological changes caused by sustained atrial tachycardia, hoping to gain insights into the atrial electrophysiological changes caused by AF in man.

Experimental and clinical studies have shown that sustained AF decreases the atrial effective refractory period (AERP). AERP changes occur over a period of days to weeks, but AF can decrease AERP over a time interval as short as several minutes. Although the AERP reduction caused by AF favours arrhythmia maintenance, it cannot be the only factor involved because AF-induced AERP alterations become maximal well before AF-promoting effects stabilize. One of the AF-promoting effects is tachycardia induced atrial conduction slowing. It has a slower time course than AERP changes, probably due to structural changes and could account for at least a part of the continued development of AF promotion after AERP changes have stabilized.

Tieleman and coworkers found that the AERP shortened with loss of the normal rate adaptation in response to 24 hours of rapid atrial pacing in goats. Here the tendency of the atria to fibrillate increased. With resumption of sinus rhythm after cessation of pacing, the refractory period normalized over a period of slightly more than 24 hours. This electrical remodeling could be modulated using several pharmacological agents. First, the calcium channel blocker verapamil reduced atrial electrical remodeling; suggesting that tachycardia-induced calcium overload might trigger the shortening of the refractory period. By contrast, digoxin delayed the recovery from electrical remodeling of the atria. This could be due to the effect of digoxin on calcium handling, preventing effective wash-out of calcium after cessation of pacing.

**Contractile dysfunction of the atrium**

Besides the electrical remodeling observed in experimental studies, clinical studies
demonstrated atrial contractile dysfunction after AF.\textsuperscript{37,38} Leistad and co-workers investigated the contractile dysfunction in an experimental model for AF.\textsuperscript{39} They demonstrated that the atrial contractile dysfunction after acute atrial fibrillation is reduced by the calcium channel antagonist verapamil, which suggests that transsarcolemmal calcium influx contributed to this dysfunction. The calcium agonist BAY K8644 increased postfibrillation atrial contractile dysfunction. A remarkable finding was that atrial contractility increased in the first seconds after atrial fibrillation before a longer period of reduced atrial contractility ensued. On the basis of these observations they hypothesised that cytosolic calcium overload due to rapid depolarization during the preceding fibrillation might be responsible for the atrial contractile dysfunction. This hypothesis was also suggested by Shapiro et al. in 1988.\textsuperscript{40} It should be noted that in the pig model of Leistad and coworkers\textsuperscript{39} atrial contractile dysfunction was observed after only five minutes of AF, indicating that contractile dysfunction, like electrical remodeling in the goat\textsuperscript{7}, starts early.\textsuperscript{39}

**Structural remodeling**

Until now, research focused on electrical and contractile remodeling. AF is also associated with structural changes.\textsuperscript{27,31,41} Ausma and coworkers described and quantified the structural remodeling in atrial myocardium due to sustained atrial fibrillation in the goat.\textsuperscript{41} They maintained atrial fibrillation in normal goats for a prolonged period of time. After 9 to 23 weeks of sustained atrial fibrillation, several areas of the right and left atria were examined by light and electron microscopy. They found that a substantial proportion of the atrial myocytes (up to 92\%) revealed marked changes in their cellular substructures, such as loss of myofibrils, accumulation of glycogen, changes in mitochondrial shape and size, fragmentation of sarcoplasmic reticulum and dispersion of nuclear chromatin. They pointed out that these changes are the same as observed in chronically hibernating myocardium. This is a condition that occurs in patients as a result of low flow ischemia caused by stenosis of one or more coronary arteries.\textsuperscript{41} The clinical condition is defined as the ability of the myocardium to adapt to chronic ischemia by down-regulating its contractile function, thereby maintaining cell viability for a prolonged period of time. Furthermore, it was found that these hibernating myocytes resembled a form of dedifferentiation as a result of chronic atrial fibrillation.\textsuperscript{42} Some of the atrial cardiomyocytes acquired a dedifferentiated phenotype, as deduced from the re-expression of $\alpha$-smooth muscle actin, the disappearance of cardiotin and the staining patterns of titin, which resembled those of embryonic cardiomyocytes.

**Considerations for the thesis**

Thus, experimentally it has been shown that contractile dysfunction starts acutely after AF onset and was reduced by verapamil but increased by BAY K8644.\textsuperscript{39} These results
strongly indicate that changes in the calcium homeostasis triggered by tachycardia induced intracellular calcium overload\textsuperscript{43-45}, play a pivotal role in the induction of atrial contractile dysfunction. Shortening of the atrial effective refractory period was an other important factor contributing to the maintenance of AF\textsuperscript{7,46}. This shortening could also be mediated by verapamil and gave a reduction of the tachycardia induced electrical remodeling of the atria.\textsuperscript{35} This finding also suggests that electrical remodeling could be due to changes in the calcium homeostasis.

**Aim of the thesis**

The main goal was to study the molecular remodeling in human atrial fibrillation. We focussed on gene expression of proteins which influence the calcium homeostasis and action potential duration in human AF. The impact of modulating systems like the natriuretic peptide system and the endothelin system were also studied.

For the purpose of the thesis, right and left atrial appendages were collected during four years from different groups of patients undergoing cardiac surgery. The patients clinical characteristics (underlying heart disease, type of AF, electrocardiograms, medication and exercise tolerance) were assessed. The result was a unique human atrial appendage collection of around 150 individual patients. Because of this amount of different atrial appendages it was possible to match AF patients with control patients in sinus rhythm for age, sex, underlying heart disease, left ventricular function and as far as possible for medication use. In this way, dissecting the effect of AF was optimized.

During the progression of the study a discrepancy between alterations in mRNA and protein expression of several ion-channels in patients with paroxysmal AF was observed. No alterations in mRNA expression of ion-channels compared to important reductions on the protein level were found in human paroxysmal AF. This new finding prompted us to explore an adaptive mechanism unknown to occur in AF. We hypothesized reduction in protein channels due to calcium activated neutral protease calpain. To test this the calpain activity, protein expression and localization were determined in tissue of patients with atrial fibrillation. Finally the role of calpain activity in ion-channel protein, structural and electrophysiological remodeling was studied.
Introduction

References

45. Schouten VJA, Morad M. Regulation of Ca2+ current in frog ventricular myocytes by the holding potential, cAMP and frequency. Pflugers Arch 1989; 415:1-11.