Molecular adaptations in human atrial fibrillation
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Calcium Homeostasis

Studies showed that AF has the tendency to become more persistent over time. A large percentage of patients with paroxysmal AF will develop persistent AF. Also, pharmacological and electrical cardioversion and maintenance of sinus rhythm thereafter become more difficult the longer the arrhythmia exists. Therefore it is important to study the underlying mechanisms which play a role in the vulnerability to AF.

Experimental studies showed that electrical and contractile remodeling occur early after the onset of AF (Figure 1A). Both remodeling processes were attenuated by blocking of the L-type Ca\(^{2+}\) channel indicating that changes in the calcium homeostasis triggered by tachycardia induced intracellular calcium overload, play a pivotal role in the induction of atrial electrical remodeling and contractile dysfunction.

We first investigated the AF induced contractile dysfunction by studying the molecular remodeling of proteins which influence calcium homeostasis (Chapter 2 and 3). The main finding was reductions in mRNA and protein expression of the L-type calcium channel and sarcoplasmatic reticulum Ca\(^{2+}\) ATPase (SR Ca\(^{2+}\) ATPase), predominantly in patients with persistent AF. Also, an increased reduction in mRNA expression was found the longer the duration of persistent AF existed. Patients with >6 months AF revealed reductions in mRNA of L-type Ca\(^{2+}\) channel, in contrast to patients with <6 months duration of AF, in whom no changes in mRNA expression were seen (Figure 1B and 2). This finding indicates that changes in mRNA expression are the consequence rather than the cause of AF. The results described in chapter 2 and 3 were confirmed by other studies which also showed that the L-type Ca\(^{2+}\) channel and SR Ca\(^{2+}\) ATPase were both reduced in AF. Unfortunately,
in one study no time dependent changes in mRNA expression could be investigated since the duration of AF was not known. Both studies were limited by differences between AF and controls with respect to the underlying heart disease, which could have influenced mRNA expression. Another limitation was that in both studies only mRNA expression of L-type Ca$^{2+}$ channel and SR Ca$^{2+}$ ATPase was measured and no protein levels, which are anticipated to represent the amount of functional proteins more adequately.

Taken together these studies indicate that changes in gene expression of proteins influencing the calcium homeostasis occur in persistent AF. These changes probably are a contributory factor for the atrial contractile dysfunction in AF.

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**Figure 1A.** Overview AF induced adaptations in experimental studies

**Figure 1B.** Overview AF induced adaptations in human AF
It is well known that an abrupt increase in heart frequency, like in AF, causes an immediate (within one action potential) and then a gradual (reaching steady state over several minutes) decrease in action potential duration (APD). These alterations in APD reduce atrial effective refractory period (AERP) and shorten the wavelength for reentry, which will facilitate the occurrence and maintenance of reentrant arrhythmias like AF. The rapid nature of these changes suggests that the short-term APD adaptation to rate is due to functional changes in ion channels. With longer periods of sustained atrial tachycardia, changes develop over the course of hours to days. Most studies in changes of ion-channel protein expression have been performed in animal experimental settings and gradually, studies have revealed that the rapid shortening of the AERP in animal experimental AF mainly involves functional changes in the L-type Ca\(^{2+}\) channel. In human AF the relationship between changes in AERP and ion channel gene expression has not been investigated previously. We studied the regulation of L-type Ca\(^{2+}\) channel and K\(^+\) channels and their relation to AERP in patients with persistent and paroxysmal AF (Chapter 5). We demonstrated a positive correlation between the ion-channel protein expression of L-type Ca\(^{2+}\) channel, Kv4.3, Kv1.5, HERG, minK and Kir3.1 and the AERP but also with the rate adaptation to AERP in patients with persistent and paroxysmal AF. Low ion-channel protein levels were associated with short AERP and poor rate adaptation. This indicates that electrical remodeling is paralleled by general ion-channel protein reductions as part of the adaptation mechanisms during AF. Since reduced ion-channel protein expression occurred due to AF we called this phenomenon ion-channel remodeling. The ion-channel protein remodeling could play an important role in the susceptibility to AF after restoration of sinus rhythm. Since shortening of AERP can be explained by decrease in L-type Ca\(^{2+}\) channel and increase in K\(^+\) channel gene expression or activity, the reductions in L-type Ca\(^{2+}\) channel could represent an explanation for the electrophysiological changes during AF.

As noted the shortening in AERP can also be explained by an increase in K\(^+\) channel activity and expression, we investigated the contribution of potassium channels in paroxysmal and persistent AF (Chapter 4 and 5). Reductions in mRNA and protein levels were found for several K\(^+\) channels in patients with persistent AF. In patients with paroxysmal AF these reductions were observed predominantly at the protein level and not at the mRNA level, suggesting the activation of a proteolytic system. The reductions in mRNA and protein amount of K\(^+\) channels do not explain the shortening of AERP, however other studies have also reported the decrease in K\(^+\) channels in AF. One study found increased I\(_{KACH}\) and I\(_{K1}\) in isolated human atrial cells of patients with persistent AF due to different underlying heart diseases. The apparent inconsistency between protein levels...
and current density can only be explained by assuming a change in single channel properties in patients with persistent AF, such as an increase of mean open-time and increase in channel conductance or a change in voltage dependency.

Unfortunately in human studies it is difficult to make a time course for remodeling processes, but experimental data can elucidate the ion-channel remodeling in more detail. Human and experimental studies showed that brief periods of experimental AF (<1 h) abbreviate AERP and favor AF induction via functional changes, including Ca\(^{2+}\) overload induced L-type Ca\(^{2+}\) current (I\(_{\text{cal}}\)) inactivation, that cause APD shortening (Figure 1A,B).\(^{15,16}\) With longer periods of sustained atrial tachycardia adaptations appear to involve alterations mainly in ion channel density that are due to modified gene expression (Figure 1A,B and 2).\(^{11,12}\) An examination of ionic current changes in atrial myocytes from dogs subjected to rapid atrial pacing for 7 and 42 days\(^{11,12}\) indicated that high rate stimulation of atrial myocytes does not change a variety of currents, including inward and delayed rectifier K\(^{+}\) currents, T-type Ca\(^{2+}\) current and Ca\(^{2+}\) dependent Cl\(^{-}\) current. Currents that show important alterations were the transient outward K\(^{+}\) current (I\(_{\text{to}}\)) and L-type Ca\(^{2+}\) current (I\(_{\text{cal}}\)), both of which are reduced by about 70% after 6 weeks of rapid atrial pacing due to reductions in protein amount.\(^{12,17}\) Other properties of I\(_{\text{cal}}\) and I\(_{\text{to}}\), like voltage, time and frequency

Figure 2. Overview L-type calcium channel alterations

- = mRNA amount human study (1, Chapter 5; 2, Chapter 3; 3, Chapter 2)
- = mRNA amount experimental study (5, Yue et al. Circ. 1997 and 1999)
- = protein amount human study (1, Chapter 5; 3, Chapter 2)
- = amount current experimental study (5, Yue et al. Circ. 1997 and 1999)
- = experimental binding study (6, Gaspo et al. CVR 1999)
dependence are unchanged. This observation suggests that the changes observed are due
to a reduction in the number of functional channels in the membrane rather than to a
change in basic channel properties. The use of pharmacological probes to mimic the effects
of reduced $I_{Ca,L}$ and $I_{To}$ on the action potential showed that reductions in $I_{Ca,L}$ are likely to
play the central role in the APD alterations caused by atrial tachycardia with the changes
in $I_{To}$ being of much less importance,\textsuperscript{11} despite the quantitatively similar reduction.

Thus, experimental and human AF studies reported important observations concerning
ion-channel remodeling. Although a difference in time course was found between human
and experimental AF. Changes in mRNA expression were observed in animal experiments
around 1 week of AF, in human AF significant changes were observed only after $> 6$
months (Chapter 3) and $>3$ months\textsuperscript{6} (Figure 2), indicating that other factors play a role in
the adaptation mechanisms in human AF, for example preconditioning.

Furthermore in AF series of changes were found, involving rapid functional alterations
and slower changes in gene expression that cause APD reduction and reduced cellular
calcium loading. These changes can be considered to reduce $I_{Ca,L}$ and thereby protect the
cell against potentially lethal $Ca^{2+}$ overload resulting from an increase in rate of action
potential generation between resting sinus rhythm and AF. This protective effect occurs,
however, at the expense of electrophysiological changes that promote the maintenance of
AF.

**Neurohumoral changes**

The cardiac natriuretic peptide system and the endothelin system play an important
role in maintaining volume homeostasis especially in conditions that affect
ehemodynamics.\textsuperscript{18,19} In Chapter 6 and 7 the local gene expression of these systems in atrial
tissue of patients with AF was studied. Persistent AF was associated with evident expression
of ANP and BNP mRNA and also endothelin-1 mRNA contents. The extent of these changes
was more pronounced in patients with concomitant valvular heart diseases, indicating that
these systems play a role in human AF and in particular in the presence of atrial pressure
or volume overload. Furthermore reductions were found in the protein expression of the
endothelin type A and B receptors during paroxysmal and persistent AF in contrast to
unchanged expression of mRNA amounts of these receptors suggesting post-transcriptional
regulation.

**Post-transcriptional regulations**

A remarkable finding during the study of mRNA and ion-channel protein remodeling
was a discrepancy between changes in mRNA and protein levels in patients with paroxysmal
AF (Chapter 4, 5 and 7). Whereas ion-channel protein levels of L-type $Ca^{2+}$ channel, Kv1.5,
Kir3.1 and minK, but also ET-A were substantially decreased, the mRNA levels were
essentially unaffected in paroxysmal AF. This discrepancy was also observed in other studies\textsuperscript{20,21} and prompted us to explore the role of an adaptative mechanism of which the influence in AF was unknown: the activation of a proteolytic system. Different proteolytic pathways could be involved in AF. Since cytosolic calcium is increased during AF\textsuperscript{22,23}, proteolysis may be invoked by calcium dependent neutral proteases, calpain I and II. Calpains are proteases which cleave mainly cytoskeletal and membrane-associated proteins into ‘limited fragments’ without further degradation.\textsuperscript{24} In cardiac cells, calpains mediate cell death in metabolically inhibited cultured rat cardiomyocytes and are involved in troponin proteolysis and cross-linking following cardiac stunning and calcium overload.\textsuperscript{25-27} In Chapter 8 an increased proteolytic activity in atrial tissue of patients with paroxysmal and persistent lone AF was described. This increase was predominantly due to elevation of calpain I activity and expression. Furthermore we observed that calpain I protein was mainly localized at the nucleus and intercalated discs of atrial myocytes (Chapter 9). The intensity of staining was low in sinus rhythm higher in paroxysmal AF and reached a maximum in persistent AF. At the intercalated discs calpain can interact with Ca\textsuperscript{2+} and thereby become an active proteinase and can degrade some important ion-channels like the Na-channel\textsuperscript{28} and Kv1.5\textsuperscript{19}, but also several proteins directly involved in excitation-contraction coupling.\textsuperscript{29} At the nucleus\textsuperscript{30} calpain can induce degenerative features leading to apoptosis, which is observed in human AF (Figure 3).\textsuperscript{31}

The role of calpain in cellular changes underlying the electrophysiological (Chapter 5), ion-channel (Chapter 5) and structural remodeling (Chapter 9) was examined. The amount of structural and ultra-structural changes in the atrial tissue was examined by light microscopy and electron microscopy, respectively. Calpain activity correlated with the

\begin{figure}[h]
\centering
\begin{tikzpicture}
  \node (AF) {AF};
  \node (calcium overload) [below of=AF] {calcium overload};
  \node (induction calpain activity) [below of=calcium overload] {induction calpain activity};
  \node (degeneration proteins) [below of=induction calpain activity] {degeneration proteins};
  \node (necrosis/apoptosis) [below of=degeneration proteins] {necrosis/apoptosis};
  \draw [arrow] (AF) -- (calcium overload);
  \draw [arrow] (calcium overload) -- (induction calpain activity);
  \draw [arrow] (induction calpain activity) -- (degeneration proteins);
  \draw [arrow] (degeneration proteins) -- (necrosis/apoptosis);
\end{tikzpicture}
\caption{AF calcium overload induction calpain activity degeneration proteins necrosis/apoptosis}
\end{figure}
expression levels of ion-channel proteins, the degree of structural changes, measured AERP and the rate adaptation coefficient of AERP. The results suggest that induction of calpain activation represents a missing link between the calcium overload observed in AF and remodeling of atrial myocytes during AF (Figure 3).

**Integrated model for remodeling processes in AF**

*Evidence for AF induced Calcium overload*

While studying the ion-channel gene expression adaptation mechanisms in human AF, it became clear that the central feature in all these processes is calcium, in particular calcium overload. Most investigations recognize the direct and indirect role of calcium and our studies support the notion of calpain activation in AF. This notion is appealing since it provides the molecular link between AF induced calcium overload and remodeling processes, which was still not identified. Several lines of evidence for the crucial role of AF induced calcium overload and subsequent calpain induction are reported below.

It has been proposed that atrial contractile dysfunction occurs after short-term and chronic AF (Figure 1A,B). Contractile dysfunction after chronic AF is most likely related to the cellular alterations in atrial myocytes reflected by structural alterations, probably induced by proteolysis. The explanation for the atrial dysfunction after short-term AF might be an increase in cytosolic Ca\(^{2+}\) due to the high rate of atrial activation. Fast successive action potentials inhibit a proper sarcoplasmic reticulum Ca\(^{2+}\) re-uptake, resulting in elevated cytosolic Ca\(^{2+}\), possibly impairing the excitation-contraction coupling and contractile function. Ausma and coworkers showed that sarcolemma-bound Ca\(^{2+}\) and Ca\(^{2+}\) deposits in mitochondria increased markedly up to 2 weeks in experimental AF and tends to regress towards normal levels at 4 and 8 weeks of AF (Figure 1A). Unfortunately, for Ca\(^{2+}\) localization they used antimonate based methods, which limit the visualization of overall Ca\(^{2+}\) load at subcellular sites like the sarcoplasmic reticulum. Other preliminary data showed that atrial tachycardia causes an immediate increase in cytoplasmic Ca\(^{2+}\) concentration, which results in impaired Ca\(^{2+}\) release and cellular contractile dysfunction after the cessation of tachycardia.

Also other signaling pathway(s) by which AF leads to changes in atrial calcium handling are involved. Recently completed experimental work suggests that T-type Ca\(^{2+}\) channels may mediate atrial tachycardia-induced electrical remodeling, because the T-type Ca\(^{2+}\) channel blocker mibefradil limits both the ERP changes and AF promotion caused by one week of rapid atrial pacing. Also in this case calcium overload would be prevented by blocking a calcium channel.

The similarity between the cellular ultrastructural changes caused by sustained AF and those seen in hibernating myocardium have led to a suggestion that atrial ischemia may play a role in triggering remodeling caused by AF. Whether ischemia occurs in AF is
still debatable, but a reduced atrial blood flow in dogs with rapid pacing induced AF was found and could result in atrial ischemia.\textsuperscript{41} A potential role for atrial ischemia is consistent with the protective effect observed with blockade of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger in short term tachycardia-induced atrial remodeling.\textsuperscript{42} In this model ischemia would give rise to a decrease in intracellular pH, which leads to an exchange of intracellular hydrogen ions for extracellular Na\textsuperscript{+} ions. Such an increase in intracellular Na\textsuperscript{+} results in a lower, or even negative, equilibrium potential for the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger, thereby leading to a greater magnitude of ‘reverse-mode’ functioning of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger and therefore an influx of Ca\textsuperscript{2+} ions.\textsuperscript{43} Alternatively, inhibition of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger may alter cellular ionic homeostasis and combat calcium overload induced by ischemia.

In chapter 7 a mechanism that potentially modulates calcium overload in AF was described. Studies on gene-expression of the endothelin system revealed that these systems play a role in AF induced remodeling especially in patients with underlying valve disease. We found that mRNA amounts of endothelin-1 are induced predominantly in persistent AF with underlying valve disease. It is known that elevated endothelin-1 levels increase intracellular calcium levels via the L-type Ca\textsuperscript{2+} channel\textsuperscript{44}, indicating that calpain activation also could play a role in AF with underlying valve disease, via different signal transduction pathways. Moreover, increased amounts of BNP (Chapter 6) could be a compensatory mechanism to reduce the intracellular calcium overload and thereby leading to relaxation of the myocardial cell. BNP modulates cardiac calcium homeostasis via reduced intracellular concentrations of cyclic adenosine monophosphate (cAMP), which inactivate the L-type Ca\textsuperscript{2+} channel and activate the acetylcholine-dependent potassium channel leading to repolarization of the action potential.\textsuperscript{18}

Thus, several lines of evidence point to a central role of cellular calcium overload in AF induced remodeling. Our work now reveals the potential role of calcium sensitive processes that lead to changes in gene-expression and structural changes. Because elevated levels of intracellular Ca\textsuperscript{2+} are known to activate proteolysis, this could result in increased breakdown of myofilaments\textsuperscript{27,45} and ion-channel proteins (Chapter 9). In turn this could be responsible for decreased contractility as well as for the vulnerability to AF.

\textit{Time course structural remodeling}

In addition to electrophysiological, functional ion-current and ion-channel gene expression changes, AF is associated with alterations in morphology. In Chapter 9 we described an increase in degenerative contraction band necrosis observed in patients with persistent and paroxysmal AF. Furthermore, we observed an increase in myocardial hibernation (loss of sarcomeres and pale nuclei) only in patients with persistent AF, which positively correlated with the duration of AF. This indicates that in human persistent AF hibernation could be the specific structural change due to AF, in accordance with
development of hibernation in the goat model for AF.\textsuperscript{40} We observed abundant degenerative features in lone, paroxysmal AF. These could represent the prelude to the vulnerability to AF by inducing dispersion of conduction (Figure 1B). Once persistent AF has been developed, hibernation (which depends on prolonged periods of sustained AF) is more abundant considered that cells liable to degeneration have now disappeared. These notions are supported by the finding that in our patients with persistent AF, hibernation increased with the duration of AF, while degeneration decreased. Possibly, hibernating myocardium is protected against degeneration, as found after ischemic preconditioning.\textsuperscript{46} The reported structural changes in human AF are in accordance with other studies. In humans structural changes occur in atrial myocytes in patients with persistent AF.\textsuperscript{47} In patients with atrial arrhythmias, myolysis and glycogen storage were only observed in a small number of cells and that changes were frequently accompanied by lysosomal degeneration. In experimental models these structural abnormalities appeared to be more pronounced when the underlying pathology was aggravated by sustained AF.\textsuperscript{48,49} The occurrence of degenerative myocardium could lead to increased dispersion of refractoriness and conduction, which was found to enhance the inducibility and spontaneous occurrence of idiopathic human AF.\textsuperscript{50,51} In addition to these defined changes in structural features, the myocytes of AF patients displayed increased heterogeneity of cell size. Taken together, the observed structural changes are indicative of a substantial deterioration of normal tissue architecture, likely to promote AF through heterogeneity of atrial refractoriness\textsuperscript{50,51} and slowed atrial conduction.\textsuperscript{9,10,52} Since extreme physical stress, in combination with sustained elevated cytosolic calcium levels, as in experimental AF\textsuperscript{22} often results in necrosis, calpain could play an important role in this condition.\textsuperscript{53}

In their model Ausma and coworkers noted mitochondrial enlargement, glycogen accumulation, loss of sarcoplasmic reticulum and contractile elements in the atria of goats subjected to chronic AF for upto 23 weeks.\textsuperscript{40} These changes resemble those observed in the hibernating myocardium of the patients we investigated (Chapter 9). Recently the time course of structural changes during AF in goats after 1-16 weeks of AF was studied.\textsuperscript{54} Here, the structural changes appeared to develop progressively, the earliest changes having been noted after 1 week of AF and related to nuclear redistribution of heterochromatin (Figure 1A). The nuclei showed a homogeneous distribution of chromatin, resembling that found in embryonic/neonatal cardiomyocytes.\textsuperscript{40,55} After 4 weeks and at later times, AF affected sarcomeres, glycogen, mitochondria and sarcoplasmatic reticulum simultaneously. The loss of sarcoplasmatic reticulum and contractile proteins surely cause a decrease in contractile force and hence atrial stunning\textsuperscript{54}, which could be mediated by calpain.\textsuperscript{25,27}

Since AF is promoted by slow conduction\textsuperscript{9,10,52,56} studies investigated the gap-junction proteins, connexins, which play an important role in homogenous wavefront propagation and conduction velocities in the heart.\textsuperscript{9,21,57,58} Gap-junctions are clusters of channels which
span the closely apposed plasma membranes, forming cell-to-cell pathways. Connexins are permeable to ions and small molecules up to 1 kDa in molecular mass, like second messengers such as inositol triphosphate, cyclic AMP and calcium.

The initial data presented on changes in intercellular connexins were contradictory. One study in the dog showed that AF increases connexin43 expression, the most abundant connexin and another in the goat suggested that connexin43 is unaltered, but the distribution of connexin40, mainly present in atrium was altered. In a recent study the gap junctional changes in relation to stabilization of AF were studied. In goats that were in sinus rhythm the distribution of connexin40, a connexin that gives high conductance, was homogeneous. After 2 weeks in AF, which was the time associated with markedly increased intracellular Ca^2+ deposition and just before AF became sustained, heterogeneity in the connexin40 distribution was observed. The connexin40 distribution pattern correlated with the occurrence of structural changes (myolysis) in atrial myocytes.

The structural changes, myolysis and heterogeneity of connexin40 distribution, possibly relate to calcium induced calpain activity and explain the slow recovery (weeks to months) after cardioversion of AF in patients, the contractile dysfunction and the electrophysiological changes during AF.

**Electrophysiological properties**

Over the past several years, AF-induced electrical remodeling and its underlying mechanisms have been studied in substantial detail. In experimental studies part of the underlying electrophysiological changes explaining the progressive nature of AF were demonstrated. 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 was termed atrial electrical remodeling. The reduction in rate adaptation of the AERP is also observed in patients with AF. All studies have shown that sustained atrial tachycardia decreases AERP and changes occur over a period of days to weeks, but AF can decrease AERP over a time interval as short as several minutes (Figure 1A,B). Although the AERP reduction caused by AF favors arrhythmia maintenance, it seems not 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 delayed onset of structural changes in the gap junctional remodeling and could account for at least a part of the continued development of AF promotion after AERP changes have stabilized. Whether gap junctional remodeling is caused by calpain induction is unknown, but it is known that at least proteasome activity underlies a connexin43 degradation.
In addition to changes in the absolute value of AERP, atrial tachycardia alters the spatial distribution of AERP. The spatial heterogeneity of AERP appears to be an important determinant in the maintenance of AF\textsuperscript{50,51,62,63} and there are indications that changed atrial autonomic innervation, i.e. norepinephrine induced atrial sympathetic innervation, plays an important role.\textsuperscript{64} Since norepinephrine causes elevation of the intracellular calcium concentration in atrial myocytes, calpains might be activated and represent the causal link to the maintenance of AF.

The combination of electrophysiological changes caused by sustained atrial tachycardia i.e. reduced AERP, diminished or reversed adaptation to rate, slowed conduction and increased spatial AERP heterogeneity, and the underlying structural changes caused by calcium overload induced calpain activity would be expected to promote AF maintenance by enhancing the number of functional reentry circuits during AF.

**Future perspectives**

*Pre-conditioning*

The specific structural change induced by AF (Chapter 9) seemed to resemble chronic hibernating myocardium.\textsuperscript{40} It is generally believed that by down-regulating their function, cardiomyocytes adapt to a lowered oxygen availability and thereby restore the oxygen supply/demand ratio. Part of the atrial cardiomyocytes acquired a dedifferentiated phenotype, by re-expression of typical embryonic proteins. Furthermore, there is indirect evidence that dedifferentiated,\textsuperscript{55} hibernating cardiomyocytes tolerate ischemia better than non-dedifferentiated cardiomyocytes.\textsuperscript{65} It could be hypothesised that endogenous protective mechanisms, such as an increased expression of certain heat shock proteins (stress induced proteins which protects the cell against damage)\textsuperscript{66}, are up-regulated in AF induced hibernating myocytes. Although direct evidence of such up-regulation is missing, it is known that ischemic preconditioning induces the efficient translation of stress proteins.\textsuperscript{46} Several of these proteins are subsequently translocated to the nucleus, possibly to protect against degradation of DNA that has become more susceptible to degradation due to a transformation of the chromatin organisation into a nuclease-sensitive conformation (as is the case in apoptosis).\textsuperscript{67,68} Heat shock proteins, such as Hsp70, Hsp27 and αB-crystallin are known to protect against ischemic cardiac damage. Unlike ischemic preconditioning, which also attenuates apoptotic cell death induced by ischemia/reperfusion in a pig model of short-term hibernation\textsuperscript{69}, mRNA expression of Hsp70 and several other apoptosis-modulating proteins was not altered in the ventricle during coronary stenosis nor during subsequent stunning.\textsuperscript{70} Still it could be worthwhile investigating the role of protective heat shock proteins in AF.
Chapter 10

Possible clinical relevance

The chance of successful chemical cardioversion and/or prevention of AF is dependent on the duration of AF. This clinically observed diminished efficacy of cardioversion therapy after long term AF cannot only be explained by the occurrence of electrical remodeling. The ion-channel protein remodeling and structural remodeling probably also affect the electrophysiological function of the atrial myocardium.

In patients with persistent AF, there is a correlation between the duration of AF and the time needed to recover atrial contractile function after cardioversion.\textsuperscript{33,71} The increase in calpain activity which could lead to structural remodeling of the atrial myocytes might give an explanation for the delay in recovery of contractile function in the atria after conversion to sinus rhythm as seen in patients with persistent AF. Interference with the calpain pathway by pharmacological intervention might represent an important new therapeutic strategy to decrease protein degradation and thereby reduce the vulnerability to AF. Calpain inhibitors as therapeutic agents are already used in nerve and muscle degeneration\textsuperscript{72}, but their potential benefit in heart diseases is not studied yet. After restoration of normal sinus rhythm it may take the cardiomyocytes a certain period to rebuild a normal amount of sarcomeres, if that is still possible at all.\textsuperscript{73} Data describing the recovery of ion-channel protein expression are lacking, but a few reports describe, in contrast to the structural remodeling, reversal of electrical remodeling in human AF after cardioversion.\textsuperscript{74,75} Since AF induces remodeling in the atria it is essential to restore sinus rhythm as soon as possible, thereby preventing the continuation of the atrial structural, ion-channel protein and electrical remodeling.

Furthermore, differences in adaptation mechanisms which were found between patients with lone AF and AF with underlying heart disease suggest the need for different pharmacological treatment. For example, patients with elevated levels of endothelin could be treated with an endothelin receptor antagonist, which has been shown to normalize alterations in expression of various cardiac genes (like normalization of ryanodine receptor, sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase, angiotensin-converting enzyme, angiotensin II type I receptor and prepro-endothelin 1) in failing myocardium.\textsuperscript{76}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{agarose_gel.png}
\caption{Example of an agarose gel. Here the L-type calcium channel $\alpha_1$ subunit and the GAPDH are given. H9c2 cells were stimulated 0, 12, 24 and 36 hours and 36 hours stimulation combined with 48 hours recovery. The amount of L-type Ca\textsuperscript{2+} channel increased during the different stimulation protocols.}
\end{figure}
New Experimental model for AF

To mimic human AF experimental models were developed. AF is studied in more detail in different animal models. The dog and goat models for AF are well studied and characterized. For studying molecular and cellular mechanisms the animal models have disadvantages. First, in the animal a lot of (unknown) parameters can interfere with the results. Second, animal models take time and are expensive and the third important disadvantage is for the animal itself. These are reasons to think of a different experimental model for AF, especially for studying the fundamental calcium sensitive pathways. A possible new model could be the electrical stimulation of myocardium cells. Pilot experiments have been done for H9c2 cells, which are rat myoblast cells and are able to differentiate into myotubes. H9c2 cells appear to be unique in that they express the cardiac isoforms of the L-type Ca$^{2+}$ channel alpha 1-subunit mRNA (data not shown). Another good candidate cell line is the immortalised HL-1 cells. These are contracting mouse atrial cells and when electrically stimulated could mimic human AF.

For the electrical stimulation experiment a special culture flask was developed by Popta and Henning. Pilot experiments were done to investigate changes in L-type Ca$^{2+}$ channel mRNA expression and for measuring the calpain activation during electrical stimulation in H9c2 myotubes. Therefore, the cells were stimulated for 12, 24 and 36 hours followed by three days recovery (0.5 Hz, 25V). Figure 4 shows that in stimulated H9c2 cells the mRNA expression of the L-type Ca$^{2+}$ a1 subunit was increased compared to unstimulated H9c2 cells. Thus the H9c2 cell line may prove to be useful when studying the regulation of subtype-specific Ca$^{2+}$ channel gene expression. The second set of experiments were done to measure the calpain activity with a calpain specific fluorogenic substrate. The calpain activity increased significantly after 20 and 30 hours of stimulation (Figure 5). This activity could be reduced by 65% by a specific inhibitor of calpain I. These two sets of pilot experiments reveal that these cell models could represent excellent models for studying the signaling pathways activated by electrical stimulation and the potential benefits of pharmacological intervention in AF.

![Figure 5](image_url)

Calpain activation measurement in H9c2 cells. The cells were stimulated 0, 20 and 30 hours. An increase in calpain activity was found in stimulated cells and inhibited by calpain I inhibitor by 65%.
In conclusion, the combination of electrophysiological, ion-channel protein and structural remodeling caused by sustained AF would be expected to promote AF maintenance by increasing the calcium overload induced calpain activity leading to induction of the number of functional reentry circuits during AF. New cell models could be beneficial for studying the signaling pathways activated by electrical stimulation and pharmacological intervention in AF.

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