Abstract

Atrial fibrillation is the most common cardiac arrhythmia with an overall prevalence of almost 1%. Increasing prevalence and associated risks such as stroke and mortality have increased the need for better and more reliable therapeutic treatment. This has stimulated research to elucidate the pathophysiological mechanisms underlying atrial fibrillation. Atrial fibrillation is primarily characterised by electrical remodelling and functional deterioration. Both phenomena are reversible but after prolonged duration of atrial fibrillation, a discrepancy occurs between rapid electrical remodelling and slow recovery of contractile function. Recent studies have indicated that morphological remodelling might underlie this incongruity. In experimental models of lone atrial fibrillation, the remodelling involves cellular changes that are reminiscent of dedifferentiation and are characterised by cellular volume increase, myolysis, glycogen accumulation, mitochondrial changes and chromatin redistribution. The absence of clear signs of degeneration in these models points towards cardiomyocyte adaptation or a mechanism of programmed cell survival. In patients with atrial fibrillation cardiomyocyte degeneration does occur along with dedifferentiation which might be the result of underlying cardiac pathologies or longer duration of atrial fibrillation. In this review we focus on structural remodelling during atrial fibrillation. The different aspects of histological and ultrastructural changes as well as their role in atrial dysfunction and cardiomyocyte survival are discussed. We briefly describe the underlying molecular remodelling, and possible mechanisms responsible for remodelling involving calcium overload and stretch are presented. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Atrial function; Contractile apparatus; Electron microscopy; Remodelling; Supraventricular arrhythmia

1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia observed in western civilisation, with an overall prevalence of almost 1%. Based on four major population studies, Feinberg et al. calculated that the prevalence of AF rises with increasing age, ranging from <1% in young adults to 2.3% in people over 40 years and >5% in those older than 65 years [1]. In addition, the prevalence is strongly influenced by the presence of underlying diseases like hypertension, valve disease, myocardial infarction and ischemic heart disease [2,3]. The growing prevalence of AF together with the increased risk of stroke [4] and mortality [5] will increase the burden on the medical health care at the beginning of the new century. However, the development of safe and more effective medical treatments requires a better understanding of the pathophysiological mechanisms underlying AF.

Because AF is primarily characterised by irregular and rapid electrical activity, research has been mainly focused on its electrophysiological aspects. In animal models it was shown that AF changes the electrophysiological properties of the atrium which facilitates the maintenance of AF [6]. However, this electrical remodelling is reversible and a return to normal electrophysiological properties is reached within 3 days after restoration of sinus rhythm [6]. The
quick rescue of electrophysiological properties is in contrast with the delayed recovery of contractile function after cardioversion [7,8], which indicates that apart from electrical remodelling other factors are involved in atrial dysfunction during AF.

Recent studies have shown that, in addition to electrical remodelling, cardiomyocytes undergo dramatic structural alterations during AF [9,10]. In experimental models of AF this remodelling is predominantly of an adaptive nature and suggestive of a mechanism of cardiomyocyte protection against environmental stress. In addition, the structural remodelling might explain the slow improvement of contractile function following restoration of sinus rhythm.

This review focuses on the morphological remodelling during chronic AF. The different aspects of structural alterations in experimental models of AF as well as patients with AF are described and their role in atrial dysfunction and cardiomyocyte survival discussed. Furthermore, the molecular remodelling possibly underlying structural remodelling is briefly described. Finally, we present possible mechanisms responsible for morphological remodelling involving calcium overload and stretch.

2. Atrial dysfunction during AF

AF is characterised by severe contractile dysfunction that is caused by rapid and irregular atrial activity. In experimental models it was established that during the first days of pacing induced AF, the atrial effective refractory period is markedly shortened (electrical remodelling) [6,9,11–13]. Within the same period, the atrial contractility measured during slow rate pacing, is completely abolished [14]. After short periods of AF, both phenomena are completely reversible and normal values are reached within 2–3 days [14]. It takes approximately 1–2 weeks of pacing induced AF before the arrhythmia becomes self-sustaining [6,11]. After longer periods of AF (weeks to months), the atrial effective refractory period normalises within a few days [6,15,16] and atrial activation recovers within a few weeks [17–19]. However, recovery from contractile dysfunction may take several weeks to months [7,8,13,20]. The duration of AF also determines the success of therapeutic intervention [21–23]. After 4 months of AF in a goat model the efficacy of pharmacological cardioversion was reduced compared to 1 month (30 vs. 78%) [24]. The success rate of pharmacological cardioversion in patients with AF for less than 1 year was also higher compared to patients in which AF persisted for more than 1 year [23].

Apart from a relationship with the duration of arrhythmic activity, the stabilisation of AF also appears to be related to atrial size. It is generally accepted that atrial dilatation increases the vulnerability to AF [21,25,26]. Atrial dilatation is often the result of an underlying disease, e.g. valve disease [27,28], but it also occurs as a result of persistent lone AF [9,29,30]. Similar to the duration of AF, atrial size is a prognostic factor for the therapeutic success [21–23]. Once sinus rhythm has been re-established, atrial dilatation slowly decreases [28].

Thus, besides an early time window of atrial remodelling, which involves rapid and reversible electrical and contractile remodelling, a second time window exists during AF. This second time window is characterised by a relatively rapid reversibility of electrical remodelling but also by a considerably slower functional recovery following cardioversion. This discrepancy suggests the involvement of a second factor that plays a role in stabilisation of AF [31] and that is probably related to the structural remodelling which occurs during chronic AF.

3. Structural remodelling during AF

3.1. Light microscopy

One of the first studies which associated structural alterations in the atria with the occurrence and maintenance of AF was published in 1977 by Thiedemann and Ferrans [32]. Light microscopic evaluation of atrial tissue from patients with mitral valve disease revealed variable degrees of fibrosis. In addition, all atria showed substantial numbers of hypertrophied cells as well as cells in which myolysis had occurred. Nuclei were changed in size and shape and occasionally cells with more than one nucleus were observed. It was found that 90% of patients with severe structural alterations suffered from AF. In an animal model, Boyden et al. [33] described the histological changes in the enlarged atria of dogs with mitral valve disease. The volume of the left atrium increased up to six times the normal volume and most of the animals suffered from atrial arrhythmias. Atrial remodelling was characterised by decreased wall thickness, disarrangement of cell bundles, cellular hypertrophy and an increase in connective tissue. The atria in a dog model of congestive heart failure, which promotes the induction of AF, also displayed extensive interstitial fibrosis as well as cellular hypertrophy and cells with disruption of sarcomeres and loss of myofibrils [34]. A relationship between morphological changes and AF was also described by Mary-Rabine et al. [35] in patients with different types of cardiac disease. Again, patients with AF showed considerable atrial dilatation. In addition, the presence of AF and atrial dilatation was associated with the degree of morphological abnormalities like cellular hypertrophy and myolysis. Similar alterations were more recently described in fibrillating atria from patients with atrial dilatation and mitral valve disease [36–38]. Increased fibrosis, cardiomyocyte hypertrophy, myolysis and glycogen accumulation were the major histological findings in the atria. Schotten et al. also found a strong correlation between myolysis and contractile force [38]. However, although the overall amount of myolysis...
was slightly larger during AF, the changes were also observed in patients with mitral valve disease in the absence of AF [36,38].

Recently, Wouters et al. reported on cellular changes in patients with valvular disease and AF. Besides cardiomyocyte degeneration, cellular changes in these patients mainly consisted of cardiomyocyte hypertrophy, depletion of sarcomeres and accumulation of glycogen. Remarkably, the localisation of glycogen differed between two groups of patients with different underlying heart disease and was either found throughout the cytoplasm (patients with coronary heart disease) or confined to large vacuoles (patients with rheumatic heart disease) (Fig. 1) [37]. This difference in localisation as well as the presence of vacuoles was suggested to be the result of the different underlying diseases [37]. Atrial vacuolisation was also observed in patients undergoing coronary artery bypass grafting. In these patients, the size and frequency of the vacuoles correlated with the occurrence of postoperative AF. In fact, 90% of the patients with large perinuclear vacuoles in most of the atrial cells developed AF whereas AF occurred in only 5% of the patients with no, or mild, vacuolisation [39]. In a postmortem study Pirolo et al. found reversible vacuolisation of cardiomyocytes in the borderzones of myocardial infarcts. They suggested that reversal of regional ischemia might be involved in the observed reversibility of vacuolisation [40].

Many studies in which atrial remodelling is related to AF report similar observations. Common characteristics are atrial dilatation, cardiomyocyte volume increase, myolysis, accumulation of glycogen and increased extracellular space [32,35–38]. However, most studies were performed in patients with cardiac disease and this underlying disease might to some extent account for the observed atrial remodelling [32,36,38]. Reports on atrial remodelling in patients suffering from lone AF are scarce. Again, atrial dilatation is often observed, but additional histological examination of the cellular morphology is usually lacking. Frustaci et al. reported in more detail on the atrial abnormalities in patients with lone AF. Histological evaluation of the atria revealed signs of atrial myocarditis in most of the patients. However, the atria of two patients without signs of inflammatory infiltration contained hypertrophied cardiomyocytes in which vacuoles were visible. The presence of these atrial vacuoles was associated with myolysis up to 60% along with replacement fibrosis [41]. Extensive myolysis was also recently reported by Brundel et al. in patients with persistent lone AF [42].

The development of animal models of lone AF offered tools to study the morphological aspects of atrial remodelling. In 1995, Morillo et al. described the electrophysiology in dogs after rapid pacing induced AF [9]. Besides shortening of the atrial effective refractory period and the atrial fibrillation cycle length, they observed several structural changes after 6 weeks of rapid atrial pacing. The size of both atria was considerably increased and correlated with the inducibility of AF. In addition, signs of early and focal hypertrophy were visible [9]. A more detailed analysis of atrial remodelling during pacing induced AF was performed in a goat model. In 1995, Wijffels et al. established the principle of ‘AF begets AF’ with this model, although, at that time no structural correlate was described [6]. Two years later, Ausma et al. reported on the morphological remodelling which could be observed in the atria of these goats [10]. Light microscopic analysis of different sites of the atria showed changes that were comparable to those seen in patients with AF. The atrial myocytes were enlarged and exhibited depletion of sarcomeres which was accompanied by accumulation of glycogen (Fig. 2) [10]. These changes progressively increased during AF and occurred without any signs of cellular degeneration [43,44] or apoptosis [45]. In addition, there were no alterations in the content or distribution of the connective tissue [10]. Three important observations were made concerning the morphological changes: (1) there was a resemblance to the histological changes seen in chronic ischemic but viable myocardium, the so-called hibernating myocardium [46–48]. (2) Many of the morphological features were similar to those seen in foetal cardiomyocytes. (3) Although cellular structure changed dramatically, the cells appeared to be viable and cellular degeneration was hardly visible. Based on these features, Ausma et al. hypothesised that in the experimental goat model, AF results in cardiomyocyte adaptation through dedifferentiation [10]. This concept of cellular adaptation rather than degeneration was not only based on light microscopic observations but also on findings at the immunohistochemical and the ultrastructural levels.

### 3.2. Electron microscopy

Cardiomyocytes show distinct changes at the ultrastructural level during chronic AF. Different authors have reported on these changes and although similar observations have been described, their interpretation is diverse. In a comprehensive study on the ultrastructural changes in the goat model, Ausma et al. found additional evidence for the hypothesis of AF-induced dedifferentiation of cardiomyocytes. As expected on basis of light microscopic observations, ultrastructural findings consisted of depletion of contractile elements and accumulation of glycogen (Fig. 3) [10]. Myolysis was illustrated by the presence of residual sarcomeric structures like clumps of Z-band material. Within the myolytic areas the sarcoplasmic reticulum lost its organisation and numerous mitochondria were visible. These mitochondria displayed remarkable changes in size and shape, but their cristae appeared normal and did not show any signs of degeneration (Fig. 3). The intercalated discs also appeared to be normal but there were less T-tubular membrane invaginations of the sarcolemma [10]. Time course experiments revealed a...
progressive increase in these changes and after 16 weeks of AF half of the cells suffered from myolysis and subsequent alterations [43,44]. A striking observation which could be noted even after 1 week of AF was the redistribution of the heterochromatin within the nucleus (Fig. 3) [10,44]. In contrast to condensation of heterochromatin, which is usually observed during cellular degeneration and apoptosis, the chromatin was homogeneously distributed throughout the nucleus. Like the glycogen storage, loss of sarcomeres, mitochondrial shape changes
and absence of sarcoplasmic reticulum, this homogeneous distribution of chromatin is a characteristic feature of foetal cardiomyocytes [10]. The morphological similarities with foetal cardiomyocytes combined with the absence of any ultrastructural signs of cellular degeneration further supported the concept of cardiomyocyte dedifferentiation. Earlier ultrastructural studies had not recognised cardiomyocyte dedifferentiation. Thiedemann et al. interpreted the ultrastructural changes that they observed in patients with mitral valve disease as cellular degeneration. However, many of the ultrastructural features in these patients were similar to those in the goat model and most of the patients with such alterations also suffered from AF. The cardiomyocytes showed loss of contractile material together with Z-band abnormalities. The myolytic area was filled with cytoplasmic components like cytoskeletal filaments, glycogen particles, free sarcoplasmic reticulum profiles and mitochondria. Again, the mitochondria varied in size and shape and the majority did not show signs of degeneration [32]. Besides these changes, which may be regarded as dedifferentiation rather than degeneration, there were severely affected cells with clear signs of degeneration. These cells displayed large amounts of myelin figures together with dense bodies without any clear substructures. Atrophic cells surrounded by fibrotic material were also observed, and in many of these cells hardly any organelles were visible [32]. Several other authors have described similar observations concerning cellular degeneration during AF. Cellular atrophy, the fragmentation of mitochondrial membranes, and the presence of myelin figures and dense bodies are common features of these degenerative cells [9,33,35,41]. However, other ultrastructural changes that were found could also be looked upon as cellular adaptation rather than degeneration. Hypertrophic cells with extensive myolysis and glycogen accumulation were frequently present [33,35,41]. High numbers of abnormally shaped, but viable, mitochondria and homogeneous redistribution of chromatin were also described [13,33]. Cellular hypertrophy, loss of sarcomeres and increased glycogen content was also observed by Frustaci et al. in patients with paroxysmal lone AF. The remaining sarcomeres were organised normally and although some mitochondrial degeneration was observed, there were no major signs of cellular degeneration [41]. Wouters et al. also reported the presence of cellular degeneration besides cellular adaptation in patients with mitral valve disease (Fig. 4) [37]. These authors could make a clear distinction between dedifferentiation and
degeneration and suggested that although some cellular degeneration does occur, it is not the result of ongoing dedifferentiation [37].

All these phenomena indicate that part of the cardiomyocytes appear to dedifferentiate to a survival state in order to endure the altered environmental conditions during AF. In experimental models of AF, cardiomyocytes mainly adapt to a survival phenotype whereas in patients
Fig. 4. Electron microscopic pictures of cardiomyocyte degeneration in patients with mitral valve disease and atrial fibrillation. (a) Detail of severe degeneration comprising necrotic remnants, cytosolic blebs filled with vesicles, and the presence of inclusion bodies of unknown origin; magnification: ×2500. (b) Detail of a possibly apoptotic cardiomyocyte. A huge vacuole containing glycogen (gl) is surrounded by normally structured sarcomeres (s) and mitochondria (m), while the horse-shoe shaped nucleus (n) shows marked clumping of the heterochromatin (arrows); magnification: ×7700. (Reproduced from [37] with permission of Pulsus Group).

with AF both cellular degeneration and cellular adaptation occur. This difference might be due to species variance but is more likely related to differences in duration of AF and underlying cardiac pathology.

4. Molecular remodelling during AF

The observed histological and ultrastructural changes show that cardiomyocytes undergo dramatic remodelling
during AF. The altered morphology as well as the diminished functionality is a reflection of transitions that take place at the molecular level. The cardiomyocytes have to cope with new circumstances and consequently change the expression and organisation pattern of proteins involved in activation, conduction, and contraction.

4.1. Channel proteins

The most obvious outcome of molecular adaptation might be the electrical remodelling because it occurs at the onset of AF. Ions like calcium, sodium and potassium are important players in the excitation–contraction cycle, and the expression patterns of their channels and other proteins involved in ion homeostasis have been extensively studied. Several authors have found altered mRNA and/or protein levels of ion channels during AF [49–54]. Downregulation has been described for the L-type calcium channel [50,51,55], the sarcoplasmic reticulum calcium ATPase [50,51,55], and several potassium channel subunits [49,50,52–55], whereas the expression of other proteins involved in calcium handling like the sodium/calcium exchanger, phospholamban, calsequestrin and the ryanodine receptor was not altered [50,51,55]. The decreased level of L-type calcium channel expression was correlated with the shortening of the atrial effective refractory period [53] but was not influenced by atrial dilatation [51]. Previously, Van Wagoner et al. had shown that in AF patients with a reduced density of outward potassium current, the expression of the potassium channel subunit Kv1.5 was decreased [49]. This indicates that the current densities are influenced by alterations at the channel protein expression level, an observation that was also described in a dog model of chronic AF [56–58]. It appears that the cardiomyocytes use diverse and complex pathways to regulate channel expression, alter channel properties, and co-ordinate channel interactions during AF.

4.2. Gap junctional proteins

Changes in protein distribution and expression are also noted for gap junctional proteins (gap junctional remodelling). Persistent AF had been shown to affect connexin40 distribution in the goat model of AF [59]. A time course experiment in this model revealed that from 2 weeks onward, the heterogeneity in connexin40 distribution increased leading to a reduction in the levels of connexin40. The localisation of connexin43 remained unaltered but the changes in connexin40 distribution correlated with AF stability [60]. In contrast, rapid atrial pacing induced AF in dogs resulted in increased expression of connexin43 [61]. Unfortunately, in the latter study the connexin40 levels were not addressed. Patients with persistent AF also displayed heterogeneous distribution of connexin40 and, to a lesser extent, of connexin43 [62]. A recent clinical study indicated that the heterogeneous distribution of connexin40, as observed in goats during AF, is a common feature in humans irrespective of the presence of AF. Furthermore it was shown that patients susceptible to postoperative AF had elevated levels of connexin40 [63]. However, AF patients with complex atrial activation (fully remodelled atria) had lower levels of connexin40 compared to patients with sustained induced AF (no history of AF, non remodelled atria) [64]. All in all, there appear to exist some discrepancies concerning the gap junctional remodelling during AF. This might be due to interspecies differences, variations in experimental set-up in the case of animal models or differences in underlying heart disease whenever patients were involved.

4.3. Contractile and structural proteins

The expression of contractile and structural proteins has been extensively studied in the goat model of AF. Immunohistochemical staining was used to assess the response of proteins on AF and to establish the nature of cardiomyocyte remodelling. As expected, the myolytic cardiomyocytes in goats lost several of their structural proteins like myosin, tropomyosin, actin and α-actinin during AF. Only at the periphery of the cells, where the remaining sarcomeres resided, normal cross-striation patterns could be observed [65]. Within the myolytic areas the distribution of desmin appeared disorganised and other proteins adapted a foetal expression pattern, i.e. titin and cardiotin [65]. The fact that these changes occurred without signs of apoptosis [45] and were accompanied by re-expression of α-smooth muscle actin is indicative of cardiomyocyte dedifferentiation [65,66]. In line with the structural observations, the remodelling of structural proteins became apparent after 1–2 weeks of AF and progressively increased [66,67].

5. Mechanisms of remodelling

One of the main actors in the complex regulation of electrical and morphological remodelling appears to be calcium, a common messenger in signalling pathways [68]. Calcium overload has been shown to downregulate sodium channel expression [69], which could explain the reduced sodium current density in atrial myocytes from dogs with pacing induced AF [56]. Several authors have shown that the calcium antagonist verapamil was able to reduce the electrical remodelling [70,71] and attenuate the contractile dysfunction [8,72,73] during short-term AF. However, after a longer duration of AF, the protective effect of verapamil disappears [74]. At the onset of AF the high rate of atrial activation causes an excessive influx of calcium [73]. As a result, calcium overload occurs and atrial contractile function becomes depressed. The diminished contractile function will lead to increased passive stretch, which in turn promotes AF [75,76]. The consequence of
this course of events may be a vicious circle of AF induced stabilisation of AF via atrial stretch.

Recently, the first direct evidence of calcium overload during AF was described [77,78]. In the goat model, Ausma et al. found increased levels of sarcolemma bound and mitochondria bound calcium within the first 2 weeks of AF. After 4 weeks of AF the calcium level started to normalise and control values were reached at 16 weeks of AF [78]. Because this transient calcium overload coincided with the morphological changes, Ausma et al. suggested that morphological remodelling was the consequence of activation of proteolytic pathways by calcium overload [78]. Recent studies in patients with persistent and paroxysmal AF support this view. In these patients, Brundel et al. found increased levels of the calcium activated proteolytic protein calpain I, located in the nucleus, the cytoplasm and at the intercalated discs [42]. They also showed that induction of calpain activity correlated with shortening of the atrial effective refractory period, increased structural alterations in the atrial myocytes, and decreased protein levels of channel proteins like MinK, Kir3.1, Kv1.5, and the L-type calcium channel [42]. The effect on L-type calcium channel expression might explain the lack of L-type calcium channel blocker diltiazem to protect against atrial electrical remodelling after longer duration of AF [79]. Degradation of calcium channel proteins [80] and contractile/structural proteins by calpain has also been observed by others [81–84].

These data not only show that calcium overload does occur during AF but also that calcium is a likely candidate for induction and maintenance of protein degradation and for regulation of protein expression.

6. Conclusions

The atrial myocardium undergoes many changes during atrial fibrillation. One of these changes involves structural remodelling which is generally characterised by cardiomyocyte volume increase, loss of sarcomeres, accumulation of glycogen and mitochondrial abnormalities [10,13,32,33,35,37,41,85]. Many of these features had previously been interpreted as cellular degeneration. However, there appears to be a discrimination between degenerative and non-degenerative changes. In experimental models of lone AF, the absence of clear signs of cellular degeneration [10,36,45] and the phenotypic resemblance with foetal cardiomyocytes point towards cardiomyocyte dedifferentiation. However, in patients with AF, both cardiomyocyte dedifferentiation and degeneration occur. The observation that many of these features also occur during other cardiac diseases [10,37,46,48,65] suggests a uniform primary response mechanism of cardiomyocytes in jeopardy. The adaptive response occurs apart from necrosis and apoptosis or programmed cell death [37,45] and might actually be considered as programmed cell survival, as it appears to render the cardiomyocytes able to survive stress conditions including ischemia and stretch [86]. This would also explain the capacity of cells to regain function, although delayed, once environmental conditions have become normalised [7,8,13,20,47].

It is conceivable that the changes in distribution and expression of proteins involved in contraction, excitation, and conduction affect the electrical and contractile properties of the atrium during AF. The reduction of sarcomeric proteins is likely to affect the contractile properties of the atrium. Altered distribution and expression of channel proteins probably interferes with ion handling and excitation–contraction coupling. Likewise, alterations in gap junctions will influence the characteristics of atrial conduction. Calcium overload and stretch appear to be important regulating mechanisms of structural remodelling.

Whether the morphological remodelling caused by AF is completely reversible still needs to be established. Nevertheless, the concept that ‘AF begets AF’ [6], might not hold true when the morphological remodelling is extended.

References


