Mechanisms of atrial structural changes caused by stretch occurring before and during early atrial fibrillation

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Abstract
Structural remodelling occurring before, due to the underlying heart disease, and during atrial fibrillation (AF) sets the stage for permanent AF. Current therapy in AF aims to maintain sinus rhythm in symptomatic patients, but outcome is unfortunately poor. Stretch of the atria is a main contributor to atrial remodelling. In this review, we describe different aspects of structural remodelling as seen in animal models and in patients with AF, including atrial enlargement, cellular hypertrophy, dedifferentiation, fibrosis, apoptosis, and loss of contractile elements. In the second part, we describe downstream signals of mechanical stretch and their contribution to AF and structural remodelling. Ultimately, knowledge of mechanisms underlying structural remodelling may help to identify new pharmacological targets for AF prevention.

Keywords Atrial fibrillation • Atrium • Remodelling • Stretch

This article is part of the Review Focus on: New Insights into the Molecular Basis of Atrial Fibrillation

1. Introduction
Atrial fibrillation (AF) is the most common cardiac arrhythmia, having a prevalence of 1% in young patients up to 9% in patients at the age of 75 years.1 It is estimated that the prevalence will increase about three-fold by 2050.2 AF is not benign. It increases cardiovascular morbidity, including stroke and heart failure and doubles mortality.3 AF causes electrical and structural remodelling.4,5 Both are important contributors for initiation and persistence of AF. Electrical remodelling however is beyond the scope of this review, it has been extensively reviewed by others.6 In addition, the complex atrial architecture may also play a role in initiation and maintenance of AF.7 AF in relatively young patients without underlying heart disease (lone AF) is often induced by triggers in the pulmonary veins. AF caused by underlying diseases is thought to have a different mechanism. Risk factors for AF include hypertension, heart failure, and mitral valve disease,8 which all are complicated by haemodynamic overload of the ventricles and the atria. Pressure or volume overload of the atria causes elongation of the cardiomyocytes, i.e. increased stretch. AF promoting changes, atrial remodelling, occur in atria from patients with heart failure, hypertension, and mitral valve disease, before the first episode of AF.9–11 as such creating a substrate for AF. It is thought that once AF develops the remodelling process further deteriorates (Figure 1).4,12 Thus, atrial remodelling in patients with AF is caused by both the associated diseases and AF itself (Figure 2) and has been investigated in several experimental models5,13–28 and in patients with AF.9,29–36

Long-term maintenance of sinus rhythm in AF is difficult to achieve.37,38 The amount of remodelling importantly affects success of rhythm control therapy. To achieve pharmacological rhythm control in AF patients, drugs that target ion channels are first choice. These antiarrhythmic drugs do not affect the underlying structural remodelling process, i.e. they do not take away or reduce the AF substrate. Furthermore, these drugs can cause severe side effects, such as ventricular proarrhythmia, especially in patients with underlying heart disease. Early prophylactic treatment strategies that modulate structural remodelling may improve long-term maintenance of sinus rhythm and atrial function and possibly also reduce cardiovascular events.12,39,40 Our aim is to describe structural changes caused by stretch occurring before and early after start of AF and the mechanisms of these alterations in a search for improvement of pharmacological rhythm control therapy.

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2. Structural remodelling

Structural remodelling is seen as the main contributor for initiation and persistence of AF and might be present before start of AF caused by associated diseases.9–11 Atrial structural changes observed in animal models of AF with or without underlying diseases include (i) atrial enlargement, (ii) cellular hypertrophy, (iii) dedifferentiation, (iv) fibrosis, (v) apoptosis, and (vi) loss of contractile apparatus (myolysis), and changes in size and shape of the mitochondria, disruption of the sarcoplasmatic reticulum, and homogeneous distribution of nuclear heterochromatin (Table 1).5,13–20,26,28 The first structural changes occur after 1 week of AF and structural remodelling progresses over time.41 In atrial biopsies of patients with AF, the same alterations have been observed as described in animal models (Table 2).9,29–36 A schematic representation of structural remodelling is shown in Figure 3.

2.1 Atrial dilatation

Dilated atria have been observed frequently in different animal models.14,16–20 Schoonderwoerd et al.14 observed no atrial enlargement after rapid atrial pacing (240 b.p.m.) for 4 weeks with controlled ventricular rate (80 b.p.m.) in goats, in contrast to the goats that were rapidly paced in both the atria and ventricles. This suggests that not rapid pacing in the atria alone, but an additional high ventricular rate, resulting in congestive heart failure (CHF) and haemodynamic overload is an important determinant in atrial dilatation. In patients with AF, atrial dilatation is generally present,9,30–36 being related to both the severity of the underlying disease and the AF burden.29,36,42

2.2 Atrial cardiomyocyte hypertrophy

In the pacing-induced AF goat model, atrial cardiomyocytes are increased in size after 9–23 weeks of AF.5 Also, in models with rapid atrial pacing hypertrophy of atrial cardiomyocytes has been observed.15,16,18 Rapid atrial pacing in the presence of a controlled ventricular rate for 1 week did not result in hypertrophy,13 whereas pacing for a longer time period did.15 In different heart failure models13,19 and in AF patients hypertrophied atrial cardiomyocytes have been demonstrated.9,29,30,32,33

Figure 1  Hypothetical representation of how underlying diseases, such as hypertension and/or heart failure can induce atrial substrate remodelling long before onset of AF. When AF starts substrate remodelling deteriorates. Adapted with permission from Cosio et al.12 AF, atrial fibrillation; ECV, electrical cardioversion; SR, sinus rhythm.

Figure 2  Flow chart showing the series of events caused by stretch. Hypothetical scheme of stretch induced by hypertension, heart failure and possibly extreme endurance exercise leading to calcium overload, activation of the renin–angiotensin–aldosterone system (RAAS) and release of different factors, resulting in structural remodelling and finally in AF.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Model</th>
<th>n</th>
<th>Animal</th>
<th>Underlying disease, duration model</th>
<th>Enlarged atria</th>
<th>Hypertrophy</th>
<th>Fibrosis</th>
<th>Dedifferentiation</th>
<th>Cell death</th>
<th>Myolysis</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rapid atrial pacing, controlled ventricular rate</strong></td>
<td>Li et al. (1999)</td>
<td>13</td>
<td>Dog</td>
<td>None, 1 week</td>
<td>n.a.</td>
<td>–</td>
<td>–</td>
<td>n.a.</td>
<td>–</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>Schoonderwoerd et al. (2004)</td>
<td>14</td>
<td>Goat</td>
<td>None, 4 weeks</td>
<td>–</td>
<td>n.a.</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>Anne et al. (2007)</td>
<td>15</td>
<td>Sheep</td>
<td>None, 14 weeks</td>
<td>n.a.</td>
<td>+</td>
<td>–</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Rapid atrial pacing, uncontrolled ventricular rate</strong></td>
<td>Ausma et al. (1997)</td>
<td>17</td>
<td>Goat</td>
<td>None, 9–23 weeks</td>
<td>n.a.</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Morillo et al. (1995)</td>
<td>16</td>
<td>Dog</td>
<td>None, 6 weeks</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>Everett et al. (2000)</td>
<td>17</td>
<td>Dog</td>
<td>Mitrval regurgitation, &gt; 8 weeks</td>
<td>+</td>
<td>–</td>
<td>n.a.</td>
<td>+</td>
<td>n.a.</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Anne et al. (2007)</td>
<td>15</td>
<td>Sheep</td>
<td>Tachycardiomyopathy, 14 weeks</td>
<td>n.a.</td>
<td>+</td>
<td>+</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>Bauer et al. (2004)</td>
<td>18</td>
<td>Pig</td>
<td>CHF, 20 days</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>n.a.</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>Rapid atrial and ventricular pacing</strong></td>
<td>Schoonderwoerd et al. (2004)</td>
<td>14</td>
<td>Goat</td>
<td>CHF, 4 weeks</td>
<td>+</td>
<td>n.a.</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>Moe et al. (2008)</td>
<td>19</td>
<td>Dog</td>
<td>CHF, 2 weeks</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Rapid ventricular pacing</strong></td>
<td>Li et al. (1999)</td>
<td>13</td>
<td>Dog</td>
<td>CHF, 5 weeks</td>
<td>n.a.</td>
<td>+</td>
<td>+</td>
<td>n.a.</td>
<td>+</td>
<td>+</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>Shimano et al. (2008)</td>
<td>20</td>
<td>Rabbit</td>
<td>CHF, 4 weeks</td>
<td>+</td>
<td>n.a.</td>
<td>+</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Overload models and associated diseases</strong></td>
<td>Kistler et al. (2006)</td>
<td>21</td>
<td>Sheep</td>
<td>Hypertension, 4–5 years</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Lau et al. (2010)</td>
<td>22</td>
<td>Sheep</td>
<td>Hypertension, 5, 10 and 15 weeks</td>
<td>+</td>
<td>n.a.</td>
<td>+</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>Remes et al. (2008)</td>
<td>23</td>
<td>Goat</td>
<td>LA overload, 4–5 weeks</td>
<td>+</td>
<td>n.a.</td>
<td>–</td>
<td>n.a.</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
2.3 Dedifferentiation

Another adaptive mechanism contributing to the remodelling process involves a change in gene expression profile towards a more foetal phenotype, dedifferentiation. Genes that are expressed during development are re-expressed, including skeletal α-actin, β-myosin heavy chain (MHC), atrial natriuretic peptide (ANP), titin and desmin, and adult isoforms are downregulated. Re-expression of, for example, β-MHC might increase survival in the changed conditions by using energy more effectively. Re-expression of the foetal gene programme is a hallmark of ventricular hypertrophy and failure and has been extensively studied. In the AF goat model, both changes in quantity and localization of the above-mentioned proteins were observed. In biopsies from patients with AF, increased expression of β-MHC and re-expression of α-smooth muscle actin has been shown. Structural signs of dedifferentiation are myolysis, glycogen accumulation, mitochondrial changes, dispersion of chromatin, and loss of sarcoplasmic reticulum, and have been documented in AF models and models of associated diseases. Dedifferentiation, therefore, seems to be an adaptive mechanism for high-energy demanding processes in AF, in analogy to the well-studied situation in ventricular disease.

2.4 Fibrosis

Another hallmark of structural remodelling and an important substrate of AF is fibrosis, the formation of excessive extracellular matrix consisting of mainly fibroblasts and elastic and collagen fibres. Figure 4 provides an overview of factors involved in the accumulation of extracellular matrix. In the goat model of AF and in rapid atrial pacing models with controlled ventricular rate, no fibrosis could be shown. One study showed atrial fibrosis after 3 months of AF with controlled ventricular rate in dogs that was more pronounced with an uncontrolled ventricular rate. In models induced by rapid atrial pacing without controlled ventricular rate, and heart failure models fibrosis has been documented consistently. Therefore, a high ventricular rate resulting in increased atrial stretch seems to play a role in the development of more extensive fibrosis. In human AF, fibrosis has been found frequently.

2.5 Apoptosis

Apoptosis is a natural process of self-destruction, i.e. programmed cell death. Typical changes include cell shrinkage, loss of cell shape, membrane blebbing, protein fragmentation, degradation of the chromatin, and nuclear fragmentation. Caspases are important proteases involved in prosecuting apoptosis. Necrosis, in contrast, is not a well-regulated process in which cell death is caused by external factors. In AF-related models, cell death has been described, but it is not always clear whether apoptosis or necrosis is present. In vivo, tachypacing of atrial HL-1 cardiomyocytes increased caspase activity, indicating apoptosis. In animal models, no signs of cell death in the atria were observed in rapid atrial pacing models with controlled ventricular rate and in the AF goat model. In contrast, in rapid atrial pacing models with induction of CHF and CHF models, atrial cell death was observed. This implies that a high ventricular rate is an important factor for the occurrence of cell death in AF. In biopsies from patients with lone AF, degeneration of atrial myocytes and necrosis were found. Thiedemann and Ferrans observed accumulation of lysosomal degradation products suggesting necrosis.
### Table 2  Atrial structural remodelling in AF, data from human tissue biopsies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Underlying cardiac disease</th>
<th>n</th>
<th>Biopsy</th>
<th>Duration (months)</th>
<th>Enlarged atria</th>
<th>Hypertrophy</th>
<th>Fibrosis</th>
<th>Cell death</th>
<th>Myolysis</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frustaci et al. (1997)</td>
<td>Paroxysmal lone AF</td>
<td>Ctrl: 11, AF: 12</td>
<td>RAS</td>
<td>3–108</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Inflammation</td>
</tr>
<tr>
<td>Thiedemann and Ferrans (1977)</td>
<td>Valve disease</td>
<td>Ctrl: 4, AF: 10</td>
<td>LAW</td>
<td>6–396</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Anne et al. (2005)</td>
<td>Mitrail valve disease</td>
<td>Ctrl: 10, AF: 9</td>
<td>RAA,LAA</td>
<td>46.8 ± 15.6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>n.a.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Han et al. (2008)</td>
<td>Mitrail valve replacement</td>
<td>Ctrl: 17, AF: 15</td>
<td>RA</td>
<td>48.9 ± 42.1</td>
<td>+</td>
<td>n.a.</td>
<td>n.a.</td>
<td>+</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>Arré-Sempé et al. (1999)</td>
<td>Coronary artery disease, valve disease or congenital heart defects</td>
<td>Ctrl: 35, AF: 17</td>
<td>RAA</td>
<td>1–240</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Rucker-Martin et al. (2002)</td>
<td>Coronary artery disease, valve disease or congenital heart defects</td>
<td>Ctrl: 14, AF: 10</td>
<td>RAA</td>
<td>n.a.</td>
<td>+</td>
<td>n.a.</td>
<td>+</td>
<td>n.a.</td>
<td>+</td>
<td>Dedifferentiation</td>
</tr>
<tr>
<td>Ke et al. (2008)</td>
<td>Coronary artery disease, paroxysmal lone AF, mitral valve disease</td>
<td>Ctrl: 13, AF: 14</td>
<td>RAA, LAA</td>
<td>–</td>
<td>–</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Ke et al. (2008)</td>
<td>Coronary artery disease, persistent lone AF, mitral valve disease</td>
<td>Ctrl: 13, AF: 17</td>
<td>RAA, LAA</td>
<td>0.1–56</td>
<td>+</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

RAS, right atrial septum; RA, right atrium; RAA, right atrial appendage; RAFW, right atrial free wall; LAW, left atrial wall; LAA, left atrial appendage. +, increased compared with control; −, no changes compared with control; n.a., not available. Column others: +, increased compared with control; −, reduced compared with control; =, no changes compared with control. RECK, reversion-inducing cysteine-rich protein with Kazal motifs.
Myolysis is a term used to describe the loss of cellular myofibril structure. In animal models of rapid atrial pacing with controlled ventricular rate, no loss of myofilaments has been shown.13,14 In contrast, myolysis has been observed in atria of AF goats and animals with associated diseases.5,13,14,17,18 In patients with AF, also loss of contractile lysis has been observed in atria of AF goats and animals with associated diseases.5,13,14 In contrast, myolysis is a term used to describe the loss of cellular myofibril structure.

2.6 Myolysis

Myolysis is a term used to describe the loss of cellular myofibril structure. In animal models of rapid atrial pacing with controlled ventricular rate, no loss of myofilaments has been shown.13,14 In contrast, myolysis has been observed in atria of AF goats and animals with associated diseases.5,13,14,17,18 In patients with AF, also loss of contractile elements has been observed.9,29,30,33,34,36 In right atrial appendages from patients with persistent AF a 14% decrease in sarcomere content,51 and decreased levels of the contractile proteins troponin T, I, and C have been demonstrated.36 In paroxysmal lone AF myolysis has not been found consistently.29,36 Thus, myolysis seems to be a general characteristic of atrial structural remodelling, which is more consistently seen in models with moderate or severe CHF than models of lone AF with a controlled ventricular rate and in patients with more ‘severe’ AF.

2.7 Summary

Structural remodelling, including atrial dilatation, hypertrophy, fibrosis, dedifferentiation, apoptosis, and myolysis, are related to the severity of the underlying disease and duration of AF. In experimental models, an uncontrolled ventricular rate seems often essential to mimic the clinical situation of AF occurring in association with heart diseases. All points to the fact that longstanding atrial overload, i.e. stretch, sets the stage for atrial remodelling and AF.

3. Atrial myocardial stretch

Diseases associated with AF, including hypertension, heart failure and mitral valve disease,6 are often complicated by increased haemodynamic load of the atria, i.e. increased stretch. In animal models of underlying diseases, rapid atrial and/or ventricular pacing often results in heart failure, making it difficult to dissect effects of haemodynamic overload of the atria from other effects, including ventricular overload. Models to more specifically investigate the effects of atrial stretch are cell stretching in vivo on flexible membranes and ex vivo using isolated hearts with increased pressure or volume. In vivo models include transverse aorta constriction and models of mitral valve disease and hypertension.21–28

Increased atrial stretch results in increased vulnerability to atrial arrhythmias as shown ex vivo using isolated guinea-pig hearts subjected to increased atrial volumes,52 and rabbit hearts subjected to increased atrial pressures.53 Furthermore, in vivo, hypertension, mitral regurgitation and models of left atrial overload also caused increased AF vulnerability.21–25,27,54 Notably, no spontaneous AF occurred in these animals. This suggests the presence of an AF substrate in the absence of spontaneously occurring AF triggers. In cats with cardiomyopathy and dogs with mitral valve fibrosis spontaneous AF was seen, possibly caused by the longer duration of disease and more severe structural remodelling.26,28

In vitro stretch of ventricular cardiomyocytes has been associated with hypertrophy and apoptosis.45,55 In atrial cardiomyocytes, less data are available from in vitro experiments. Data suggest that stretch leads to hypertrophy, dedifferentiation and extracellular matrix remodelling, as well as to electrical remodelling.26–50 In vivo, an increased atrial size is generally seen upon stretch.21–28 Cellular hypertrophy is seen in most models.21,24,26,28 except in a model of mitral regurgitation with a relatively short duration of stretch.25 This suggests a role for the duration of overload in causing cellular hypertrophy. Fibrosis was not observed in shunt overload models of goat and sheep,21,24 but it was seen in models of hypertension, mitral regurgitation, mitral valve disease, and transverse aorta constriction.21,22,23–28 Cell death has only been described in the chronic ovine hypertension model after 4–5 years.21 Also dedifferentiation21,26,28 and myolysis21,24,26,28 have only been described in animals with longer disease duration. In human, structural changes related to stretch are difficult to investigate. Tissue biopsies are obtained from patients with underlying heart disease. In addition, studies in patients are hindered by the use of drugs like inhibitors.
of the renin–angiotensin–aldosterone system (RAAS). Furthermore, biopsies used are often from right atrial appendages. Whether these biopsies represent changes induced by the haemodynamic situation in the left atrium is unclear.

3.1 Summary

Atrial stretch increases atrial susceptibility to arrhythmias, probably by inducing atrial structural remodelling. Although data are limited, data suggest that atrial stretch is an important mediator for atrial remodelling, in addition to a high ventricular rate. Future research should focus on investigating direct effects of stretch on atrial structural remodelling, including time-course of structural remodelling caused by stretch, in both in vitro and in vivo models, and prevention of these effects by drugs, e.g. angiotensin-converting enzyme (ACE)-inhibitors.

4. Downstream signals of stretch

4.1 Calcium overload

In atria from AF patients changes in Ca\(^{2+}\) handling proteins, including reduced levels of the \(\alpha_1\)-subunit of L-type Ca\(^{2+}\)-channels (LTCCs), as well as a reduction in L-type calcium current (I\(_{\text{CaL}}\)) have been observed. Alterations in calcium handling in AF have extensively been reviewed elsewhere. Figure 5 provides a hypothetical overview of calcium handling in cardiomyocytes during AF. Stretch has been linked to changes in intracellular calcium handling. In vitro experiments show increased intracellular calcium levels upon stretch of ventricular cardiomyocytes, most likely mediated via LTCCs, stretch-activated channels (SAC) and ryanodine receptors (RyR). In addition, hypertrophy and apoptosis induced by stretch of ventricular cardiomyocytes were reduced by inhibition of LTCC, Na\(^+\)/H\(^+\)-exchanger and reverse mode of the Na\(^+\)/Ca\(^{2+}\)-exchanger (NCX) and LTCC and RyR, respectively. From atrial less data are available. In human atrial myocytes stretch reduced I\(_{\text{CaL}}\), which was inhibited by application of a Ca\(^{2+}\)-chelator. In vivo left atrial stretch also reduced I\(_{\text{CaL}}\) in atrial cardiomyocytes. In addition, a reduced I\(_{\text{CaL}}\) was found in cells isolated from dilated human atria. Targeting calcium overload using the calcium channel blocker verapamil improved atrial contractile function and reduced electrical remodelling in AF models and reduced electrical remodelling in an ex vivo stretch model. In contrast, treatments that increased intracellular calcium concentrations worsened contractile function, electrical remodelling and inducibility of AF. Treatment of spontaneously hypertensive rats with the dihydropyridine calcium channel blocker azelnidipine reduced atrial cardiomyocyte hypertrophy and fibrosis. These studies suggest involvement of calcium

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**Figure 5** A schematic representation of the calcium homeostasis and events that may take place due to calcium overload induced by AF. Depolarization of the cardiomyocyte leads to inflow of calcium into the cell via (i) L-type Ca\(^{2+}\)-channels, (ii) reverse mode Na\(^+\)/Ca\(^{2+}\)-exchanger, and (iii) T-type Ca\(^{2+}\)-channels. This induces calcium-induced-calcium release from the sarcoplasmic reticulum via ryanodine receptors (RyR) into the cytoplasm. Calcium, subsequently, binds to contractile elements and initiates contraction. In the diastole, calcium leaves the cytoplasm via sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA), which is regulated by phospholamban (Pln), and via the Na\(^+\)/Ca\(^{2+}\)-exchanger and plasma membrane Ca\(^{2+}\)-ATPase. Ions can also leave and enter the cell via stretch-activated channels (SAC). During AF calcium overload can contribute to an altered signal transduction. Activation of Ca\(^{2+}\)-dependent proteins such as calpain, calcineurin, and calcium/calmodulin-dependent protein kinase II (CaMKII) may be increased. Activation of calpain may result in degradation of muscle proteins (myolysis). Increased calcineurin activation activates NFAT (nuclear factor of activated T-cells) by dephosphorylation and CaMKII activates myocyte enhancer factor 2 (MEF2) signalling. Both lead to altered gene expression such as increased ANP and BNP expression and hypertrophy. Calsarcin, a stretch-sensitive protein localized to the Z-disk, is an inhibitor of calcineurin. The plasma membrane calcium-ATPase fine tunes diastolic calcium levels and inhibits calcineurin via direct binding.
overload in electrical and structural remodelling in the atria, leading to increased AF inducibility and vulnerability as well as contractile dysfunction. In contrast to the positive effects in animal experiments, verapamil alone could not prevent recurrences of AF in humans. In addition to the effects of calcium levels on contractility and AF inducibility, in particular diastolic calcium can contribute to signal transduction that itself can lead to further remodelling, which will be discussed in the following section.

4.1 Calcineurin

Evidence from a porcine model of AF and from right atrial appendages of persistent AF patients suggests activation of calcineurin during AF. In addition, calcineurin is implicated as a regulator of L_{Ca,1} in an in vitro model of atrial tachycardia. Calcineurin is a Ca^{2+-}calmodulin-activated protein phosphatase activating the transcription factor NFAT (nuclear factor of activated T-cells) by dephosphorylation and nuclear translocation. Calcineurin has been implicated as a major regulator of ventricular cardiac hypertrophy and may be involved in stress-sensing via the Z-disk protein calsarcin. In addition to its role in hypertrophy, calcineurin is also involved in calcium signalling, in particular diastolic calcium can contribute to signal transduction. In atrial myocytes static stretch induced calcineurin protein expression, calcineurin activity, myocytes enriched calcineurin interacting protein gene expression, and DNA binding activity of NFATc. In this model inhibition of calcineurin and NFAT reduced stretch-induced changes in matrix metalloproteinases (MMPs) and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs). In vivo experiments using ventricular cardiomyocytes suggest activation of the calcineurin/NFAT pathway upon stretch. Calcineurin inhibition prevented stretch-induced hypertrophy, suggesting involvement of calcineurin in structural remodelling. In atrial myocytes static stretch induced calcineurin protein expression, calcineurin activity, myocytes enriched calcineurin interacting protein gene expression, and DNA binding activity of NFATc. In this model inhibition of calcineurin and NFAT reduced stretch-induced changes in matrix metalloproteinases (MMPs) and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs). In vivo, Shiroshita-Takeshita et al. could not demonstrate any effect of calcineurin inhibition by cyclosporine A on atrial remodelling and AF progression in dogs subjected to rapid atrial pacing with controlled ventricular rate. Thus, uncertainty about the role of calcineurin in atrial remodelling remains. In vitro models of stretch, however, suggest involvement of calcineurin in atrial remodelling upon stretch, i.e. with structural heart disease.

Increased intracellular levels of Ca^{2+} may also affect calpain and calcium/calmodulin-dependent protein kinase II (CaMKII). Greiser et al. showed increased activated CaMKII expression in an AV-block-induced model of atrial dilatation. Otherwise, these proteins have not been linked to stretch, but studies suggest contribution to atrial structural remodelling and AF.

4.2 Activation of the renin–angiotensin–aldosterone system

Activation of local RAAS in AF patients is suggested by increased atrial mRNA expression of ACE and changes in the expression of angiotensin receptors. Activation of RAAS may be related to increased fibrosis. Stretch may also induce activation of RAAS. In vitro experiments using stretched ventricular cardiomyocytes showed increased mRNA expression of angiotensinogen and ACE. The angiotensin II (ATII) receptor blocker (ARB) losartan reduced, while application of All amplified the stretch response on brain natriuretic protein (BNP) gene promoter activity. In addition, mechanical stretch is capable of activating the AT1 receptor in the absence of All. In neonatal rat atrial cardiomyocytes losartan reduced all stretch-induced phenomena including changes in ion currents resulting in shortened action potential duration, hypertrophy, and changes in expression and activity of MMPs and TIMPs. These studies suggest involvement of localized tissue RAAS, and specifically the AT1 receptor, in the response to stretch-induced atrial structural remodelling. In heart failure models systemic inhibition of RAAS using ACE-inhibitors and ARBs showed reduced atrial structural remodelling and less AF. In patients, ACE-inhibitors and ARBs may be effective in the prevention of AF. A large randomized clinical trial, however, showed that the ARB, valsartan, was ineffective in reducing AF incidence.

4.3 Endothelin-1

Plasma endothelin-1 (ET-1) levels are increased in permanent AF and heart failure when compared with heart failure patients in sinus rhythm. In addition, in right atrial appendages of AF patients protein levels of the ET receptor type A and B (ETA and ETB) were reduced. ETs are widely expressed throughout the body. ET-1 is the predominant form and is produced by vascular endothelial cells, smooth muscle cells, fibroblasts, and cardiac myocytes and is a very potent vasoconstrictor. In vitro studies show release of ET-1 upon stretch in ventricular cardiomyocytes. In addition, stretching of ventricular myocytes increased mRNA expression of prepro ET-1 and ET-1, whereas gene expression of ET-converting enzyme-1b and ETA was decreased. Data from atrial cardiomyocytes are limited: ETA and ETB receptor blockers could not alter stretch-induced increase in MMP-2 activity. The effects of increased ET-1 levels caused by stretch may be associated with hypertrophy and fibroblast proliferation. Interestingly, atria and ventricles seem to respond differently to ET-1. Atrial fibroblasts proliferate more upon stimulation with ET-1 when compared with ventricular fibroblasts. Furthermore, in vivo the ETA/ETB receptor antagonistbosentan inhibited stretch-induced increase in BNP expression only in the atria and not in the ventricles. The above suggests a role for ET-1 in the remodelling process but the available data are yet scarce.

4.4 Natriuretic peptides

In AF patients, plasma levels of ANP increase immediately after start of AF, and decrease again after cardioversion. In patients with AF and heart failure plasma levels of ANP decline after longstanding AF, possibly due to depletion of the ANP stores. Concerning plasma levels of BNP in AF conflicting data exists. Furthermore, increased expression of pro-BNP and decreased expression of the natriuretic peptide receptor A (NPRA) have been found in right atrial appendages of patients with persistent AF. ANP and BNP are endogenous hormones that activate the NPRA resulting in natriuresis, vasorelaxation, inhibition of aldosterone and renin, and anti-fibrotic, anti-hypertrophic, anti-apoptotic, and anti-inflammatory effects. ANP and BNP are released upon stretch and mRNA expression is increased, as shown in in vitro experiments using stretched ventricular cardiomyocytes. Stretch of atrial cardiomyocytes leads to increased mRNA expression and release of ANP and BNP are increased in both heart failure and AF, suggesting that this compensatory mechanism may not be sufficient to stop the atrial remodelling process.
4.5 Inflammation and oxidative stress

Inflammation has also been linked to AF. Inflammatory infiltrates have been shown in atrial biopsies from lone AF patients. Furthermore, serum levels of inflammatory markers are increased; both in patients with lone AF, as well as in AF with underlying diseases. A recent study showed accumulation of myeloperoxidase (MPO), a component of neutrophils, in atria from AF patients. Furthermore, MPO infusion in MPO-deficient mice induced fibrosis and increased AF vulnerability, suggesting MPO as a major mediator in atrial fibrosis. Several observations suggest a link between stretch and inflammation. In atrial tissue of two different hypertension models in sheep, inflammation was observed in one, whereas it was absent in another. Atrial tissue of two different hypertension models in sheep, inflammation was observed in one,22 whereas it was absent in another.21 Whether inflammation in this model causes atrial fibrosis warrants further investigation. A causal link between inflammation and atrial remodelling has been suggested by studies using prednisone or statins to inhibit atrial remodelling induced by tachypacing.83,109,110 In addition, in a pressure overload model a role of mast cells in causing atrial fibrosis and increasing AF inducibility was shown.77

Also oxidative stress, the presence of an increased amount of oxidants, reactive oxygen and nitrogen reactive species, seems to play a role in AF.31,111 Furthermore, oxidative stress is linked to atrial structural remodelling. In mice, overexpression of Rac1, a regulator of NAPDH oxidase activity and as such resulting in the generation of reactive oxygen species, resulted in atrial remodelling including atrial fibrosis and cellular hypertrophy.112 A link with stretch is provided in ventricles. In vitro, stretch of ventricular cardiomyocytes resulted in increased superoxide anion production.55 Furthermore, reactive oxygen species scavengers reduced stretch-induced hypertrophy and apoptosis. Concerning atrial stretch, increased serum oxidative stress was reported in patients with mitral regurgitation.113 An association between oxidative stress and atrial remodelling is provided by animal studies using antioxidant drugs. Probucol and simvastatin reduced atrial remodelling in tachypacing models.109,110,114 Treatment with vitamin C and E had no effect on atrial remodelling.110

Thus, there might be a link between stretch and inflammation and oxidative stress. This might be linked to atrial remodelling as suggested from ventricular tissue and studies using anti-inflammatory drugs and antioxidants.

4.6 Matrix metalloproteinases and tissue inhibitors of metalloproteinases

The formation of fibrosis is linked to the release of MMPs and its endogenous inhibitors, TIMPs. MMPs are secreted from fibroblasts, smooth muscle cells, endothelial cells, and myocytes.115 In AF patients, changes in the expression of MMPs and TIMPs have been reported.35,116 Interestingly, also changes in serum levels of markers of collagen turnover have been documented in AF patients.117,118 An association between MMPs, TIMPs and stretch has been suggested. Stretch of ventricular cardiomyocytes resulted in increased expression and activity of MMP-9 and MMP-14, without changes in TIMP-2.119 Stretch of atrial cardiomyocytes increased MMP-2 and MMP-9 protein expression and activity.58 Stretch also increased TIMP-1 expression, but the amount of MMP-2/MMP-9 bound to TIMP-1 was reduced, suggesting increased MMP activation. In an ovine AF model, changes in MMP-2 and TIMP-2 seem to depend on a high ventricular rate.15 Furthermore, in patients with mitral valve disease, MMP-1 protein expression and MMP-9 activity were decreased in left atrial samples compared with biopsies from patients undergoing coronary artery bypass surgery, without a difference between mitral valve disease with and without AF, suggesting changes in MMPs upon increased atrial stretch. These in vitro, in vivo, and patient data suggest an association between MMPs and TIMPs and stretch.

4.7 Transforming growth factor β1

Transforming growth factor β1 (TGFβ1) is a pro-fibrotic cytokine, which is in the heart mainly produced by fibroblasts and macrophages.120 TGFβ1 functions through TGFβ-receptor type I and type II on (myo)fibroblasts, phosphorylating Smad protein transcription factors leading to increased extracellular matrix production. In patients with permanent AF increased atrial protein levels of TGFβ1 and its signal transduction proteins have been documented compared with patients in sinus rhythm, though not in all studies.121 Data on stretch and TGFβ1 activation are limited, only one study showed release of TGFβ1 into the culture medium upon stretch of ventricular cardiomyocytes.122 Interestingly, TGFβ1 has been linked to atrial remodelling, this was shown by cardiac-specific overexpression of constitutively active TGFβ1 in mice.123 These mice showed increased atrial fibrosis and increased AF inducibility. This suggests that TGFβ1 can be a mediator in structural remodelling in AF.

4.8 Heat shock proteins

Heat shock proteins (HSPs) are stress-induced chaperones that function in protein folding and degradation. Increased expression of HSPs has a protective effect in the heart.124 In AF, increased protein levels of inducible HSP70 have been found in atria from AF goats after 16 weeks of AF, returning back to normal values after 8 weeks of sinus rhythm.125 In AF patients, increased expression in right atrial appendages of HSP27 and HSP70 have been documented126,127 as well as changes in HSP60 expression.126,128 Induction of HSPs reduced AF vulnerability and maintenance in an atrial tachypacing model129. Furthermore, AF duration induced by burst pacing in a model of acute atrial ischaemia was reduced by induction of HSPs.130 Reduced export of HSP70 mRNA to the cytoplasm due to deficiency of NUP155, an essential nucleoporin gene, was associated with AF,131 also providing evidence that induction of HSPs may have protective effects in AF. Stretch has been shown to increase ventricular expression of HSP70 upon pressure overload but data on atrial stretch are yet not available.132

4.9 Summary

Downstream signals of stretch include calcium overload, activation of RAAS, release of ET-1, natriuretic peptides, MMPs andTIMPs, TGFβ1, and HSPs. Evidence for an association between stretch ET-1, TGFβ1 and HSPs is only available from stretch of ventricular cells. Care has to be taken that results from ventricles are not directly translated to atria as the response from atrial and ventricular tissue is not always the same.99,133 More research is needed to firmly establish a link between atrial stretch and these possible downstream signals. Furthermore, expression differences of for instance AT1 receptor, ETA,
Funding has received more and more attention. Clinical evidence, however, is substrate in order to prevent or reduce atrial remodelling and thus AF remodelling and putative therapeutics. Upstream therapy targeting the previously unknown targets serving as biomarkers of atrial structural far most research on possible mechanisms has focused on putative therapeutics. 

5. Conclusions

Atrial structural remodelling is the main contributor for initiation and persistence of AF. Structural remodelling that is consistently seen in models of AF and in patients with AF includes atrial enlargement, cellular hypertrophy, dedifferentiation, fibrosis, apoptosis, and myolysis. Stretch is thought to be an important mediator in activating various signals, leading to structural remodelling, before and during AF. So far most research on possible mechanisms has focused on putative candidates. Future research will most likely add proteomics to identify previously unknown targets serving as biomarkers of atrial structural remodelling and putative therapeutics. Upstream therapy targeting the substrate in order to prevent or reduce atrial remodelling and thus AF has received more and more attention. Clinical evidence, however, is still lacking. Identifying new targets will allow animal models to test putative treatments and to define optimal timing. Elucidating the mechanisms and signals involved in atrial structural remodelling will ultimately improve therapy, i.e. (primary) prevention of AF and consequently reduce in cardiovascular morbidity and mortality.

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References


3. Camm AJ, Kirchhof P, Lip GY, cell stretching, data from animal overload models and targeting structural remodelling by inhibiting these downstream signals. These signals activated by atrial stretch and leading to atrial structural remodelling may be good targets for upstream therapy. However, future research is warranted in stretch specific models, i.e. using cell stretching, or animal models such as pressure overload, without underlying heart failure, to confirm a link between atrial stretch, its downstream signals and structural remodelling. Information on timing of these effects is also needed to specifically target signals that occur first. Using animal models the time-course of remodelling can be investigated, including the timing of therapy. This to investigate up to which time-point upstream therapy can still reverse remodelling and when upstream therapy can only stop further deterioration of remodelling. In addition, the involvement of the described downstream signals of stretch should be more in debt investigated using specific inhibitors.


Structural changes caused by atrial stretch


