Atrial remodeling due to atrial tachycardia and heart failure
Schoonderwoerd, Bas Arjan

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Chapter 5

Atrial Ultrastructural Changes During Experimental Atrial Tachycardia Depend on the High Ventricular Rate

Bas A. Schoonderwoerd, Jannie Ausma (*), Harry J.G.M. Crijns (*), Dirk J. van Veldhuisen, Engbert H. Blaauw (#) and Isabelle C. van Gelder

From the Department of Cardiology, Thoraxcenter, University Hospital Groningen, Cardiovascular Research Institute Maastricht (*) and the Department of Cell Biology and Electron Microscopy, University of Groningen (#), the Netherlands

Submitted
ABSTRACT

Background Atrial structural and electrophysiologic changes occur during atrial tachycardia. The role of the high ventricular rate in these processes remains yet to be established.

Methods and Results Six goats were subjected to 4 weeks of rapid atrioventricular (AV) pacing with an atrial and ventricular rate of 240 bpm resulting in the development of congestive heart failure (CHF). In another 5 goats, AV block was created after which they were subjected to 4 weeks of atrial pacing, also at 240 bpm while the ventricular rate was kept low and regular at 80 bpm (A-paced). Pacing was only interrupted for measurement of atrial effective refractory periods (AERP) and right atrial diameter (RAD). The ultrastructure of both atria was examined by light- and electron microscopy, including quantification of the percentage of atrial extracellular matrix (%ECM). A group of 6 goats served as controls.

In the AV-paced group severe structural remodeling occurred in the atria, including severe loss of sarcomeres, glycogen accumulation, disruption of sarcoplasmic reticulum, the appearance of numerous small mitochondria and nuclei with homogeneously distributed chromatin. In contrast, in the atria of A-paced goats structural changes were virtually absent. Only a redistribution of nuclear chromatin and the appearance of numerous mitochondria was observed. The ultrastructure was normal in control animals. Furthermore, %ECM was increased in AV-paced goats (29%) when compared to A-paced animals (18%) and controls (17%) (p<0.05). Finally, RAD increased by 51% in AV-paced goats while in A-paced goats RAD was unchanged (p<0.05). In both experimental groups AERP shortened during pacing.

Conclusions Structural remodeling during chronic atrial tachycardia is related to the concomitant presence of a high ventricular rate and hence the occurrence of CHF rather than the high atrial rate. Furthermore, electrical remodeling can occur in the absence of significant structural changes.
INTRODUCTION

Atrial fibrillation (AF) is the most common arrhythmia. From clinical practice it is known that the arrhythmia has a progressive character, a process which seems at least in part to be independent of progression of underlying heart disease. In patients with paroxysmal AF, duration and frequency of episodes tend to increase in time and finally the arrhythmia usually becomes chronic (“permanent AF”). AF induces changes in atrial electrophysiology and structure that possibly play a role in this phenomenon, including shortening of the atrial refractory period \(^1\) and dilatation of the atria. \(^2\) Furthermore, experimental \(^3\)-\(^6\) as well as clinical \(^7\)-\(^9\) studies have shown that AF leads to predominant histopathological changes in the atria, including dedifferentiated and degenerative changes in the myocytes themselves and their surrounding extracellular matrix. The cellular changes include a variety of alterations in the myocytes with as main features the loss of contractile material and the accumulation of glycogen. Besides these cellular changes, changes in the surrounding extracellular matrix occur. Atrial fibrosis has been described in experimental models \(^10\) as well as in patients. \(^9\) These changes may form a substrate for AF and thus may play a key role in the perpetuating character of this arrhythmia.

The mechanisms underlying these structural changes, however, have not been elucidated. On one hand, the increased atrial rate may be an important factor. On the other hand, since AF usually leads to a rapid ventricular response with possible development of congestive heart failure (CHF), this so-called tachycardiomyopathy may be contributive. CHF, also in the absence of AF, causes changes in atrial structure comparable to those found in AF. \(^7\),\(^11\),\(^12\) Recently, we have shown that atrial dilatation during atrial tachycardia only occurs in the presence of a rapid ventricular rate. \(^13\) The aim of the present study is to evaluate the role of a concurrent high ventricular rate, resulting in CHF, during atrial tachycardia in the development of atrial (ultra)structural remodeling.

METHODS

Our methods have been previously described. \(^13\) In short, we used a total of 16 female goats. Three experimental groups were studied. Five uninstrumented animals served as controls (control group). We instrumented the other 11 goats. Four custom made felt electrode arrays were sutured on the right and left atrial appendage and the right and left ventricular lateral wall. Two pairs of piezoelectric transducers were placed on the right atrium (RA) and the left ventricle (LV), respectively, for measurement of RA and LV diameters. Additionally, in five goats, a DDDR pacemaker in VDD mode was
implanted with epicardial pacemaker leads on the RA and right ventricle (RV) after which total AV block was created by radiofrequency catheter ablation via the external jugular vein.

Experimental protocol
RA and LV diameters were measured simultaneously during sinus rhythm over a period of 10 seconds. AERP was measured from one pair of electrodes on each atrium at three different basic cycle lengths (BCL) of 400, 300 and 200 ms.

Pacing protocol
After a baseline study, the instrumented goats were subjected to AV pacing during 4 weeks. Pacing was performed at the RA and the RV. These 11 goats were divided in two groups. The five goats with AV block (A-paced group) were subjected to 3:1 AV pacing with a rapid atrial pacing cycle length of 250 ms (240 bpm) and a ventricular pacing cycle length of 750 ms (80 bpm), which resembles the physiological heart rate of a goat during sinus rhythm. The other six animals (AV-paced group) were subjected to rapid 1:1 AV pacing with an atrial and ventricular pacing cycle length of 250 ms (240 bpm). In both groups the AV delay was 100 ms.

Pacing was only interrupted for measurement of the RA and LV diameters and AERPs at t=4, 8, 12, 24, 30, 36, 48, 60 hours and 3, 7, 10, 14, 17, 21, 24, 28 days (4 weeks). Continuous capture during pacing was confirmed by randomly performed 24-hour Holter registrations.

Tissue processing
At the end of the experimental period, the goats where anaesthetized and thoracotomy was performed. The heart was quickly removed from the thorax and tissue samples from both atria where immediately fixed for at least 24 hours in 3% glutaraldehyde in 90 mM KH$_2$PO$_4$ buffer (pH =7.4) and then washed with KH$_2$PO$_4$ buffer containing sucrose, postfixed with OsO$_4$ in 50 mM veronal acetate buffer for 1 hour, dehydrated through graded ethanol series and routinely embedded in epoxy resin Epon.$^{14}$ For electron microscopy, ultrathin sections (60 nm) cut from each Epon sample were counterstained with uranium acetate and lead citrate. The sections were examined with a Philips 201 electron microscope operating at 60 kV. For light microscopy, sections were cut from the Epon blocks. These sections were stained with periodic acid Shiff (PAS) and 0.1% toluidine blue according to the method preciously described.$^{3}$

Light microscopic quantification of changes in extracellular matrix
To assess the amount of connective tissue in the myocardium, morphometry was carried out with the aid of a grid of vertical and horizontal lines providing 494 (26 x 19)
intersections (points). In accordance with the principles of morphometry, counting of the number of points overlying a certain structure results in quantitative determination of the surface of the structure under investigation in relation to the surface of the entire tissue under the square grid. The total number of points was defined as 100%, and the points counted in the extracellular matrix (ECM) were expressed as a percentage of the entire tissue within the limits of the grid. The same procedure was repeated on different areas of the same section. Blood vessels, perivascular interstitial cells and fat tissue were excluded from the connective tissue quantification. Morphometry was carried out by one investigator (B.A.S.) in a blinded and random fashion.

Statistical analysis
Data are presented as mean ± standard deviation. Kruskal-Wallis test and Wilcoxon rank-sum test (two-tailed) were used to evaluate the differences between the ECM values in AV-pacing, A-pacing and the control group. Initially Kruskal-Wallis tests were performed to explore differences between the three groups. In addition, subgroup analysis using Wilcoxon rank-sum test was performed to further explore differences in sets of two groups separately. A p-value of < 0.05 was considered statistically significant. The p-values resulting from the hypothesis testing should be interpreted as explorative in nature, rather than indicating statistical significance. The analyses were performed using commercially available computer software (Statistical Analysis System version 6.12, SAS Institute, Cary, NC).

RESULTS

Light microscopy
Atrial myocardium from the control goats (sinus rhythm) contained regularly organized bundles of myocytes surrounded by a regular amount of perimysial connective tissue and individual myocytes that were surrounded by a relatively small amount of endomysial connective tissue (Figure 1 panel A). The sarcomeres within the myocytes were visible by extensive staining with toluidine blue whereas PAS positive glycogen staining was virtually absent.

However, in the AV-paced goats, the myocyte bundles were less organized and showed separation of myocytes by an increased amount of connective tissue. Especially the amount of endomysial connective tissue surrounding the myocytes was elevated (Figure 1, panel B). Within the individual myocytes loss of sarcomeric material (myofilaments) was prominent. In these myolytic areas accumulation of glycogen was observed (PAS positive material), an array of changes previously described as the hallmarks of dedifferentiation.15
In addition, changes of a degenerative nature were observed in a small number of atrial myocytes such as cytoplasmic vacuolization and the presence of dark nuclei. Loss of myocytes might be a contribution to the relatively increased amount of connective tissue.

In contrast to these marked changes in AV-paced goats, there were no abnormalities seen in the A-paced goats. (Figure 1, panel C) Both extracellular matrix and the myocytes resembled those seen in the control goats.

Electron microscopy
In the control goats (sinus rhythm) atrial myocytes had a highly organized structure, in longitudinal sectioning visible as cross-striated rods of myofilaments with rows of mitochondria in between. The nuclei displayed a normal distribution of heterochromatin clustering along the nuclear membrane. (Figures 2, 3 and 4, panel A)

In contrast atrial myocytes from AV-paced goats showed a high degree of disorganization. These included severe loss of sarcomeres, glycogen accumulation, disruption of sarcoplasmic reticulum, the appearance of numerous small mitochondria and nuclei with homogeneously distributed chromatin (Figures 2, 3 and 4, panel B). In addition, changes of a degenerative nature were observed in a small number of these myocytes such as cytoplasmic vacuolization, lysosomal structures and clumping of nuclear chromatin.
Figure 2. Electron microscopy of atrial myocardium in overview. Magnification: X 1850. (A) Atrial myocardium of a control goat. Regularly organized sarcomeres with clear cross striations and normal mitochondria are present within the unaffected atrial myocytes. N=nucleus, S=sarcomeres. (B) Atrial myocardium from an AV-paced goat showing severe myolysis, accumulation of glycogen (G) and remnants of sarcomeres (S). N=nucleus. (C) Atrial myocardium from an A-paced goat showing normal organization of sarcomeres and an increased amount of mitochondria (M). The nucleus (N) shows a homogenous distribution of chromatin.

Figure 3. Electron microscopy of mitochondria. Magnification: X 7400. (A) Detail of atrial myocardium from a control goat showing normal amount of mitochondria (M) located between the sarcomeres (S). Mitochondria are of normal size. (B) Atrial mitochondria from an AV-paced goat. Mitochondria (M) of variable sizes are present, especially small mitochondria (m) appear as a result of the AV-paced induced dedifferentiation. (C) Atrial-paced myocardium showing an increased amount of mitochondria (M) which are of normal shape and size.
In A-paced goats only mild ultrastructural changes were seen when compared to control animals. The contractile apparatus remained intact as could be concluded from the presence of intact myocytes containing organized myofilaments visible as cross-striated myofilaments in longitudinal sections. The most eye catching alteration was the presence of a huge number of mitochondria between the myofilaments. Furthermore, a homogenous distribution of nuclear chromatin was observed which was comparable to the AV-paced situation. Clumping of chromatin or degenerative changes were absent. (Figures 2, 3 and 4, panel C)

Table 1. Mean Percentage of Extracellular Matrix

<table>
<thead>
<tr>
<th></th>
<th>RA ECM(%)</th>
<th>LA ECM (%)</th>
<th>Total (RA+LA) ECM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>21.0±16.9</td>
<td>15.6±2.9</td>
<td>17.4±8.4</td>
</tr>
<tr>
<td>AV-pace</td>
<td>29.0±8.8</td>
<td>29.1±1.9 *</td>
<td>29.1±5.8 *</td>
</tr>
<tr>
<td>A-pace</td>
<td>21.0±7.9</td>
<td>15.9±6.3</td>
<td>18.4±7.3</td>
</tr>
</tbody>
</table>

*p<0.05 when compared to A-pace or Controls.

Figure 4. Electron microscopy of nuclei. Magnification: X 13500. (A) Detail of part of a nucleus from an atrial myocyte of a control goat showing clear clumping of nuclear chromatin along the nuclear membrane (arrows). (B) Detail of a part of a nucleus of an AV-paced goat showing homogenous distribution of nuclear chromatin. Arrow indicates the nucleolus. (C) Detail of a part of a nucleus of an A-paced goat showing a homogenous distribution of nuclear chromatin. Clumping of chromatin along the nuclear membrane is absent. Arrowhead indicates part of the nucleolus.
Quantification of the amount of extracellular matrix
In Table 1, the mean amount of ECM in the three groups expressed as a percentage of total amount of myocardial tissue is given. In control goats the mean amount of ECM in the right and left atria was 21.0% and 15.6%, respectively. This was comparable to the quantity of ECM in the A-paced goats (21.0% and 15.9% respectively, p=NS for both). However, in the AV-paced goats there was an increase of ECM with the presence of fibrosis in both the left and right atrium. The percentage of atrial ECM in the AV-paced groups was 29.0% (p=NS) and 29.1% (p<0.05 when compared to controls and A-paced goats).

Changes in atrial diameter
The time course of changes in right atrial diameter in both experimental groups have been described previously. After four weeks of pacing, mean right atrial diameter reached 151% of baseline in the AV-paced animals. In contrast, in the A-paced goats right atrial diameter was unchanged after four weeks.

Atrial electrical remodeling
The time course of left and right atrial effective refractory periods (AERP) in both experimental groups have been described previously. At baseline, right AERP at a BCL of 400 ms were 144±19 ms and 157±10 ms in the AV-paced and A-paced animals, respectively. In both groups, AERP shortened rapidly after initiation of pacing and reached values of 102±12 ms and 79±7 ms after 4 weeks of pacing, respectively (p<0.05 vs. baseline for both groups). Within each group the time course of right AERP at other BCL and of left AERP were similar.

DISCUSSION

Main results
We evaluated the influence of a high ventricular rate and the consequent development of heart failure on atrial (ultra)structural remodeling during chronic experimental atrial tachycardia.

In the absence of a high ventricular rate, a chronic high atrial rate does not result in predominant changes in atrial (ultra)structure. Only minor alterations such as an increased number of mitochondria and a homogeneous distribution of nuclear chromatin were observed. In contrast, a high atrial rate in combination with a high ventricular rate induces severe damage to the atrial ECM as well to the individual atrial myocytes. These include a variety of cellular structural changes with as main features the loss of contractile material and sarcoplasmic reticulum, the accumulation of glycogen and chan-
ges in shape and size of the mitochondria. This was accompanied by an increase of perimysial and endomysial connective tissue, including the development of severe fibrosis. These changes are compatible to those found in patients with persistent AF.16

Furthermore, we demonstrate that atrial electrical remodeling due to high atrial rates, characterized by shortening of AERP is not necessarily accompanied by (ultra)structural changes and/or atrial dilatation. This indicates that these processes must, at least in part, have different underlying mechanisms which, however, have not yet been elucidated.

Several mechanisms may be responsible for the observed (ultra)structural changes in the AV-paced goats. First, a rise in the ventricular rate results in a rise in atrial pressure and thus in an increased stretch of the atrial wall which in time leads to atrial dilatation as we observed in the AV-paced animals.13 Previously, it was shown that changes in atrial ultrastructure during chronic AF are most prominent in those areas where the atrial wall is relatively thin i.e. most vulnerable to stretch.14,17 Furthermore, other conditions which are associated with elevated atrial pressures such as heart failure,11,12,17 atrial septal defect18 or mitral valve insufficiency19 are, also in the absence of AF, associated with changes in atrial architecture and atrial myocytes such as found in the present study.

Second, immediately after the onset of AF the energy demand of the atria increases due to the high rate of atrial activation, resulting in an immediate increase in atrial coronary blood flow.20 In contrast, when the arrhythmia persists for months atrial perfusion has been shown to be reduced when compared to sinus rhythm.21 It is unknown, however, whether this reduced perfusion is a cause of atrial structural and functional deterioration or a consequence of a decreased atrial demand due to hibernation (i.e. adaptation of the atrial cells to the energy delivery/demand mismatch). Also, in patients with chronic underperfusion of the left ventricle i.e. chronic hibernating myocardium is associated with decreased ventricular contractility.22

In our model, the high atrial rate will increase the energy demand of the atria. However, in the AV-paced animals this does not lead to dedifferentiation or degeneration. In the absence of a high ventricular rate, the coronary flow reserve in combination with the observed increase in atrial mitochondria may be able to cope with this increased demand. Only if high atrial pressures and eventually atrial dilatation, as were present in the AV-paced goats, ensues due to the unfavorable hemodynamics of the high ventricular rate, the atrial rate becomes temporary (i.e. during the development of the dedifferentiation) too high. This turns on cell survival programs leading to the picture of dedifferentiation in the atria of AV-paced goats. Later on energy consumption may decrease again, accompanied by a decrease in coronary perfusion (perfusion metabolism feedback) without the opportunity for the atrial cells to escape that situation and built up again normal architecture since that would again lead to a mismatch of energy requirements and energy delivery.

Third, at the onset of AF, the high atrial rate itself induces an increased influx of
calcium.\textsuperscript{23} As a consequence of this increased calcium influx atrial contractile function diminishes within the first three days, thereby enhancing the increased stretch on the atrial wall.\textsuperscript{24} Concomitant ischemia during AF, when blood flow supply and demand do not match, might also induce Ca\textsuperscript{2+}-overload.\textsuperscript{25} Also in this situation calcium-overload might further depress the contractile function and promote atrial stretch. This atrial stretch might in turn further enhance the Ca\textsuperscript{2+}-influx, leading to a cascade of reactions, since Ca\textsuperscript{2+}-overload was hypothesized to induce proteolytic activity by calpain activation leading to myolysis and structural remodeling.\textsuperscript{26} Therefore, calcium induces and maintains protein degradation and changes in regulation of protein expression during long-term AF. In the present study, an isolated high atrial rate did not lead to structural changes. Somehow, the rate dependent rise in atrial intracellular calcium apparently is not potent to cause structural changes. Of note, however, in both groups atrial electrical remodeling occurred,\textsuperscript{13} a process also attributed to intracellular calcium overload,\textsuperscript{27} but occurring within several hours to days after onset of the high atrial rate. Clearly, structural changes are either not only caused by calcium-overload or depend on more and/or severe exposition.

Finally, it is well known that the development of tachycardia induced CHF is associated with activation of the renin-angiotensin system (RAS)\textsuperscript{28} resulting in increased levels of renin and angiotensin II. The latter neurohormone has been proposed to be responsible for the development of cardiac fibrosis. Previously, Li et al. demonstrated inhibition of the development of fibrosis by administration of the angiotensin converting enzyme inhibitor Enalapril during the induction of pacing induced CHF in dogs.\textsuperscript{29} We have shown that in our AV-paced goats, the levels of circulating renin progressively increased while this neurohormone remained unchanged in the A-paced animals.\textsuperscript{30} This may explain the development of extensive atrial fibrosis and possibly cellular structural remodeling in the AV-paced goats while no changes in atrial ECM was observed in the A-paced group.

Clinical relevance
Since the above mentioned changes have been previously shown to be largely irreversible,\textsuperscript{6,10,31} rate control during AF is essential to prevent structural damage to not only the ventricles but also to the atria. This may also inhibit progression of structural changes favoring thrombo-embolic complications and the domestication of AF in patients in which the arrhythmia is paroxysmal. In this respect, however, it remains unclear which level of rate control should be targeted.

Limitations
The duration of the present experiment was four weeks while in the clinical situation the arrhythmia may be present for many months. Nevertheless, four weeks of rapid atrioventricular pacing was sufficient to induce severe structural abnormalities.
ACKNOWLEDGEMENTS

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