Aldosterone promotes atrial fibrillation

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Aims

Hyperaldosteronism is associated with an increased prevalence of atrial fibrillation (AF). However, it is unclear whether this is the consequence of altered haemodynamics or a direct aldosterone effect. It was the aim of the study to demonstrate load-independent effects of aldosterone on atrial structure and electrophysiology.

Methods

Osmotic mini-pumps delivering 1.5 μg/h aldosterone were implanted subcutaneously in rats (Aldo). Rats without aldosterone treatment served as controls. After 8 weeks, surface electrocardiogram, the inducibility of AF, and atrial pressures were recorded in vivo. In isolated working hearts, left ventricular function was measured, and conduction in the right atrium (RA) and the left atrium (LA) was mapped epicardially. The atrial effective refractory period (AERP) was determined. Atrial tissue was analysed histologically.

Results

Neither systolic nor diastolic ventricular function nor atrial pressures were altered in Aldo rats. All Aldo (11/11) showed inducible atrial arrhythmias vs. two of nine controls (P = 0.03). In Aldo, the P-wave duration and the total RA activation time were longer. Prolongation of local conduction times occurred more often in Aldo, whereas the AERP did not differ between both groups. In Aldo, atrial fibroblasts and interstitial collagen were increased, active matrix metalloproteinase 13 was reduced, and atrial myocytes were hypertrophied. The connexin 43 content was unaltered.

Conclusions

Aldosterone causes a substrate for atrial arrhythmias characterized by atrial fibrosis, myocyte hypertrophy, and conduction disturbances. The described model imparts atrial proarrhythmia directly to aldosterone, since ventricular haemodynamics appeared unaltered in this model. This mechanism may have therapeutic impact for primary and secondary prevention of AF.

Keywords

Aldosterone • Atrial fibrillation • Structural remodelling • Atrial conduction disturbances • Ventricular function

Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia with a prevalence that is projected to increase in the future.1 Apart from triggers, AF development and perpetuation depend on electrical and structural remodelling of the atria.2,3 Most patients with AF suffer from structural heart disease. Under these conditions, the renin–angiotensin–aldosterone system (RAAS) is activated.4 Furthermore, primary aldosteronism is associated with a 12-fold higher prevalence of AF compared with essential hypertension.5 In patients with AF, the expression of the mineralocorticoid receptor is increased, augmenting the genomic effects of aldosterone.6 In patients with heart failure, serum aldosterone is elevated, as is the AF prevalence. In experimental models of hypertension induced by chronic aldosterone treatment in rats, ventricular and atrial fibrosis has been

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documented as well.\(^7,8\) In some heart failure models, blockade of aldosterone attenuates formation of fibrosis and suppresses spontaneous AF.\(^7\) These findings indicate a potential role of aldosterone in the pathogenesis of AF. However, it is presently unclear whether altered atrial electrophysiology is the result of haemodynamic changes (i.e. increased atrial load) or a direct aldosterone effect. A novel technique that combines epicardial mapping of rat atria with haemodynamic measurements in the working heart mode was used. This enabled us to impute atrial structural remodelling and disturbed electrical conduction as an AF substrate directly to aldosterone, independent of haemodynamic changes.

**Methods**

For more detailed methods, see Supplementary material online.

**Animal studies**

In 8-week-old Sprague–Dawley rats, osmotic mini-pumps (ALZET pump 2ML4, Durect Corporation, Cupertino, CA, USA) were implanted subcutaneously delivering 1.5 \(\mu\)g aldosterone/h (Aldo) for 8 weeks \((n = 43)\). The control group did not receive aldosterone \((n = 40)\). Both groups were fed with normal rat chow (V1536, Ssniff, Soest, Germany) containing 0.9% NaCl. The blood pressure was measured non-invasively every week. After 8 weeks, a surface electrocardiogram (ECG) was recorded and a transesophageal atrial burst stimulation to induce AF was performed in 11 Aldo and nine controls. Thereafter, the hearts were excised and connected to a working heart apparatus for epicardial mapping (see below). For in vivo left and right heart catheterization, 10 animals per group were investigated additionally. Another 10 Aldo and 10 controls were anesthetized and the hearts were isolated for a ‘working heart’ preparation to perform haemodynamic measurements. Furthermore, 12 Aldo and 11 controls were used for either histological biochemical or morphological analysis. The study was approved by the animal Ethics Committee of the Saarland University and animal handling was performed according to the European directive on laboratory animals (86/609/EEC).

**‘Working heart’ preparation**

For ‘working heart’ preparation,\(^10,11\) besides aortic cannulation, the left atrium (LA) was cannulated through the pulmonary vein with a steel cannula \((\text{length } 1.5 \text{ cm, } 1.5 \text{ mm inner diameter, } 2 \text{ mm outer diameter})\) sewed in by a tobacco pouch suture (Ethicon Prolene 7.0). This cannula was connected to a heated preload column, resulting in a myocardial temperature of 37 °C when the heart was working.

For haemodynamic analysis, two platinum electrodes were attached to the right atrium (RA) to pace the hearts at 340 b.p.m. Left ventricular (LV) systolic and diastolic function was recorded via a high-fidelity conductance catheter (Millar 1.4 F SPR-839, 12 mm spacing, Millar, Houston, TX, USA) inserted into the LV cavity by apical puncture. End-systolic pressure–volume relationship (ESPVR) and its slope \(E_{\text{es}}\) (end-systolic ventricular elastance) were assessed by a sudden increase in afterload pressure from 90 to 150 mmHg while preload was kept constant at 5 mmHg and a heart rate of 340 b.p.m. During this procedure, the peak LV end-systolic pressure (LVESP) was assessed. The maximal end-systolic pressure value minus the LVESP at an afterload of 90 mmHg defined the preload-dependent maximal pressure amplitude \((P_{\text{Am}})\). \(P_{\text{Am}}\) is a contractile parameter defining contractility reserve. Further parameters like ejection fraction (EF) and stroke volume were also measured at maximal developed LVESP.

End-diastolic pressure–volume relationship (EDPVR) measurement was displayed by sudden decrease in preload pressure from 15 to 0 mmHg due to evacuating the preload column with a large 50 mL syringe at a constant afterload of 90 mmHg and a heart rate of 340 b.p.m. Cardiac inflow \(=\text{cardiac output (CO)}\) and aortic flow were recorded continuously by inline ultrasonic transit time probes and were used to calibrate the volume measurement by the conductance catheter. Parallel conductance was determined by the saline dilution method, which involved injecting a 15 \(\mu\)L bolus of hypertonic \((5%)\) saline into the LA cannula, causing a transient change in the conductivity of Krebs–Henseleit buffer in LV. During steady state, load-dependent parameters of cardiac function were measured at a preload of 5 mmHg, an afterload of 90 mmHg, and a heart rate of 340 b.p.m.

For mapping experiments, the pulmonary artery and the vena cava inferior and posterior were occluded to adequately load both atria. An RA pressure of \(\sim 0–2 \text{ mmHg}\) was obtained by leaving the vena cava superior open.

**Data analyses of pressure–volume measurements**

In pressure–volume (PV) loops, LV pressure was plotted as a function of instantaneous LV volume (figure 1B). When ventricles were made to eject against increasing levels of afterload (‘aortic occlusion’), end-systolic pressure–volume coordinates (left shoulder of the loops) are connected by the red or blue straight line in figure 1B and fit to the equation: \(P_{\text{es}} = E_{\text{es}}(V_{\text{es}} - V_{\text{o}})\), where \(P_{\text{es}}\) is end-systolic pressure, \(V_{\text{es}}\) is the end-systolic volume, and \(V_{\text{o}}\) is volume axis intercept. \(E_{\text{es}}\) is the slope of ESPVR and indexes LV end-systolic elastance \((E_{\text{es}}, \text{mmHg}/\mu\text{L})\), a measure of LV contractility and systolic stiffness. Stroke work (SW) \((\text{mmHg}/\mu\text{L})\) is the numerically integrated area within each PV loop. Mechanical efficiency can be calculated by SW divided by potential and SW. Ejection fraction was calculated by the equation: \(\text{EF} = (V_{\text{ed}} - V_{\text{es}}V_{\text{ed}} - V_{\text{o}}) \times 100\%\), where \(V_{\text{ed}}\) is the end-diastolic volume.

When ventricles were made to eject against falling levels of preload (‘evacuating of the preload column’), EDPVR was assessed. End-diastolic pressure–volume relationship coordinates formed a curvilinear function (lower boundary of PV loops) fitting the equation: \(P_{\text{ed}} = k +xe^{\beta V}\), where \(V\) is the end-diastolic volume \((V_{\text{ed}})\), \(\beta\) the chamber stiffness coefficient, and \(k\) the fitting constant. The latter two parameters mainly determine LV diastolic compliance. Left ventricular compliance can also be expressed by the end-diastolic volume at a defined preload (e.g. 5 mmHg) to measure LV capacitance.

**Electrophysiological measurements**

To record the surface ECG in vivo, rats were anesthetized and lead II was recorded using 25 G subcutaneous needle electrodes in the limbs. To test inducibility of atrial tachycardias, repetitive LA burst stimulation \((50\text{ Hz}; 2\text{ s}; 4\times\text{threshold, 12 stimulation cycles per minute over 3 min})\) was performed via a 4 F quadripolar diagnostic catheter (Bard Electrophysiology, Lowell, MA, USA) in the esophagus, where a high and sharp atrial electrogram amplitude could be recorded. Atrial tachyarrhythmia was considered to be inducible when a rapid self-sustained atrial activation lasting more than 30 s was provoked.

In isolated hearts, conduction on the LA and RA was mapped epicardially during sinus rhythm (SR) and rapid atrial stimulation \([\text{cycle length (CL) }75\text{ ms}]\) using a 64-polar custom-made electrode to record unipolar electrograms \((\text{interelectrode distance }0.5\text{ mm})\)\(^{10,12}\). Both atria were stimulated from the cranial \((A)\) and anterior free wall \((B)\).
Maps from five consecutive beats were analyzed and the results were averaged. Local activation time below each electrode was automatically defined as the time point of the steepest negative deflection of the unipolar electrogram using a custom-made software. Total activation time (TAT) was calculated as the difference between the earliest and latest local activation time under the mapping electrode. Local activation time differences (‘conduction times’) were calculated between neighboring electrodes. Conduction times of \( \geq 2 \) ms (apparent conduction velocity, \( \approx 25 \) cm/s) were considered as being prolonged. Because normal conduction velocity in rat atria is \( \approx 50 \) cm/s, using these criteria is unlikely to overestimate the amount of intra-atrial conduction disturbances. To measure anisotropy (direction dependence) of conduction, the absolute difference in TAT and the absolute difference in the percentage of prolonged conduction times between pacing from Site A vs. B were calculated for both atria.

The atrial effective refractory period (AERP) was measured at pacing intervals of 150 ms at the free cranial and anterior wall of RA and LA (Sites A and B). Single premature stimuli (2× threshold, with a threshold of \(<50 \mu A\) at 1 ms impulse duration) were interpolated after every fifth interval, starting at a coupling interval shorter than the AERP. The coupling interval (steps of 2 ms) resulting in a propagated response was taken as the AERP.

**Statistics**

Continuous data are presented as mean \( \pm \) SD. To determine differences between two groups, an unpaired t-test or non-parametric test (Mann–Whitney U-test) was used, as appropriate. All calculated P-values are two-sided. Categorical variables were compared using Fisher’s exact test. Comparisons with \( P < 0.05 \) were considered to be statistically significant. All analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

**Results**

**Haemodynamics**

To study effects of aldosterone on ventricular systolic and diastolic function or atrial load, left and right heart catheter measurements in vivo as well as pressure–volume analysis in isolated working hearts were performed. In vivo, there were neither differences in LV and RV end-systolic (LVESP, RVESP) and end-diastolic (LVEDP, RVEDP) pressures nor RA pressures (Table 1 and Figure 1D). Right ventricular and LV contractility and relaxation in vivo also did not differ between both groups (Table 1). Pressure–volume analysis of isolated working hearts showed no difference in systolic and diastolic parameters in both groups (Table 1). The ESPVR was characterized by its slope (\( E_{es} \)) as a load-independent index of systolic function (Figure 1B). \( E_{es} \) was not reduced in Aldo (Table 1). Furthermore, load-dependent parameters of systolic function (CO, EF, and \( dP/dt_{max} \)) were similar in both groups. Left ventricular compliance characterized by the end-diastolic volume at a preload of 5 mmHg was also similar in both groups (Figure 1C). Furthermore, the stiffness constant \( \beta \) derived from the EDPVR, a load-independent diastolic parameter, did not
Table 1  Haemodynamic data

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<th>Control</th>
<th>Aldo</th>
<th>P-value</th>
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<tr>
<td><strong>In vivo measurements</strong> (n = 10)</td>
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<td>Heart rate (b.p.m.)</td>
<td>306 ± 20</td>
<td>287 ± 30</td>
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<td>LVESP (mmHg)</td>
<td>103.2 ± 14.5</td>
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<td>LVEDP (mmHg)</td>
<td>4.0 ± 2.2</td>
<td>1.9 ± 1.4</td>
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<tr>
<td>RVESP (mmHg)</td>
<td>36.2 ± 6.9</td>
<td>31.5 ± 6.7</td>
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<td>RVEDP (mmHg)</td>
<td>3.7 ± 2.4</td>
<td>2.4 ± 2.6</td>
<td>0.37</td>
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<td>RA pressure (mmHg)</td>
<td>6.0 ± 1.1/0.6 ± 0.3</td>
<td>6.0 ± 1.6/0.6 ± 0.6</td>
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<td>Relaxation constant τ (ms) RV</td>
<td>11.9 ± 2.0</td>
<td>12.9 ± 5.6</td>
<td>0.62</td>
</tr>
<tr>
<td>Relaxation constant τ (ms) LV</td>
<td>9.4 ± 1.2</td>
<td>9.6 ± 1.8</td>
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<tr>
<td>dp/dt\text{max} (LV)</td>
<td>6452 ± 2052</td>
<td>6360 ± 1536</td>
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<td>dp/dt\text{min} (LV)</td>
<td>−6214 ± 2697</td>
<td>−4794 ± 810</td>
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<tr>
<td>dp/dt\text{max} (RV)</td>
<td>2817 ± 1700</td>
<td>2038 ± 561</td>
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<td>dp/dt\text{min} (RV)</td>
<td>−1955 ± 884</td>
<td>−1775 ± 620</td>
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<th>Control</th>
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<tr>
<td><strong>Pressure–volume analysis (in vitro)</strong></td>
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<td>Systolic parameters (isolated heart, n = 10; heart rate 340 b.p.m.)</td>
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<tr>
<td>LVESP (mmHg)</td>
<td>93.9 ± 4.3</td>
<td>96.6 ± 5.3</td>
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<tr>
<td>EF (%)</td>
<td>54.3 ± 6.4</td>
<td>56.8 ± 4.9</td>
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<td>CO (mL/min)</td>
<td>62.1 ± 9.2</td>
<td>73.5 ± 14.7</td>
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<tr>
<td>E\text{es} (mmHg/μL)</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.76</td>
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<td>dp/dt\text{max} (mmHg/s)</td>
<td>4178 ± 1225</td>
<td>4096 ± 516</td>
<td>0.60</td>
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<tr>
<td>PA\text{max} (mmHg)</td>
<td>35.8 ± 8.1</td>
<td>32.6 ± 9.7</td>
<td>0.58</td>
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<tr>
<td>Mechanical efficiency</td>
<td>0.80 ± 0.02</td>
<td>0.70 ± 0.05</td>
<td>0.58</td>
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<td>Diastolic parameters (isolated heart)</td>
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<tr>
<td>Stiffness constant β (μL\textsuperscript{-1})</td>
<td>0.008 ± 0.005</td>
<td>0.010 ± 0.004</td>
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<td>Fitting constant k</td>
<td>1.2 ± 1.2</td>
<td>0.4 ± 0.3</td>
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<td>V\text{es} (μL)</td>
<td>370 ± 81</td>
<td>430 ± 133</td>
<td>0.41</td>
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<tr>
<td>Relaxation constant τ (ms)</td>
<td>13.9 ± 3.8</td>
<td>13.4 ± 10</td>
<td>0.93</td>
</tr>
<tr>
<td>dp/dt\text{min} (mmHg/s)</td>
<td>−3065 ± 930</td>
<td>−3203 ± 483</td>
<td>0.67</td>
</tr>
<tr>
<td>Peak filling rate (μL/ms)</td>
<td>10 434 ± 5084</td>
<td>11 302 ± 3181</td>
<td>0.76</td>
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</table>

EF, ejection fraction; CO, cardiac output; E\text{es}, end-systolic elastance; V\text{es}, end-diastolic volume at preload 5 mmHg; RA, right atrium, LA, left atrium, RV, right ventricle, LV, left ventricle; ESP, end-systolic pressure; EDP, end-diastolic pressure. Load-dependent parameters of ventricular function were measured at preload 5 mmHg, afterload 90 mmHg, heart rate 340 b.p.m.

Serum aldosterone concentration was approximately four-fold increased in Aldo accompanied by a significant reduction in potassium serum concentration compared with controls. Magnesium and calcium serum concentration was similar in both groups (Table 2). Non-invasive blood pressure measurements revealed a moderate blood pressure increase after ≥3 weeks of aldosterone treatment (Figure 1A). Heart rate was slightly reduced in Aldo compared with controls (340 ± 22 vs. 373 ± 30 b.p.m., P < 0.003).

Electrophysiological effects

Surface ECG in anaesthetized Aldo revealed a slower heart rate compared with controls (R-R intervals: 189 ± 24 vs. 165 ± 11 ms, P < 0.01). There was a significant prolongation of the P-wave duration indicating atrial conduction disturbances (25.6 ± 2.2 vs. 20.1 ± 3.1 ms, P < 0.001). Furthermore, in Aldo rats, atrioventricular (AV)-nodal conduction was prolonged as was ventricular depolarization and repolarization (Figure 2). To estimate whether aldosterone predisposes to atrial arrhythmias, oesophageal burst pacing was performed in 11 of 11 Aldo, and sustained atrial arrhythmias could be induced compared with two of nine controls (P = 0.03; Figure 3).

differ in both groups (Table 1). Parameters of active myocardial relaxation, such as the relaxation constant τ and dp/dt\text{min}, were also not significantly different between the groups. Thus, parameters of systolic or diastolic ventricular function as well as RA or LA load did not differ in Aldo compared with controls. Additionally, the maximal developed LVESP values during aortic occlusion for ESPVR measurements were similar as well as PA\text{max} assessments (Table 1). The sudden increase in afterload was associated with marked but nearly identical decrease in stroke volume as well as EF in both groups compared with steady-state conditions of 90 mm-Hg (see Supplementary material online, Table S1). All other parameters of systolic and diastolic function at maximal developed afterload did not differ significantly between both groups (see Supplementary material online, Table S1).

Serum aldosterone concentration was approximately four-fold increased in Aldo accompanied by a significant reduction in potassium serum concentration compared with controls. Magnesium and calcium serum concentration was similar in both groups (Table 2).
To reveal mechanisms underlying the increased susceptibility for atrial arrhythmias, we measured epicardial atrial conduction using a custom-made 64-polar electrode. In Aldo rats, conduction was substantially disturbed (Figure 4). As can be seen from representative recordings (Figure 4C–F), the propagation of the depolarization wave was slower and spatially heterogeneous in Aldo compared with control rat atria (see crowding of isochrones in Figure 4D with corresponding increased local conduction times in Figure 4F). Overall, TAT of the mapping area during SR or rapid stimulation from the superior free wall (Figure 5A, CL 75 ms) was prolonged in the RA, but not in the LA of Aldo (SR: RA: 10.6±1.8 ms, P<0.01; LA: 5.8±1.4 ms, P=0.23; rapid atrial pacing: RA-A: 11.3±1.9 vs. 7.9±2.2 ms, P<0.05; LA-A: 6.3±1.9 vs. 6.2±1.3 ms, P=0.90; Figure 5A). In contrast, rapid stimulation from the anterior free wall did not show a significant difference on both atria (RA-B: 10.2±3.1 vs. 8.6±1.0 ms; P=0.13; LA-B: 5.88±1.5 vs. 6.63±1.8 ms; P=0.57). Additionally, in Aldo local conduction times ≥2 ms occurred more often in RA and tended to occur more often in rapidly paced LA (SR: RA: 14.8±14.2 vs. 2.6±3.0%, P<0.01; LA: 0.6±1.8 vs. 1.7±2.7%, P=0.31; rapid atrial pacing: RA-A: 16.7±6.8 vs. 6.4±4.9%, P<0.01; LA-A: 7.5±2.8 vs. 4.5±3.6%, P=0.08; RA-B: 10.6±7.5 vs. 6.5±4.7%, P=0.18; LA-B: 5.2±4.1 vs. 2.6±1.8%, P=0.05; Figure 5B).

Anisotropy was determined to indicate direction dependence of local conduction as an index of atrial structural remodelling. Aldo showed a significant increase in anisotropy when prolonged local conduction times were compared on both atria (Figure 5C). To determine whether electrical remodelling (a shortening of atrial refractoriness) is responsible for an AF substrate in Aldo, AERP were measured. They did not differ between both groups (Figure 5D, RA-A: 35.4±20.8 vs. 31.6±11.5 ms, P=0.59; RA-B:
36.3 ± 11 vs. 33.3 ± 13.1 ms, P = 0.62; LA-A: 58.0 ± 10.2 vs. 53.1 ± 14.4 ms, P = 0.46; LA-B: 55.8 ± 10.0 vs. 48.3 ± 13.4 ms, P = 0.22). Furthermore, inter-atrial AERP difference, as a measure of heterogeneity of refractoriness, was not altered in Aldo rats (23.7 ± 15.9 vs. 23.4 ± 16.3 ms, P = 0.91).

**Histological and morphological studies**

To reveal the mechanisms that underlie the disturbed atrial conduction, atrial structure and its determinants were analysed. Aldo led to a moderate increase in heart weight and the heart weight/tibia length ratio (Table 2). Consistent with our haemodynamic data, there were no signs of cardiac decompensation or heart failure (lung weight, 14.4 ± 2.7 vs. 13.1 ± 1.9 g, P = 0.23; liver weight, 1.9 ± 0.2 vs. 1.9 ± 0.8 g, P = 0.87). Additionally, Brain natriuretic peptide (BNP) expression did not differ in both groups neither in the atria nor in the LV (Table 2). Atrial size tended to be increased in Aldo (Table 2). Interstitial fibrosis was increased in Aldo compared with controls in both atria (Figure 6A–C). Similarly, the collagen content was augmented in both ventricles of Aldo (Table 2). Atrial and ventricular myocytes were hypertrophied in Aldo compared with controls (Figure 6D and Table 2). The number of cardiac myocytes/mm² of both atria was decreased in Aldo (Table 2). Atria of Aldo showed more fibroblasts/mm² (Table 2). Protein expression of the most important collagen degrading enzyme in rodents, active matrix metalloproteinase 13 (MMP13), was reduced in LA and RA tissue in Aldo compared with controls, while constitutive MMP13 was reduced in Aldo without reaching significance (Figure 6E and F). The amount of tissue inhibitor of matrix metalloproteinase 4 (TIMP4) was similar in both atria (RA: 0.18 ± 0.06 vs. 0.18 ± 0.04 IOD, P = 0.95; LA: 0.16 ± 0.01 vs. 0.15 ± 0.05 IOD, P = 0.95). Relative aldosterone receptor mRNA and protein expression was not significantly altered in both groups (Figure 6G and H).

In membrane preparations of LA and RA tissue, the amount of connexin 43 and phosphorylated connexin 43 was similar in Aldo vs. controls (Figure 7A–C). Qualitative assessment of connexin 43 distribution by immunohistology showed no difference between both groups (Figure 7D).

**Discussion**

By using in vivo measurements and a novel technique that combines haemodynamic measurements and electrophysiological recordings with a 64-polar epicardial mapping electrode in working rat hearts, we demonstrated that chronic aldosterone treatment substantially disturbs atrial conduction without compromised ventricular haemodynamic function and electrical remodelling. These alterations in conduction properties were induced by structural atrial remodelling and created a substrate for the perpetuation of AF. In humans, AF is predominantly associated with structural heart disease accompanied by neurohumoral activation including the RAAS. Patients with primary aldosteronism show a 12-fold higher prevalence of AF compared with patients with essential
hypertension. In experimental heart failure models and a randomized controlled study in heart failure patients, aldosterone blockade reduces atrial fibrosis and/or suppresses AF. In these studies, haemodynamic alterations and their consequences (atrial dilatation and fibrosis) may be an essential factor for the development or prevention of atrial arrhythmias. Therefore, it was the aim of the study to demonstrate the pure endocrine effects of aldosterone on the atria unaffected by ventricular systolic and diastolic dysfunction.

**Generation of a rat model with elevated serum aldosterone and normal atrial load**

In heart failure, ventricular dysfunction is one factor inducing atrial structural and electrophysiological changes. In a dog model of tachycardiomyopathy, atrial conduction defects occurred. This was observed in the presence of severe LV systolic dysfunction and markedly elevated filling pressures (LVEDP of 20 mmHg). To study endocrine mechanisms without ventricular dysfunction or elevated atrial pressures, we established a novel rat model with elevated systemic aldosterone levels. In contrast to the ‘classic’ model characterized by severe hypertension, we did not perform unilateral nephrectomy and fed rats with normal diet containing 0.9% sodium. Subcutaneous delivery of 1.5 mg/h aldosterone over 8 weeks resulted in approximately four-fold increase in serum aldosterone levels (inadequate for normal salt intake) and an only mild systolic and diastolic hypertension. These levels seem to be of clinical relevance because patients with the Conn syndrome showed a four-fold increase and those with systolic heart failure a two-fold increase in aldosterone serum concentrations compared with healthy controls.

Mild hypertension was shown in awake Aldo after 8 weeks of aldosterone administration compared with control rats (Figure 1A). Heart rate was slightly reduced in Aldo (<10%).
When haemodynamic measurements were performed under general anaesthesia in vivo, blood pressure dropped to subnormal values (ca. 100 mmHg), revealing thereafter no differences between both groups (Table 1). This phenomenon might be explained by reduced ‘adrenergic drive’ due to anaesthesia in the studied animals. Decreased heart rate of Aldo was documented only by trend presumably signalling minor biological importance.

In vivo, LV and RV end-diastolic pressures were not elevated in Aldo compared with controls. Furthermore, parameters of ventricular contractility and relaxation (LVESP/RVES, dp/dt\textsubscript{max}, dp/dt\textsubscript{min}, as well as the relaxation constant τ) were similar in both groups revealing no difference in left and right ventricular systolic and diastolic function between Aldo and controls in vivo (Table 1). Similar haemodynamic results were obtained in pressure–volume analysis of isolated working hearts at an afterload of 90 mmHg close to the haemodynamic results were obtained in pressure–volume analysis in vivo and LV and RV end-diastolic pressures were not elevated in Aldo.

Aldosterone affects atrial electrophysiology and structure

In vivo, a prolonged P-wave duration was measured and transesophageal burst stimulation induced sustained atrial arrhythmias in Aldo. epicardial mapping of isolated hearts revealed a locally disturbed atrial conduction. The activation time under the mapping electrode was clearly increased on the RA of Aldo and prolonged local conduction times occurred more often on both atria of Aldo. Additionally, in Aldo rats, the occurrence of prolonged local conduction times depended more on the direction of propagation than in control animals. Thus, hyperaldosteronism increased atrial anisotropy of conduction. This can favour reentry phenomena thereby stabilizing atrial arrhythmias.
findings tended to be pronounced on the RA compared with the LA. This further supports the conclusion that mild arterial hypertension in this model did not affect atrial remodelling. The observed conduction defects were associated with structural remodelling characterized by atrial fibrosis and hypertrophy and likely represent the substrate for atrial arrhythmias induced by aldosterone. In a dog model of tachycardiomyopathy, stability of AF was associated with interstitial fibrosis and locally disturbed conduction.19 Consistently, in patients with congestive heart failure, conduction abnormalities and atrial enlargement could be observed.28 These abnormalities were accompanied by an increased inducibility and stability of AF. In a goat model of ventricular bradycardia (total AV block), atrial enlargement and hypertrophy of atrial cardiomyocytes alone (i.e. in the absence of atrial fibrosis) were associated with a substrate for AF.14 In the present study, we showed for the first time that aldosterone induces a substrate for atrial arrhythmias with locally disturbed conduction, independent of increased atrial load and characterized by atrial fibrosis and myocyte hypertrophy.

Left atria and RAs of Aldo rats contained increased numbers of fibroblasts, while the total number of atrial myocytes was likely not different in both groups. Although the number of

Figure 6 (A and B) Photomicrographs from sections of right atrial (RA) of a control and an Aldo rat. Sirius red was used to stain collagen. The Aldo rat showed significantly more interstitial fibrosis. (C) Relative interstitial collagen content of RA and left atrial (LA) in Aldo and controls. (D) Myocyte diameter in Aldo and controls. (E) Representative immunoblot of constitutive and active matrix metalloproteinase 13 (MMP13) as well as glyceraldehyde 3-phosphate dehydrogenase (GAPDH). (F) Total constitutive MMP13 protein expression (left side) and active MMP13 protein expression (right side). (G) Representative immunoblot of aldosterone receptor. (H) Total aldosterone receptor protein expression of the LA and RA of Aldo and controls (left side) and total aldosterone receptor mRNA expression of the LA and RA of Aldo and controls (right side).
cardiomyocytes/mm² was significantly lower in both atria of Aldo, multiplying this number with the atrial surface results in similar absolute numbers of cardiomyocytes (data not shown). Therefore, these results indicate a moderate de novo fibrosis rather than replacement of myocytes by connective tissue. Furthermore, in Aldo, the amount of atrial active MMP13 was reduced compared with controls, whereas TIMP4 was similar in both groups indicating reduced collagen degradation due to aldosterone. In chronic haemodynamic overload of the atria, remodelling of gap junction has been described. Interestingly, in our model—without evidence of increased atrial load—connexin 43 and phosphorylated connexin 43 protein levels of atrial membrane preparations were similar in Aldo and controls and were even similarly distributed in myocytes of both groups, suggesting that the observed conduction disturbances were independent of a severely reduced amount of these pore-forming proteins. This is in agreement with data in a dog model of tachycardiomyopathy, in which atrial conduction disturbances and AF promotion were primarily caused by atrial fibrosis without relevant contribution of the amount, distribution, or phosphorylation of atrial connexins.30

Aldosterone reduces significantly serum potassium levels to the lower normal range. In general, lowering of potassium serum levels reduces cellular resting membrane potential thereby favouring increased automaticity. Additionally, it prolongs repolarization thereby promoting early afterdepolarizations. Both mechanisms are stimuli that can trigger AF or reentry tachycardias. Since we did not observe spontaneous atrial arrhythmias, the arrhythmogenic relevance of decreased potassium serum levels in our model seems to be low at least. Frequent atrial arrhythmias would have resulted in electrical remodelling (e.g. shortening of AERPs) which was not observed. According to our results, aldosterone perpetuates rather than triggers atrial arrhythmias. A vicious circle may be initiated: when primary aldosteronism has created a substrate for atrial tachyarrhythmias, these can occur more easily and in turn can induce an atrial up-regulation of the mineralocorticoid receptor, thereby augmenting atrial genomic aldosterone effects. This could further stabilize the arrhythmia. On the other hand, atrial tachyarrhythmias itself could start this circle, thereby inducing structural remodelling and conduction disturbances. Our study was performed at the beginning of this potential vicious circle without up-regulation of the aldosterone receptor mRNA and protein.

Besides conduction disturbances, shortening of the AERP has been shown to sustain AF as well. However, in the present study, AERP measured at two different positions on each atrium and spatial heterogeneity of AERP did not differ between the groups. Therefore, changes in atrial refractoriness are not a mechanism by which aldosterone predisposes to atrial arrhythmias.

**Limitations**

Invasive pressure measurements were not performed in awake animals in vivo. Measurements of LV haemodynamics were conducted in anaesthetized rats in vivo or in isolated working hearts in vitro. Isolated hearts of both groups could not develop higher LV pressures than 130 mmHg during sudden afterload increase in vitro. However, at all pressure levels - during aortic occlusion up to LV depression - systolic and diastolic function did not differ in both groups. Nevertheless, small changes in atrial load due to Aldo treatment cannot be ruled out completely, because in awake animals, systolic blood pressure was moderately increased.
However, atrial and ventricular BNP levels remained unchanged after Aldo treatment.

Conclusion

Aldosterone caused a substrate for the perpetuation of AF characterized by conduction disturbances, interstitial atrial fibrosis, and myocyte hypertrophy, despite normal atrial pressures. Changes in atrial refactoriness did not occur. Thus, aldosterone can play an important causal role in the pathogenesis of AF, even under normal haemodynamic conditions. These data indicate potential benefit and clinical relevance of mineralocorticoid receptor antagonists in the primary or secondary prevention of AF, warranting prospective clinical trials.

Supplementary material

Supplementary material is available at European Heart Journal online.

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