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Aldosterone induces electrical remodeling independent of hypertension

Theresa Dartsch [a], Robert Fischer [b,c], Andrej Gapelyuk [c,d], Marco Weiergraeber [e], Dennis Ladage [a], Toni Schneider [f], Alexander Schirdewan [b], Hannes Reuter [a], Jochen Mueller-Ehmsen [a], Carsten Zobel [a,*]

[a] Department of Internal Medicine III, University of Cologne, Cologne, Germany
[b] Department of Cardiology and Pneumology (Charité Centrum 11), Charité-University Medicine Berlin, Germany
[c] Max-Delbrueck-Center for Molecular Medicine, Berlin-Buch, Germany
[d] Department of Physics, Humboldt-University, Berlin, Germany
[e] Bundesinstitut für Arzneimittel und Medizinprodukte, Bonn, Germany
[f] Institute for Neurophysiology and Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

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ABSTRACT

Background: Treatment of heart failure patients with aldosterone antagonists has been shown to reduce the occurrence of sudden cardiac death. Therefore we aimed at determining the consequences of chronic exposure to aldosterone and the aldosterone antagonists eplerenone and spironolactone on the electrophysiological properties of the heart in a rat model.

Methods and results: Male Wistar rats were chronically treated (4 weeks) with aldosterone (ALD) via an osmotic minipump. Spironolactone (SPI) or eplerenone (EPL) was administered with the rat chow. ALD treated animals developed left ventricular hypertrophy, prolonged QT-intervals, a higher rate of ventricular premature beats and non-sustained ventricular tachycardia despite normal blood pressure values. Spironolactone and eplerenone were both able to inhibit the alterations. Left-ventricular mRNA expressions of Kv4.2 and Kv4.3 (I_{to}), Kv1.5 (I_{Kur}), Kir2.1 and Kir2.3 (I_{K1}) and of $Ca_v1.2$ (L-type Ca^{2+} channel) were significantly down-regulated in ALD. Correspondingly, the protein expressions of subunits Kv1.5, Kir2.3 and Cav1.2 were significantly decreased. A diminished calcineurin activity and mRNA expression of the Aß subunit of calcineurin were found in ALD, which was insensitive to aldosterone antagonists.

Conclusions: Chronic aldosterone-overload induces blood pressure independent structural and electrical remodeling of the myocardium resulting in an increased risk for malignant ventricular arrhythmias.

1. Introduction

Sudden cardiac death (SCD), to a large extent caused by ventricular tachyarrhythmias, remains one of the major challenges for the treatment of patients with compromised cardiac function. Such arrhythmias have been documented in up to 85% of patients with severe congestive heart failure and SCD is accounting for up to 50% of deaths depending on disease severity [1,2]. It is believed that electrical remodeling as part of overall cardiac remodeling during the course of heart failure development is contributing to the pathophysiological basis for these arrhythmias [3]. The renin-angiotensin-aldosterone system (RAAS) is intimately involved in the development of electrical remodeling [4]. Particularly aldosterone levels have been reported to correlate significantly with the risk of cardiovascular events [5]. The role of aldosterone is further underlined by two major heart failure trials. Both the RALES [6] as well as the EPHESUS [7] trials demonstrated a significant reduction of SCD in a heart failure population after therapy with aldosterone antagonists. The cardiac action potential is generated by the highly orchestrated activity by a number of depolarizing and repolarizing potassium currents. In various studies heart failure was associated with suppression of repolarizing potassium currents (I_{to}, I_{K1}, I_{kur}) and proteins accounting for these currents (Kv4.2 and Kv4.3, Kir2.1 and Kir2.3 and Kv1.5)[8]. Suppression of repolarizing potassium currents leading to action potential prolongation provides an explanation for the QT prolongation/dispersion thereby accounting for a major mechanism of arrhythmogenesis. Despite the clinical data demonstrating the protective effect of aldosterone antagonists against SCD in patients with heart failure, little is known about the effects of aldosterone itself on the ionic basis of electrical remodeling in the heart. The effects of unspecific (spironolactone) and specific (eplerenone) blockade of the mineralocorticoid receptor (MR) on the electrical properties of cardiomyocytes are also mostly unknown.

Abbreviations: SCD, Sudden Cardiac Death; CON, Control; ALD, Aldosterone; EPL, Eplerenone; SPI, Spironolactone; LVH, Left ventricular hypertrophy; VT, ventricular tachycardia; MCG, magnetocardiogram; MR, Mineralocorticoid receptor; RAAS, Renin-Angiotensin-Aldosterone-System; RALES, Randomized Aldactone Evaluation Study; EPHESUS, Eplerenone Post-acute myocardial infarction Heart failure Efficacy and Survival Study; SBP, Systolic Blood Pressure; SQUID, superconducting quantum interference devices; PPP3CB, Calcineurin subunit Aß; VPB, Ventricular premature beat; GR, Glucocorticoid receptor.

* Corresponding author at: Department of Internal Medicine III University of Cologne 50924 Cologne, Germany. Tel.: +49 22132401.
E-mail address: carsten.zobel@UK-koeln.de (C. Zobel).

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Therefore the aim of this study was to determine the consequences of chronic aldosterone exposure on the electrical characteristics of the rat heart. In detail, we studied the effects of aldosterone on expression and function of the cardiac ion channels (I_{to}, I_{K1}, I_{kur}, I_{Ca}) as well as alterations in signal transduction pathways involved in aldosterone mediated signaling. Furthermore we investigated the electrophysiologic effects of the MR antagonists spironolactone and eplerenone individually and in aldosterone treated animals.

2. Materials and methods

2.1. Animal model and implantation of osmotic minipumps

Male Wistar rats (48 rats, mean body weight 211 g ± 18 g) (Charles River) were treated with aldosterone (Sigma-Aldrich) or solvent (polyethylene glycole 400, PEG 400) (Sigma-Aldrich) over a period of 4 weeks via an implanted osmotic minipump (ALZET, Pump Model 2004). Aldosterone was dissolved in PEG 400 (aldosterone release 1 μg/h). Pumps were implanted subcutaneously under anesthesia using 70 mg/kg ketamine (Ketavet®, Sanofi) and 5 mg/kg xylazine (Rompun®, Bayer). Rats received either spironolactone (100 mg/kg^{-1} body weight/day^{-1}) (Roche) or eplerenone (100 mg/kg^{-1} body weight/day^{-1}) (Pfizer) with the rat chow (Altromin). Control animals were fed with rat chow without additives (Altromin). Rats were separated into 6 groups of 8 animals each: Solvent control (CON), aldosterone (ALD), eplerenone (EPL), spironolactone (SPI), aldosterone + eplerenone (ALD + EPL), aldosterone + spironolactone (ALD + SPI). The animals had free access to food and water and were maintained in a constant environment with a conventional 12 h/12 h light–dark cycle starting at 6 am. All experiments were carried out in accordance with the guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the local authorities on animal care (Bezirksregierung Cologne, K 47, 03/05).

2.2. Blood pressure measurement

Systolic blood pressure (SBP) was determined before implantation procedure (day 0) and after 4 weeks of drug treatment (day 28) with a non-invasive tail cuff method (Rat Tail Blood Pressure, Harvard Apparatus). Rats were allowed to habituate to this procedure by restraining them in the tubes for 10–20 min/day for 5 days prior to recording blood pressures. Measurements were repeated three times calculating the mean values.

2.3. Measurement of serum aldosterone

Serum Aldosterone was measured using an aldosterone radioimmunoassay kit (Coat-a-Count Aldosterone, Siemens Medical Solutions Diagnostics), where sample aldosterone competes with ^{125}I-labeled aldosterone.

2.4. Electrocardiogram (ECG)

Some rats (n = 3 in each group) were additionally equipped with a telemetry device on day 0 to obtain long-term electrocardiograms (ECG) from conscious and freely moving animals. ECG transmitters were implanted subcutaneously with a standard lead II position (TA10 ETA-F20, Data Sciences International) to record the ECG. The ECG of each animal was recorded 24 h after 3 weeks after implantation procedure. Sampling rat was 1 kHz. ECG data were analyzed with the Dataquest A.R.T. Analysis Program, Version 4.1 (Data Sciences International) and the incidence of aberrations in the ECG was quantified for every animal group. The number of ventricular premature beats was evaluated in every treatment group and interpreted as number of ventricular premature beats per hour. Arrhythmias were diagnosed according to the Lambeth Convention criteria [9]. To separate VPBs from supraventricular ectopic beats with aberrant conduction we analyzed the corresponding RR-intervals, since ventricular premature beats in contrast to supraventricular ectopic beats rarely disturb the underlying sinus rhythm.

2.5. Magnetocardiography

On day 28, six animals of each group were anesthetized with 2% isoflurane and the rat heart magnetic field was recorded contactless over the anterior chest wall. The examinations were performed in a magnetically shielded room using seven channel magnetic measurement system (Cryoton Ltd.) based on low-temperature superconducting quantum interference devices (SQUIDs) coupled with second order axial gradiometers. Measurements were done sequentially at 4 positions corresponding of 28 magnetocardiographic leads (7 × 4). Signal averaging technique was applied to improve signal to noise ratio and to obtain representative cardiocycle. Interval between onset of Q-wave and offset of T-wave was considered for further analysis. The magnetocardiogram supplies a detailed display of the excitability of the heart, as the surrounding tissue does not interferes during the measurement in comparison to ECG recordings. The magnetic field exactly correlates with the electrophysiologic excitation of the heart.

2.6. Western-blot

Total proteins were isolated from left ventricles (n = 8) and Western Blot analysis was performed as prescribed previously [10]. The samples were subjected to 10% SDS-polyacrylamide gel electrophoresis (PAGE) (Hoefer Scientific Instruments), transferred to nitrocellulose membrane (Bio-Rad Laboratories), and blocked in 5% milk. Primary antibodies were incubated over night at 4 °C in buffer according to the manufacturer's instructions followed by an anti-rabbit α-peroxidase secondary antibody (Sigma Aldrich) to allow visualization by chemiluminescence (Lumi-Light Western Blotting Substrate, Roche Diagnostics) on a X-ray film (Curix, AGFA Healthcare,). Polyclonal antibodies used included: Anti-Kir2.1, Anti-Kir2.3, Anti-Kv4.2, Anti-Kv4.3, Anti-Kv1.5 and Anti-Ca_v1.2 (Alomone) at dilutions of 1:200. GAPDH (1:6000) (Abcam) was used as control for protein loading.

2.7. Calcineurin activity assay

The calcineurin phosphatase assay was performed according to the manufacturer's instructions (Enzo Life Sciences International). Lysates of left ventricular tissue (1 μg/μl) were incubated for 30 min at 30 °C with the calcineurin enzyme. After addition of Biomol Green™ reagent samples were analyzed after 30 min on a plate reader at 620 nm.

2.8. Statistics

Data are presented as means ± SE in figures unless otherwise specified. One-way ANOVA and Newman-Keuls Post-hoc test were applied to identify significant effects. All statistical analyses were performed using SPSS for Windows (Version 11.0, SPSS, Chicago, IL). A value of $p<0.05$ was considered statistically significant.

3. Results

3.1. Cardiac hypertrophy and hemodynamic parameters

Aldosterone treatment in male Wistar rats resulted in myocardial hypertrophy with a significant increase in relative heart weight (heart weight/tibia length) compared to control ($p<0.05$, Table 1). Aldosterone treated animals showed no signs of congestive heart failure like pale or discolored extremities, shallow, rapid breathing, decreased appetite or rapid weight gain. Spironolactone and eplerenone were able to prevent the development of hypertrophy ($p<0.01$ vs. ALD). Tibia length was unaffected by aldosterone and the aldosterone antagonists (data not shown). SBP values did not vary during drug treatment nor between groups (ALD: 116 ± 4 mmHg (day 0) and 119 ± 4 mmHg (day 28) to CON: 110 ± 6 mmHg (day 0) and 119 ± 5 mmHg (day28); n = 8, Table 1). Potassium levels were not altered in a significant manner in all treatment groups compared to control (Table 1). Only animals fed with eplerenone had significantly elevated potassium levels compared to CON. Sodium and chloride concentrations in the blood did not change during the time course of the experiment as well as creatinine levels.

Serum aldosterone approximately doubled after four weeks in aldosterone treated animals (ALD, $p<0.01$, Table 1) and in those additionally receiving spironolactone (ALD + SPI, $p<0.001$) or eplerenone (ALD + EPL, $p<0.05$) compared to the control group. Control animals fed with an aldosterone antagonist also developed increased aldosterone levels compared to CON (EPL, $p<0.05$; SPI, $p>0.05$).

3.2. Electrical alterations induced by aldosterone

After we established that aldosterone induced hypertrophy independent of hypertension in our experimental setting, we studied electrophysiological alterations in the heart. The results indicated a significant increase in the R-amplitude ($p<0.05$) in ALD compared to CON, whereas spironolactone and eplerenone attenuated this effect (Fig. 1A) underlining the aldosterone dependent left ventricular hypertrophic phenotype. More important, ALD showed a significant prolongation of the QRS complex and a prolonged QT interval in CMFM recordings (Fig. 1B and C). Heart rate was similar in all studied groups (data not shown) therefore the uncorrected QT-interval was analyzed. In contrast, aldosterone treated rats that received spironolactone had a significant shorter QRS duration than CON ($p<0.05$). Both aldosterone antagonists were able to impede the prolongation of