Cellular mechanisms of metabolic syndrome-related atrial decompensation in a rat model of HFpEF

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ABSTRACT

Heart failure (HF) with preserved ejection fraction (HFpEF) is present in about 50% of HF patients. Atrial remodeling is common in HFpEF and associated with increased mortality. We postulate that atrial remodeling is associated with atrial dysfunction in vivo related to alterations in cardiomyocyte Calcium (Ca) signaling and remodeling. We examined atrial function in vivo and Ca transients (CaT) (Fluo4-AM, field stim) in atrial cardiomyocytes of ZSF-1 rats without (Ln; lean hypertensive) and with metabolic syndrome (Ob; obese, hypertensive, diabetic) and HFpEF.

Results: At 21 weeks Ln showed an increased left ventricular (LV) mass and left ventricular end-diastolic pressure (LVEDP), but unchanged left atrial (LA) size and preserved atrial ejection fraction vs. wild-type (WT). CaT amplitude in atrial cardiomyocytes was increased in Ln (2.9 ± 0.2 vs. 2.3 ± 0.2 F/F_0 in WT; n = 22 cells/group; $p < 0.05$). Studying subcellular Ca release in more detail, we found that local central cytosolic CaT amplitude was increased, while subsarcolemmal CaT amplitudes remained unchanged. Moreover, Sarcoplasmic reticulum (SR) Ca content (caffeine) was preserved while Ca spark frequency and tetracaine-dependent SR Ca leak were significantly increased in Ln. Ob mice developed a HFpEF phenotype in vivo, LA area was significantly increased and atrial in vivo function was impaired, despite increased atrial CaT amplitudes in vitro (2.8 ± 0.2; $p < 0.05$ vs. WT). Ob cells showed alterations of the tubular network possibly contributing to the observed phenotype. CaT kinetics as well as SR Ca in Ob were not significantly different from WT, but SR Ca leak remained increased. Angiotensin II (Ang II) reduced in vitro cytosolic CaT amplitudes and let to active nuclear Ca release in Ob but not in Ln or WT.

Summary: In hypertensive ZSF-1 rats, a possibly compensatory increase of cytosolic CaT amplitude and increased SR Ca leak precede atrial remodeling and HFpEF. Atrial remodeling in ZSF-1 HFpEF is associated with an altered tubular network in-vitro and atrial contractile dysfunction in vivo, indicating insufficient compensation. Atrial cardiomyocyte dysfunction in vitro is induced by the addition of angiotensin II.

1. Introduction

Heart failure with preserved ejection fraction (HFpEF) is characterized by symptoms of heart failure (HF) in patients with structural heart disease and preserved EF and can be separated from heart failure with reduced EF (HFrEF). It is currently believed that HFpEF describes a heterogeneous clinical syndrome often associated with metabolic syndrome. Cardiac remodeling in HFpEF and HFpEF preceding stages (i.e. during hypertensive heart disease) is accompanied by remodeling of the atria (i.e. atrial enlargement) and recent evidence suggests that atrial remodeling and function in patients with HFpEF differs from patients with HFrEF [1].

An increase in left atrial (LA) volume indicating atrial remodeling and also a decline in LA contractility are strong predictors of new onset HF, AF and mortality [2–4]. About one third of patients with HFpEF develop AF [5], associated with increased mortality (hazard ratio of 1.6 to 2.7) [6,7]. However, even in the absence of AF, atrial dysfunction is common in patients with HFpEF [8] and higher LA ejection fraction has been associated with reduced mortality in HFpEF possibly due to the importance of the atrial "booster pump" function and the release of

natriuretic peptides in HF [1,9].

A variety of mechanisms have been linked to atrial remodeling in HFpEF including increased left ventricular diastolic pressure and neurohumoral activation with consecutive cardiomyocyte hypertrophy and activation of cardiac fibroblasts [10,11]. Activation of the renin-angiotensin-aldosterone system has been associated with hypertension, the development of HFpEF and atrial dysfunction [11,12] and RAS inhibition might contribute to alleviating disease progression and mitigate atrial remodeling [9].

Our knowledge on atrial cardiomyocyte function in HFpEF and preceding stages (i.e. during hypertensive heart disease) is sparse. In the present paper, we establish a quantitative correlation between atrial contractile dysfunction in vivo and intrinsic cardiomyocyte function in a lean rat model of hypertensive heart disease as well as an obese rat model that mimics important clinical features of HFpEF and explore underlying cellular mechanisms.

2. Methods

All experiments were approved by the local Ethics Committee (TVA G0212/15 and G0276/16) and performed in agreement with the Guidelines for the Care and Use of Laboratory Animals (National Institute of Health, U.S.A.). Animals were housed in a 12-hour light/dark regime under conventional conditions in the local animal facility with free access to food and water.

2.1. Echocardiography

Transthoracic echocardiography was performed in anesthetized rats with a high-resolution micro-imaging system equipped with a 17.5-Mhz linear array transducer (Vevo770TM Imaging System, VisualSonics, USA) using standard 2D- and M-mode imaging and analysis was performed in a blinded fashion. Animals were anesthetized with 5% isoflurane and 1.6 l/min oxygen in an induction chamber for 1–2 min and afterwards placed on a heated plate to maintain body temperature at 37.5 °C. Heart rate was continuously measured by ECG electrodes. Anesthesia during echocardiography was reduced to 1–2% isoflurane and same oxygen flow rate administered via an anesthetic mask. Measurements were performed to assess changes in cardiac function and dimensions (left ventricle (LV) end-diastolic diameter (LVEDD) and left atrium (LA) size) from at least three consecutive cardiac cycles under stable conditions. LA and LV ejection fraction (EF) and LV mass were calculated using standard formulas [13].

2.2. Invasive hemodynamic measurements

PV Loop measurements were performed as previously described [13]. All animals were in sinus rhythm during the final experiments.

2.3. Myocyte isolation and heart failure model

LA myocytes were isolated from ZSF-1 lean (ln; $ZSF^{+/-}$), obese (ob; $ZSF^{+/+}$) and wild type rats (WT, Charles River; Wistar Kyoto) after 21 weeks [14]. In addition LA myocytes were isolated from WT and Ob at 27 weeks. The ZSF-1 rat model is based on a leptin receptor mutation (heterozygote in lean and homozygote in obese animals). All animals were fed with high caloric diet (Formulab Diet 5008*). Animals were euthanized using isoflurane anesthesia and cervical dislocation. Hearts were excised, mounted on a Langendorff apparatus, and perfused with nominally Ca-free Tyrode solution for 5 min followed by enzyme solution containing 20 μM Ca and 22.5 μg/ml Liberase Blendzyme TH (Roche Applied Science, Indianapolis, IN) for 20 min at 37 °C. The LA was removed from the heart; tissue was minced, filtered, and washed. Isolated cells were kept in normal tyrode solution with 50 μM Ca at room temperature (20–24 °C) until indicator dye loading and subsequent experimentation.

All procedures and protocols involving animals conform to German and European regulations on animal experimentation and were approved by the local authorities (LAGeSo, TVA G 0212/15).

2.3.1. Solutions and chemicals

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless noted otherwise. The fluorescent calcium indicator Fluo-4 was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Tyrode solution contained (in mmol/L; mM): 130 NaCl, 4 KCl, 1 CaCl2, 1 MgCl2, 10 D-glucose, 10 HEPES; pH 7.4 with NaOH. All cells were plated on laminin-coated glass coverslips and exposed to perfusion with 1 mM Ca Tyrode solution. To prevent movement of cells during confocal imaging blebbistatin (10 μM; Tocris Bioscience, Bristol, UK) was added to all Tyrode based solutions.

2.3.2. Confocal and ratiometric measurements

For confocal [Ca]i measurements cells were loaded with Fluo-4/AM (5 μM, excitation at 488 nm, emission > 515 nm) for 20 min, followed by a washing period (> 10 min) in Tyrode solution. Fura-2-AM (5 μM, loading for 30 min at room temperature, followed by 15 min wash out) was used to ratiometrically (excitation at 340 nm (F_{340}) and 380 nm (F_{380}), emission collected at > 510 nm) measure averaged whole-cell $[Ca]_i$ as described previously [15]. A subgroup of myocytes was attached to laminin-coated slides and stained with 5 μM di-8-ANEPPS (Molecular Probes) for 45 min with excitation at 488 nm and emission collected at > 515 nm. The tubular network was quantified using a previously established automatic (unbiased) thresholding algorithm [16,17]. Confocal line scan images were recorded at 1250 lines/s using a 60 × oil-immersion objective lens (NA = 1.49) with a Zeiss LSM 510 system. The scan line was placed along the longitudinal or transversal axis of the cell (pixel size 0.12 μm) and either cytosolic, subsarcolemmal or nuclear regions were selected to obtain local CaT. To compare local Ca release in central and subsarcolemmal locations with little t-tubule dependence, a transversal axis of the line was chosen. To quantify global increases in bulk cytosol, a centered line parallel to the longitudinal axis of the cell was chosen. Local nuclear CaT were selected according to the oval shape of local CaT in 2D images during pacing [18].

The minimum distance from the nucleus was 4 μm for transverse line-scans. Centripetal Ca propagation velocity was calculated as previously described from transversal line-scan images as the distance between subsarcolemmal and central locations and the interval between the times when the local CaT reached time to 50% of maximal Ca release (TF50) at these respective locations [19]. CaTs were elicited by electrical field stimulation (1 and 3 Hz, resp.) of intact atrial myocytes using a pair of platinum electrodes (voltage set at ~50% above the threshold for contraction). Changes in $[Ca]_i$ are expressed as the amplitude, $\Delta F/F_0$, where F represents time-dependent Fluo-4 fluorescence and F_0 refers to diastolic fluorescence levels measured under control steady-state conditions during electrical stimulation. Tau of a monoexponential fit of the decay of CaT was obtained as a parameter of Ca removal. TF50 was used to quantify kinetics of Ca release, as it reflects atrial Ca release by the "fire-diffuse-uptake-fire" mechanism with only little influence by Ca diffusion (as opposed to time to CaT peak) [20]. Release sites along the longitudinal scan line within the cardiomyocyte with a TF50 < 10 ms were defined as early release sites, which likely reflect junctional sarcoplasmic reticulum [17,18,21]. Frequency and morphology of Ca sparks were measured using SparkMaster [22]. Rapid application of caffeine (10 mM) was used to deplete SR Ca stores and SR Ca content was derived from the amplitude of caffeine-evoked Ca transients.

SR Ca leak was assessed using a second approach: Cells were placed in normal tyrode (1 mM external Ca) and paced till steady state was reached. Pacing was stopped and 0 Na^+ 0 Ca^{2+} solution containing the RyR inhibitor tetracaine added via rapid perfusion. The delta in resting Ca observed after this intervention equals resting SR Ca leak.