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/F0 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 led 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 HFrEF 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 neuro-humoral 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 GO212/15 and GO276/16) 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 housed in a 12-hour light/dark regime under conventional conditions in the local animal facility with free access to food and water.

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 [Ca2+]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. Pura-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 (F340) and 380 nm (F380), emission collected at > 510 nm) measure averaged whole-cell [Ca2+]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 [Ca2+]i are expressed as the amplitude, ΔF/F0, where F represents time-dependent Fluo-4 fluorescence and F0 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 Ca2+ solution containing the RyR inhibitor tetracaine added via rapid perfusion. The delta in resting Ca observed after this intervention equals resting SR Ca leak.
A subset of cells CaT was recorded after treatment with angiotensin II (Ang II, Sigma) at 250 nM for 8–10 min or after treatment with Ang II (1 μM) and 2-APB (1 μM) for 2.5 h. Ca spark mass was estimated using the product of Ca spark frequency and single Ca spark amplitude. Experiments were conducted at 37 °C.

2.4. ELISA

BPN ELISA was performed according to manufacturer’s protocol (KA0979, Abnova, Taiwan) with a 1:2 dilution of the samples.

2.4.1. Western blotting

The atrial tissue samples were homogenized at 4 °C in lysis buffer with the following composition: 20 mM Tris-HCl, pH 7.4, 137 mM NaCl, 10% glycerol, 1% NP40, 20 mM NaF, 1 mM sodium orthovanadate, 1 mM sodium pyrophosphate, 50 mM β-glycerophosphate, 10 mM EDTA, 1 mM EGTA, 1 mM PMSF, 4 μg/ml aprotinin, 4 μg/ml pepstatin A, and 4 μg/ml leupeptin. For Western blotting 20 μg of tissues homogenates were run on 16.5% Tricine gels for PLB and on 4–12% Bis-Tris polyacrylamide gels for SERCA and transferred to nitrocellulose membranes for 2 h. Proteins on membrane were stained with Ponceau S. Non-specific binding was blocked with 5% dried milk in Tris-buffereed saline (pH 7.4) containing 0.1% Tween-20. Membranes were probed with anti-Phospho-Thr17 PLB, anti-Phospho-Ser16 PLB, anti-SERCA2a (Badrilla), anti-PLB (Santa Cruz) overnight at 4 °C. Anti-rabbit IgG linked with IRDye 680RD or anti-mouse linked with 800CW (LI-COR) were used as a secondary antibody. The signal was detected with Odyssey CLx System. The band intensities and total proteins stain were determined by Image Studio software (LI-COR).

2.4.2. Data analysis and statistics

Data were analyzed as previously described [16]. Statistical comparisons were made using ANOVA for paired or unpaired data with Dunn’s Multiple Comparison Test for post-hoc group comparison and statistical significance set at p < 0.05. Data are presented as individual observations or as means ± SEM of n measurements, where n is the number of cells.

3. Results

3.1. In vivo atrial function is impaired in metabolic syndrome-related HFpEF (obese ZSF-1) but not in hypertensive remodeling (lean ZSF-1)

In Ln, total body weight was increased (to 463 ± 4 g vs. 378 ± 4.8 g in WT; p < 0.05 vs. WT). LVEF was preserved and systolic blood pressure was increased as compared to WT (to 158 ± 2 vs. 127 ± 2 mm Hg; n = 8 Ln and 10 WT animals), ventricle showed hypertrophy and increased left ventricular end-diastolic pressure (LVEDP), while BNP levels and left atrial (LA) area and LA ejection fraction (EF) stayed unchanged indicating increased intracardiac pressure but normal LA function with a compensated phenotype in hypertensive remodeling (Fig. 1A–C).

In the metabolic syndrome-related model of HFpEF (Ob), total body weight was further increased (to 613 ± 10 g in 10 animals; p < 0.05 vs. WT). LVEF at 21 weeks was preserved and serum BNP levels (from 20 ± 10 and 10 ± 10 in WT and Ln to 80 ± 20 pg/ml in Ob; n = 3, 2 and 3 animals; p < 0.05) as well as systolic blood pressure (to 190 ± 1.3 mm Hg in 8 Ob animals) were significantly increased. In addition LVEDP and left ventricular mass were significantly increased (Fig. 1A–C).

In vivo, in Ob, LA area (Fig. 1Da) and LA diastolic volume were significantly increased and LA EF was significantly impaired as compared to WT and Ln (from 68 ± 2 and 64 ± 1 to 53 ± 3% in Ob; n = 10 animals/group; p < 0.05) indicating atrial mechanical decompensation in HFpEF-related remodeling.

3.2. Cytosolic Ca transients are altered in atrial cardiomyocytes during cardiac remodeling

In atrial myocytes of Ln animals, CaT amplitudes were significantly increased despite unchanged SR Ca content. Increased global CaT amplitude was associated with prolonged Ca release as time to 50% of maximal Ca release (TF50) was significantly increased in Ln (Fig. 2B). Moreover we used the area under the curve of cytosolic CaT during 1 Hz stimulation as a measure of total cytosolic Ca exposition during excitation-contraction coupling (ECC) [23]. Total cytosolic Ca was increased in Ln as compared to WT. (area under the curve of CaT S04 ± 54 a.u. in Ln vs. 303 ± 18 a.u. in WT, p < 0.05 vs. group).

Studying subcellular Ca release in more detail, we found that local central cytosolic CaT amplitude was increased, while subsarcolemmal CaT amplitudes remained unchanged and that the observed increase in TF50 could be mainly attributed to central cytosolic regions (Fig. 2C). The fraction of early (junctional) release sites was not significantly altered (to 39 ± 5 vs. 35 ± 7% and 41 ± 5% in 22 WT and 22 Ln and 26 Ob cells) and centripetal Ca propagation velocity was found to be unchanged (200 ± 32, 140 ± 28 and 198 ± 47 μm/s in 24 WT, 16 Ln and 15 Ob cells).

After the investigation of Ca release in hypertension-related remodeling, cells obtained from animals with HFpEF due to metabolic syndrome with atrial remodeling were examined:

In atrial myocytes from Ob, CaT amplitudes were also significantly increased (Fig. 2B). However, the increase of CaT amplitudes could mainly be attributed to enhanced cytosolic CaT amplitudes from central cytosolic regions (to 2.6 ± 0.2 vs. 1.9 ± 0.1 in WT), as compared to WT, while subsarcolemmal CaT amplitudes,TF50 and the % of early release sites (to 41 ± 5% in 26 Ob cells) were not significantly altered (Fig. 2C). In addition, the observed increase was not related to differences of diastolic [Ca] as the latter was found to be not significantly different between WT and Ob using ratiometric Ca imaging (F340/F380; 0.55 ± 0.03 vs. 0.54 ± 0.02 in n = 22 WT and 24 Ob cells; n.s.). Interestingly, in Ob at an age of 27 weeks the cellular CaT amplitude (F/F0) was significantly reduced to 54 ± 2% as compared to 21 weeks (to 1.5 ± 0.05 in Ob vs. 1.8 ± 0.07 in WT from n = 17 and 18 Ob and WT cells; p < 0.05), while it remained unchanged in WT. At 21 weeks, as in Ln, the Ca decay constant, tau, was unaltered (Fig. 2B).

Longitudinal/axial tubules have been shown to affect Ca release in atrial cells [24]. In support of this notion we could show in cardiomyocytes with simultaneous tubular net staining and CaT recordings, that enhanced local Ca release is associated with tubular structures (Fig. 3Ac). In the present model, 70% of WT cells, 75% of Ln cells and 82% of Ob cells (n = 66) showed a diverse tubular network including transverse and longitudinal tubules. In cells that displayed tubules, their total area occupied was not significantly different between the groups (21 ± 0.6 vs. 21 ± 0.5% in 52 WT and 55 Ob cells). To study if the distribution of the tubular net within the cells shows a difference, a 2 × 2 μm grid was superimposed on recorded images. The percentage of grids that showed a tubular (i.e. signal density per grid > 0%) net was significantly increased in Ob (74 ± 3 vs. 81 ± 1 in 52 WT and 55 Ob cells; p < 0.05). This was paralleled by a decreased standard deviation of local TF50 in Ob, indicating increased synchrony of Ca release along the (transverse) line-scan. To evaluate the short-term cellular effects of RAS activation in vivo in compensated and decompensated atrial remodeling, Ca signaling during ECC was measured after addition of Ang II. In the presence of Ang II for 8–10 min, cytosolic CaT amplitudes were significantly decreased in WT, Ln and Ob (by 30 ± 5% to 1.6 ± 0.1 in WT; by 44 ± 4% to 1.6 ± 0.1 in Ln and by 26 ± 5% to 2.0 ± 0.2 in Ob; n = 11, 8 and 8 cells). Interestingly, net SR Ca content was not significantly affected by Ang-II (to 3.5 ± 0.4, 3.8 ± 0.4 and 4.3 ± 0.7 in WT, Ln and Ob, n = 5 cells/group; n.s.). To mimic prolonged Ang II exposure on Ca homeostasis, CaT amplitudes were also assessed after 2.5 h incubation with Ang II,
yet they were not significantly different from F/F₀ after 8–10 min (2 ± 0.08, 2 ± 0.19, 1.8 ± 0.08 in n = 28, 12 and 27 WT, Ln and Ob cells; n.s.).

3.3. SR Ca leak during ECC is increased in hypertension and metabolic HFpEF

SR Ca leak is a hallmark feature of failing cardiomyocytes [25]. We tested SR Ca leak in Ln and Ob by electrically pacing cells at 3 Hz and monitor spontaneous Ca release events during a post-pacing period of 20 s (Fig. 5). While full duration at half maximum (74 ± 13 vs. 92 ± 13 vs. 94 ± 16 ms, n = 7, 18 and 11 in WT, Ln and Ob, resp.) and full width at half maximum (1.8 ± 0.2 vs. 2.5 ± 0.4 vs. 2.8 ± 0.4 μm, n = 7, 18 and 11 in WT, Ln and Ob, resp.) were unaltered, Ca spark frequency was significantly increased in Ln (to 1.9 ± 0.4 in Ln vs. 0.7 ± 0.2 in WT and 1.0 ± 0.2 Sparks/100 μm/s in Ob) but not in Ob vs. WT. However, Ca spark amplitude (0.7 ± 0.1 vs. 1 ± 0.1 vs. 1.3 ± 0.1, n = 7, 18 and 11 in WT, Ln and Ob, resp.) was significantly increased in Ob as compared to WT. This led to an increase of Ca spark mass (from 0.5 ± 0.1 in WT to 2.4 ± 0.4 in Ln and 1.2 ± 0.3 in Ob, n = 7, 18 and 11 in WT, Ln and Ob, resp.). Ca wave frequency was not different under the conditions used for the quantification of Ca sparks (0.03 ± 0.03 vs. 0.004 ± 0.003 vs. 0.007 ± 0.006 Ca waves/100 μm/s in 7, 18 and 11 WT, Ln and Ob cells; p > 0.05). In a second approach, SR Ca leak was measured using tetracaine. Interestingly, both Ln and Ob cells showed an increase in RyR-mediated SR Ca leak as compared to WT. Taken together these results indicate that Ca spark mediated SR leak and non-Ca spark mediated SR leak might be major contributors to SR Ca leak and enhanced Ca release in Ln and Ob rats, respectively.

In addition we assessed SERCA function in the model (Supplementary Fig. 3): SERCA2a/total protein was significantly decreased in Ob as compared to Ln (to 45 ± 8 vs. 103 ± 15% of WT, n = 4/group; p < 0.05), while no significant change was detected in phosphorylation of phospholamban/total phospholamban at the sites serine-16 (to 119 ± 12 vs. 98 ± 19% of WT, n = 4/group; n.s.) and threonine-17 (to 56 ± 10 vs. 120 ± 30% of WT, n = 3/group; n.s.). Moreover, SERCA activity, as derived from a mono-exponential fit to the decay of the Ca transient during electrical stimulation and during the application of Caffeine [26], was not significantly different between the groups (87 ± 3 vs. 87 ± 3 vs. 88 ± 3% in n = 9, 8 and 11 WT, Ln and Ob cells; n.s.).

3.4. Ang II leads to altered nuclear Ca during hypertensive heart disease and HFpEF

Nuclear Ca has been implicated in remodeling and hypertrophic gene program activation [27] and is known to be related to cytosolic Ca release as well as the nuclear-membrane Ca release and removal machinery [18]. In the present model, total Ca within the nuleoplasm (i.e. the area under the curve of CaT located to the nucleoplasmic region) was increased in Ln (to 424 ± 45 in Ln vs. 273 ± 21 in WT and 349 ± 28 in Ob, n = 22, 22 and 26 cells, resp.; p < 0.05) yet the ratio of nuclear to cytosolic Ca area under the curve (0.88 ± 0.05 vs.
0.89 ± 0.06 vs. 0.87 ± 0.05 a.u. in n = 22, 22 and 26 WT, Ln and Ob cells; p < 0.05) was unaltered, indicating a mere passive increase of nuclear Ca under baseline conditions. When Ang II was added, total nuclear Ca (Fig. 4) as well as the ratio of total nuclear to total cytosolic Ca were increased in Ob as compared to Ln and WT (to 0.20 ± 0.03 in 9 Ob vs. 0.13 ± 0.04 and 0.10 ± 0.02 a.u. in 8 Ln and 11 WT cells; p < 0.05 vs. Ln/WT), suggesting that Ang II promoted active Ca release from nucleoplasmic Ca stores in Ob. When the IP3-receptor blocker 2-APB was added to the experimentation chamber, nuclear CaT area under the curves were significantly decreased in Ob as compared to WT (to 123 ± 11 vs. 222 ± 33 a.u. in n = 21 Ob and 26 WT cells; p < 0.05) and not significantly changed in Ln (to 142 ± 18 a.u. in n = 18 cells).

3.5. In-vivo function is determined by left atrial area and related to CaT amplitudes

Left atrial diameter was inversely correlated with LVEF in vivo (Fig. 1A) of WT, Ln and Ob and cellular Ca signaling during ECC was altered differentially in the models. Last, we therefore correlated the results obtained by isolating single cardiomyocytes with our in-vitro findings from the same animals. In-vitro CaT amplitude was lowest in WT and associated with normal LA EF. In hypertensive atrial remodeling atrial ejection fraction was preserved and this was paralleled by an increase of CaT amplitudes, suggesting a compensatory mechanism. However in HFpEF related atrial remodeling, atrial ejection fraction was significantly decreased, indicating insufficient compensation on the cardiomyocyte level (Fig. 6).

4. Discussion

HFpEF describes a clinical syndrome associated with common systemic disease states such as arterial hypertension, diabetes mellitus or chronic kidney disease. HFpEF is likely the result of different cellular maladaptive processes associated with cardiac remodeling. As such, contribution of the atrial cardiomyocyte phenotype and cardiomyocyte function to contractile dysfunction in vivo may vary in quality and extent depending on the leading underlying systemic disease entity associated with atrial remodeling.

In the present model we show that the phenotype of hypertensive heart disease, i.e. arterial hypertension, LV hypertrophy and increased LVEDP were associated with intrinsic changes in atrial cardiomyocyte ECC even in the absence of atrial remodeling in vivo. Only in animals additionally prone to diabetes and adipositas [28] atrial remodeling in vivo was also observed. In these metabolic HFpEF rats (Ob), atrial remodeling and increased LV wall stress (as suggested by increased BNP) were associated with atrial contractile dysfunction in vivo in sinus rhythm. Reduced atrial ejection fraction in Ob was not explained - but rather (insufficiently) compensated - by an increased intrinsic cytosolic Ca transient amplitude and kinetics of the diseased atrial myocytes in physiological conditions. However, in patients with hypertensive heart disease as well as in hypertensive rats the RAS is activated and

Fig. 2. In vitro atrial function in a rat model of hypertension and metabolic HFpEF. A. Examples of local longitudinal systolic CaT at 1 Hz steady state stimulation in WT (top), lean (center) and obese (bottom) LA cells. Cartoon depicts the orientation of the respective line-scan (longitudinal). B. Quantification of CaT amplitudes at 1 Hz in the cytosolic (1st row) compartment. SR Ca content (2nd row) and time to 50% of maximal Ca release (TF50; 3rd row) as well as tau of the decay of cytosolic CaT (4th row) in the cytosolic compartment obtained from longitudinal line scans. C. Example of local CaT amplitudes in a WT and Ln cell (1st and 2nd row) and quantification (3rd row) of CaT amplitudes of local subbasal medial (SS, grey) and central cytosolic (CT, black) regions obtained from transversal line-scan images of WT, Ln and Ob cells. 4th row: Local time to 50% of maximal Ca release in WT, Ln and Ob. Cartoon depicts the orientation of the respective line-scan (transversal). *p < 0.05 vs. WT (Holm-Sidak method for comparison of CaT amplitudes in Fig. 2C). **p < 0.05 vs. Ln. ***p < 0.05 vs. Ob. n = 8 WT, 7 Ln and 10 Ob animals.
myocardial Ang II levels are increased [29,30]. By addition of Ang II we mimicked the effects of systemically increased Ang II as it is prevalent in vivo. In the presence of Ang II, contractile dysfunction as well as active release of Ca from the nuclear membrane were observable in Ob, potentially contributing to LA myopathy in vivo (Fig. 4).

ZSF-1 Ln rats develop LV hypertrophy (LVH) in response to arterial hypertension. As shown previously, Ln rats are without a relevant diabetic or nephropathic phenotype [28], and LVEF is preserved (Fig. 1). BNP was also not significantly increased in Ln vs. WT, suggesting that LA as a relevant source of BNP was able to compensate LV wall stress and balance the increased intracardiac pressure [31]. Spontaneous hypertensive rats develop no apparent atrial phenotype after 28 weeks [32] and atrial fibrosis is observed as late as 70 weeks of age [33] which indicates a rather slow development of atrial myopathy. Hamdani et al. reported unchanged diastolic function in this model. We observed first signs of diastolic dysfunction in Ln which might be related to differences in heart rate and the use of high caloric diet in all animals as opposed to Ob only in the Hamdani study [28]. Interestingly,
atrial cardiomyocyte Ca transient amplitudes were increased in Ln animals, suggesting a compensatory inotropic response to increased mechanical load. This is in agreement with results from human studies, where LV stroke volume was maintained in hypertensive heart disease due to a compensatory increase of LA emptying fraction \[34\]. In a model of hypertensive heart disease similar to ZSF Ln (i.e., spontaneous hypertensive rats; non-fat diet) an increase of central cytosolic CaT amplitudes has been observed in atrial cells \[32\] at an age of seven month, supporting the notion that Ca release is altered in hypertension and compensatory cardiac remodeling. We also observed increased SR Ca leak in Ln using two different approaches (tetracaine, sparks), suggesting that Ca leak occurs during hypertensive atrial remodeling, which may indicate a different remodeling phenotype as compared to atrial fibrillation \[35\]. In atrial cells, subsarcolemmal transients are primarily constituted of Ca influx through L-type Ca channels and RyR mediated SR Ca release. Subsarcolemmal CaT are more tightly coupled to CICR via L-type Ca channels than central CaT and the respective j-SR depletion is smaller than the non-junction counterpart. This has been shown only recently by Maxwell et al., who connected central cytosolic

Fig. 5. Ca leak in ZSF rats. A. Example recordings of Ca sparks in WT, lean and obese in a longitudinal line-scan (left). Example of single spark recording (right). B. Left. Quantification of Ca spark frequency in WT, lean and obese. Right. Amplitude of Ca sparks in WT, lean and obese. C. Left and Center. Full duration at half maximum and full width at half maximum of Ca sparks in WT, lean and obese. Right. Estimated Ca spark mass as calculated from spark frequency and amplitude. Example (C, right) and quantification (C, left) of ryanodine receptor (RyR) mediated Ca leak at obtained after steady-state stimulation and addition of 0Na/0Ca and solution containing tetracaine. \*p < 0.05 vs. WT. n = 6 WT, 5 Ln and 6 Ob animals for Ca spark data. n = 3 WT, 2 Ln and 2 Ob animals for tetracaine data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 6. In-vivo in-vitro correlation of CaT peak amplitudes (Fpeak) and left atrial ejection fraction (LA EF).
non-junctional release to a so-called “fire-diffuse-uptake-fire” mechanism, where Ca release is activated by cytosolic and luminal Ca, leading to higher SR depletion as compared to the junctional-SR. This indicates that regulation of Ca release in the n-j / central part of the cell has more adaptive capacity than SR Ca release from the j-SR and that the central cytosolic Ca release can be augmented more easily and might explain the observed increase of CaT amplitudes in Ln primarily in the cell center.

We and others have shown previously that increased cytosolic Ca leak promotes maladaptive remodeling in cardiomyocytes, most likely through Ca mediated signaling within the nucleus [36,37]. Our tetrodoin experiments suggest that diastolic Ca leak was increased in Ln vs. WT, and thus nuclear Ca exposure may have been even higher than estimated from the CaT normalized to F/F0. Indeed, in Ln total nuclear Ca was increased, likely related to increased diffusion from the cytosol throughout the cardiac cycle. As the ratio between increased cytosolic and nuclear CaT area under the curve (representing Ca exposition during ECC with potential effects on transcriptional processes [38]) remained unchanged in Ln vs. WT, we do not assume additional active release of Ca from the nuclear envelope in these diseased, but ex vivo unstimulated cardiomyocytes.

Obese ZSF rats develop metabolic syndrome and HFpEF as shown here and by other groups [28,39]: LVEF was preserved and LVEDP, BNP levels, LA size and body weight were increased as compared to Ln and WT rats (Fig. 1, see also [39,40]). LA enlargement was associated with reduced LA ejection fraction and both have been related to late stage diastolic dysfunction [41,42]. Even though LA pressure has shown to be increased in atrial hypertensive remodeling (e.g. in spontaneous hypertensive rats, [43]), LA enlargement and LA ejection fraction alone are not good predictors for diastolic dysfunction [44].

Arterial blood pressure was different in Ob as compared to Ln and WT, which is in accordance with previous reports in this model [28]. As a limitation of this study, we cannot exclude that differences in arterial blood pressure may account for some of the observed in-vivo and in-vitro differences between the models. However, systolic blood pressure did not correlate with LA area within and between the groups (Supplementary Fig. 2.), indicating that other factors related to metabolic syndrome and obesity might play a role for the observed atrial myocardial phenotype.

In the present model of metabolic HFpEF (Ob), reduced atrial ejection fraction in vivo was observed despite increased cardiomyocyte CaT amplitude. Regulation of Ca release in the n-j / central part of the cell has more adaptive capacity [20] and recent data shows that the alteration of CaT amplitude during atrial remodeling might be related to increased baseline inositol-1,4,5-trisphosphate receptor (IP3R) activity. IP3R have been shown to be more prevalent in the cell center of cardiomyocytes undergoing maladaptive remodeling [26]. Indeed, despite similarly increased cytosolic CaT amplitudes, altered Ca release in Ob at 21 weeks was different from what was observed in Ln animals: Ca spark frequency was not different from WT, while Ca spark amplitude was increased (Fig. 4). Similarly, RyR-mediated SR Ca leak was increased in Ln and Ob when quantified using an adapted version of the Shannon protocol [45]. These results would be in agreement with IP3R mediated Ca release bringing RyR closer to its activation threshold and leading to enhanced Ca release throughout the cardiac cycle [46]. Of note, SERCA activity and phospholamban phosphorylation were not significantly different between the groups. However we determined SERCA activity under steady state conditions and other regulatory proteins like sarcolipin have been shown to be pivotal for SERCA function in atrial cells [47]. Since SERCA adaptation is highly Ca sensitive [48] we still presume a higher SERCA activity during conditions of more SR Ca leak. As shown by Moirimoto et al. a higher SERCA activity per se does not necessarily lead to increased SR Ca leak [49] and changes of SERCA activity have only modest effects on SR Ca content [50] supporting the notion of altered RyR- or IP3R- mediated Ca release in Ln and Ob cells as foundation of the observed enhanced SR Ca leak.

Interestingly the CaT amplitude increase in Ob was only transient, as CaT amplitudes were significantly reduced at a later time-point of 27 weeks, further underscoring the transient compensatory nature of active atrial cardiomyocyte-dependent compensation in Ob.

Another potential mechanism for enhanced Ca release has been recently proposed by Brandenburg et al.: The group found axial tubular structures in atrial cells during remodeling forming an intracellular subdomain of Ca release [24]. In support of this notion, we were able to identify longitudinal and transversal tubular structures in atrial cells as well. They were more prevalent and more extensively organized throughout the cytosol in cells from Ob animals. Interestingly, we found that increased Ca release in Ln and Ob did not occur at the junctional SR close to the outer cell membrane, but only in the central parts of the cardiomyocyte, where we would expect tubular structures. Indeed, we were able to detect increased Ca release at sites with tubular structures and observed a more synchronous and faster (i.e. TF50 was accelerated as compared to Ln) Ca release in Ob animals (Fig. 2B and 3E). Increased synchrony has been shown to increase the global Ca transient amplitude and improves effectiveness of Ca release for the activation of myofilaments [51].

Increased cytosolic CaT amplitude and Ca leak in Ob may be implicated in cellular signaling beyond ECC. We did not observe an over proportional increase in nuclear vs. cytosolic Ca in Ob at baseline. However, the RAS is known to be over-active in Ob animals [52], and atrial cardiomyocytes from Ob responded to ATII with a significant relative increase in nuclear CaT as compared to Ln and WT, not explained by rather lower cytosol CaT (Fig. 1), suggesting active (IP3R-mediated) Ca release from the nuclear envelope. IP3R are more prevalent on the inside of the nuclear membrane [18] and the addition of 2-APB preferentially decreased nuclear CaT in Ob, further underscoring active IP3R-mediated Ca release affecting nuclear CaT in Ob.

Stimulating atrial myocytes at higher frequency (3 Hz) did not unmask alterations in the cytosolic CaT in Ob vs. WT or Ln that could have explained atrial contractile dysfunction in vivo (Supplementary Fig. 1). In animal models, but also in human congestive heart failure (mixed population of HFpEF and HFrEF), atrial remodeling could be also linked to increased atrial fibrosis, mediated by TGF-beta and/or Ang II [53,54]. In vivo contractile dysfunction might also be related to a functional (i.e. stretch-related) increase in atrial volume and therefore increased cellular distention. This in-vivo compensation despite enhanced excitation-contraction coupling adversely affects hemodynamics and potentially impacts the occurrence of atrial fibrillation and other atrial cardiomyopathy related pathologies [55]. Atrial remodeling has been shown to independently predict mortality in patients with HFpEF [1].

In conclusion, enhanced Ca release during ECC can be observed in vitro in atrial cardiomyocytes during hypertensive and metabolic HFpEF-related remodeling. Enhanced Ca release in atrial cardiomyocytes may serve as a compensatory measure to preserve atrial function in vivo during conditions of increased mechanical load. An altered tubular network might contribute to the observed atrial cardiomyocyte phenotype. In the metabolic HFpEF phenotype, atrial remodeling results in atrial contractile dysfunction despite enhanced Ca transients of atrial cardiomyocyte in vitro (Supplementary Fig. 4). During remodeling atrial myocyte nuclear Ca signaling undergoes profound changes with passive increases of nuclear Ca during hypertensive disease and active nuclear Ca release in response to RAS activation in HFpEF. Inhibition of the RAS might protect from atrial remodeling in a metabolic HFpEF phenotype.

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Conflict of interest

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Appendix A. Supplementary data

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References


