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The estrogen receptor-α agonist 16α-LE2 inhibits cardiac hypertrophy and improves hemodynamic function in estrogen-deficient spontaneously hypertensive rats

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Abstract

Objective: Cardiac mass increases with age and with declining estradiol serum levels in postmenopausal women. Although the non-selective estrogen receptor-α and -β agonist 17β-estradiol attenuates cardiac hypertrophy in animal models and in observational studies, it remains unknown whether activation of a specific estrogen receptor subtype (ERα or ERβ) might give similar or divergent results. Therefore, we analyzed myocardial hypertrophy as well as cardiac function and gene expression in ovariectomized, spontaneously hypertensive rats (SHR) treated with the subtype-selective ERα agonist 16α-LE2 or 17β-estradiol.

Methods and Results: Long-term administration of 16α-LE2 or 17β-estradiol did not affect elevated blood pressure, but both agonists efficiently attenuated cardiac hypertrophy and increased cardiac output, left ventricular stroke volume, papillary muscle strip contractility, and cardiac α-myosin heavy chain expression. The observed effects of E2 and 16α-LE2 were abrogated by the ER antagonist ZM-182780. Improved left ventricular function upon 16α-LE2 treatment was also observed in cardiac MRI studies. In contrast to estradiol and 16α-LE2, tamoxifen inhibited cardiac hypertrophy but failed to increase α-myosin heavy chain expression and cardiac output.

Conclusions: These results support the hypothesis that activation of ERα favorably affects cardiac hypertrophy, myocardial contractility, and gene expression in ovariectomized SHR. Further studies are required to determine whether activation ERβ mediates redundant or divergent effects.

Keywords: Cardiac hypertrophy; Estrogen receptor; Spontaneously hypertensive rats

This article is referred to in the Editorial by G. Schönfelder (pages 573–574) in this issue.

1. Introduction

Cardiac hypertrophy is an established and independent risk factor for the development of heart failure and sudden cardiac death [1]. The observation that female gender and sex hormones attenuate left ventricular hypertrophy in humans and in animal models has defined the myocardium as a direct target for estrogens [2–6]. The biological effects of estrogens are transmitted by two different estrogen receptors, ERα and ERβ, which are encoded by different

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genes and mediate redundant, divergent or even opposing effects in different tissues [7]. Previous studies were focused primarily on the role of non-selective ERα and ERβ agonists such as 17β-estradiol in the development of cardiac hypertrophy. Thus it is currently unknown whether either ERα or ERβ or simultaneous activation of both receptor subtypes is required to attenuate cardiac hypertrophy. The observation of distinct biological functions for ERα and ERβ in genetic mouse models lacking ERα or ERβ has guided the development of potent, isotype-selective ER ligands, which represent a novel tool to dissect the ligand-dependent function of ERα and ERβ. The present study employed the recently designed steroidal ERα agonist 16α-LE2 (also termed cpd1471). Isotype-selectivity of 16α-LE2 is thought to result from a bulky lactone ring bridging positions 16α and 17α, which is accommodated by the ligand binding pocket of ERα but not of ERβ [8]. The relative binding affinity of 16α-LE2 for human and rat ERα is 70-fold higher than for ERβ, the transcriptional selectivity is 250-fold higher from ERα than from ERβ whereas the biological potency is lower on ERα dependent functions compared to 17β-estradiol [8–10]. Isotype selectivity of 16α-LE2 for ERα has recently been demonstrated also under in vivo conditions. Ovary weight, as a functional parameter of ERβ activation, increased in hypophysectomized rats treated with 17β-estradiol or the ERβ selective agonist 8β-VE2 but remained low in rats treated with 16α-LE2 over a very broad dosage range [10]. Besides elucidating the biological function of ligand dependent ERα and ERβactivation, isotype-selective ER agonists might also have future clinical relevance to improve the efficiency and safety of non-selective ERα and ERβ ligands such as 17β-estradiol [11–15]. As we reported recently, the selective ERα agonist 16α-LE2 confers favorable effects in the vascular system [9]. Because ERα and ERβ are functionally co-expressed also in cardiac myocytes we have now tested the hypothesis that 16α-LE2 might also favorably affect cardiac hypertrophy, gene expression and function.

2. Methods

2.1. Animal model and treatment

Female SHR (Charles River™) were ovariectomized or sham operated at 6 weeks of age under isoflurane anesthesia. At day 1 after surgery animals were randomized and treated continuously for 12 weeks by daily s.c. injection with 17β-estradiol (E2; 2 μg/kg/d, Sigma), 16α-LE2 (30 μg/kg/d, Schering AG) or tamoxifen (tmx; 670 μg/kg/d, Sigma) until sacrifice at 18 weeks of age ($n=10$ animals/group). Identical studies were performed in ovx SHR co-treated with E2 or 16α-LE2 plus the ER antagonist ZM-182780 (ZM, 500 μg/kg/d, Tocris). 17β-estradiol, 16α-LE2, ZM-182780 and tamoxifen were dissolved in EtOH and injected using peanut oil as carrier, ovx control animals received EtOH / peanut oil alone. Further studies of identical design were initiated for cardiac MRI analysis and tamoxifen treatment. Total body weight, heart weight, uterus weight and tibia length were measured following hemodynamic analysis; relative heart weight was calculated from tibia length and absolute heart weight. Estradiol serum levels were measured by radio immunoassay (DPC-Biermann, Germany) using serum samples obtained after hemodynamic analysis. All protocols were reviewed and accepted by the local ethics committee and performed in accordance with the current NIH guide for the care and use of laboratory animals.

2.2. Hemodynamic analysis

Hemodynamic measurements were performed according to published protocols after 3 months of treatment at 18 weeks of age under light isoflurane anaesthesia and spontaneous respiration (isoflurane 1.5 vol.% supplemented by 0.5 l oxygen per min) [16]. Pressure curves were measured via fluid-filled PE 50 tubing connected to a microtip manometer (Millar Instruments) inserted via the right carotid artery and calibrated to mid-chest level. Left ventricular pressure curves were recorded after catheter placement in the LV cavity, systolic and diastolic blood pressure measurements were obtained upon catheter withdrawal in the thoracic aorta. During positive pressure ventilation following a midsternal thoracotomy, a calibrated flowmeter (2.5 mm; Statham) was placed around the ascending aorta for continuous measurement of aortic blood flow (cardiac output). Stroke volume was calculated from cardiac output and heart rate (SV=CO/HR), cardiac index was calculated by normalizing cardiac output to body weight (CI=CO/BW). Measurements were performed by a trained observer blinded for treatment groups.

2.3. Cardiac MRI experiments

MRI experiments were performed after 12 weeks of treatment at 18 weeks of age on a 7.05 Tesla BIOSPEC 70/21 (Bruker, Germany) under spontaneous respiration (isoflurane 1.5 vol.% supplemented by 0.5 l oxygen per min) using a rat-size whole body coil and an ECG-triggered fast gradient echo sequence (FLASH) as described before [17]. Quantitative assessment of morphology and function included 10–12 contiguous ventricular short axis slices of 2 mm thickness covering the entire heart at an in plane resolution of 390 μm. Data analysis was performed by one trained observer blinded regarding the treatment groups. Cardiac and ventricular slice volumes were determined from end-diastolic and end-systolic images by multiplication of compartment area and slice thickness (2 mm). Total volumes were calculated as sum of all slice volumes.

2.4. Isolated heart muscle preparations

Hearts were removed after hemodynamic analysis, perfused with cold Krebs–Henseleit solution (KHS) and papillary muscle strips were dissected out of the left ventricle as described before [18]. The fibers were mounted between a force transducer and servomotor (Scientific Instruments, Heidelberg FRG) and perfused with oxygenated KHS-solution at 37 °C. Force–frequency relationships were determined by measuring developed systolic force at increasing stimulation frequencies (60, 120, 200, 300 and 450/min), absolute values were normalized to the specimens cross sectional area and resting tension.

2.5. Gene expression studies

Cardiac extracts were prepared according to standard protocols from the ventricles after removing the atria. The expression of α- and β-MHC protein was analyzed by silver staining of denaturing acrylamide gels of unfractionated cardiac extracts followed by densitometric quantification based on peak height ("ScanPack-3.0" / Biometra) [5]. The expression of estrogen receptor-α and -β (ERα: SRA 100 / Stressgen 1:2.000; ERβ: CO 1531, 1:1.000, generous gift of G. Greene Univ. of Chicago), ANP (Chemicon 1:2000 rabbit polyclonal), GAPDH (Chemicon 1:3000 rabbit polyclonal) and alpha B-crystallin (Stressgen 1:6000 rabbit polyclonal) was analyzed by Western blotting using the indicated primary antibodies, horseradish peroxidase coupled secondary antibodies (anti-mouse, anti-rabbit; Amersham, 1:5.000) and the ECL detection system (Amersham). Equal gel loading was verified by Ponceau staining, GAPDH or alpha-B-crystallin expression. Recombinant estrogen receptor protein for ERα and ERβ (Panvera) was employed to verify specificity of the ERα and ERβ antibodies. Cultured neonatal cardiac myocytes were prepared as described elsewhere [5].

2.6. Statistics

Statistical significance was calculated by one-way ANOVA variance analysis followed by Student Newman–Keuls post-hoc testing in all experiments except cardiac MRI studies, which were analyzed by two-tailed Student's *t*-tests. Values are mean ± SEM, *p*-values <0.05 were considered significant.

3. Results

3.1. Ovariectomy and hormone treatment

Serum estradiol levels and uterus weight varied substantially with the estrus cycle in sham operated SHR; thus statistical analysis of uterus weight was performed also only on ovx rats (Table 1). Serum estradiol levels were lower in ovx compared to sham operated animals and associated with uterus atrophy and a significant gain of body weight. Serum E2 levels increased in SHR treated with E2 or E2 plus ZM-182780 but remained low in animals receiving 16α-LE2 (Tables 1 and 2). Uterus weight was higher and body weight was lower in estradiol and 16α-LE2 treated rats compared to ovx rats receiving placebo injections. Co-treatment with the ER antagonist ZM-182780 decreased uterus weight and increased body weight in SHR receiving E2 or 16α-LE2 (Table 2).

3.2. Cardiac hypertrophy and morphology

Absolute heart weight as well as relative heart weight normalized to tibia length was only slightly higher in ovariectomized compared to sham operated rats but reached significance upon analysis of all animals from study protocols 1–3 (Table 1; $p<0.05$). Cardiac hypertrophy was significantly reduced to equal extent in animals

Table 1
Global and hemodynamic measurements

n	sham 10	ovx 10	ovx+E2 10	ovx+16α-LE2 10
E2 serum level [pg/ml]	65±21*	31±1	121±9*	25±1
Uterus weight [mg]	338±107*	59±6	221±13(*)	164±11(*)
Body weight [g]	189±6*	239±5	200±2*	189±4*
Absolute heart weight [mg]	969±17	994±16	915±18*	924±19*
Relative heart weight [mg/cm tibia length]	343±9	363±13	315±9*	314±5*
Mean blood pressure [mm Hg]	189±9	184±4	174±4	171±5
dp/dt max [mm Hg/s]	16,171±484	14,832±793	16,133±653	15,600±1110
Heart rate [bpm]	349±9	348±11	383±7	388±7
Cardiac output [ml/min]	54±2	52±2	60±3	71±4*‡
Cardiac index [ml/min/kg]	285±9*	217±9	300±15*	375±14*‡
LV stroke volume [μl]	154±3	149±3	156±5	182±6*‡

Abbreviations: dp/dt max: maximal contraction velocity. (one-way ANOVA; *$p<0.05$ vs. ovx; ‡$p<0.05$ vs. ovx+E2); (*)significances of ovx SHR only.

Table 2
Global and hemodynamic measurements in SHR co-treated with the ER antagonist ZM-182780

n	ovx+E2 10	ovx+16α-LE2 10	ovx+E2+ZM 10	ovx+16α-LE2+ZM 10
E2 serum level [pg/ml]	87±111	28±3	146±12	28±2
Uterus weight [mg]	202±10*	193±11•	69±3	107±10
Body weight [g]	205±3*	191±3•	228±5	218±3
Absolute heart weight [mg]	889±13*	895±14•	1004±11	995±21
Relative heart weight [mg/cm tibia length]	317±9*	319±6•	364±13	359±12
Mean blood pressure [mm Hg]	163±5	164±4	173±6	171±6
dp/dt max [mm Hg/s]	15,467±600	15,200±595	15,956±1132	16,533±653
Heart rate [bpm]	334±9	330±10	348±12	334±13
Cardiac output [ml/min]	64±3*	68±4•	54±2	53±2
Cardiac index [ml/min/kg]	312±9*	356±10•	236±12	243±9
LV stroke volume [μl]	191±6*	204±6•	156±8	158±7

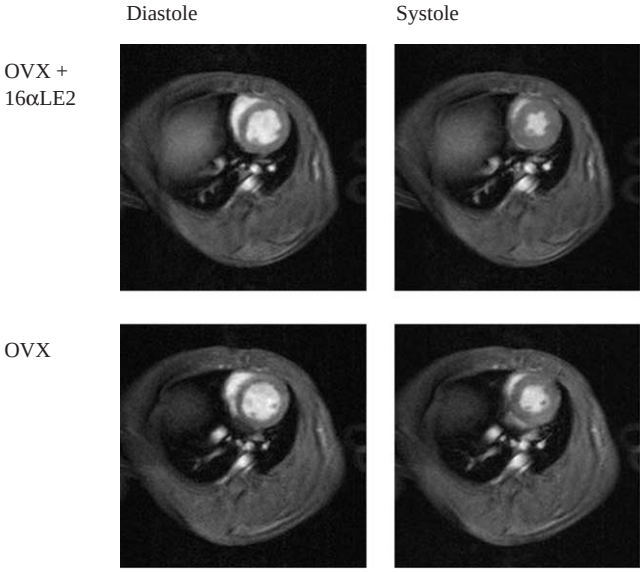
One-way ANOVA; *$p<0.05$ vs. ovx+E2+ZM; •$p<0.05$ vs. ovx+16α-LE2+ZM.

treated with the selective ERα agonist 16α-LE2 or 17β-estradiol. Co-administration of ZM-182780 increased absolute and relative heart weight in SHR treated with E2 or 16α-LE2 (Table 2).

Table 3
Cardiac MRI measurements

n	ovx 10	ovx+16α-LE2 10
Body weight [g]	235±4	203±3*
Cardiac mass [normalized to ovx]	1.00±0.03	0.89±0.01*
LV end-diastolic volume [μl]	373±12	346±7
LV end-systolic volume [μl]	158±11	125±6*
LV ejection fraction [%]	57±1	64±1*
RV end-diastolic volume [μl]	280±18	281±6
RV end-systolic volume [μl]	98±8	69±5*
RV ejection fraction [μl]	64±2	75±1*

Cardiac mass (ovx=1.0) was lower and left as well as right ventricular end-systolic volume and ejection fraction were higher in ovx SHR treated with 16α-LE2 compared to placebo treatment (two-tailed Student's *t*-test; *$p<0.05$).



3.3. Hemodynamic analysis

Cardiac output, which was similar in sham operated and ovx SHR, was significantly higher in SHR treated with 16α-LE2 compared to all other groups including animals receiving E2 (Table 1). 17β-estradiol augmented cardiac output to a lower and insignificant level. Cardiac index, which was lower in ovx compared to sham operated rats, increased upon treatment with E2 and 16α-LE2. Calculated left ventricular stroke volume was significantly higher in 16α-LE2 treated SHR compared to all other groups including rats receiving estradiol. Cardiac output, cardiac index and left ventricular stroke volume were lower in SHR co-treated with the ER antagonist ZM-

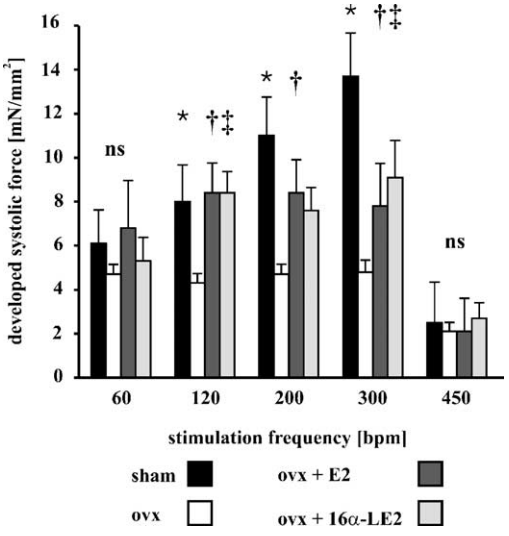


Fig. 1. Force–frequency relationship of isolated heart muscle preparations. Developed systolic force of papillary muscle preparations normalized to cross sectional area is plotted versus stimulation frequency. Heart muscle preparations from ovx animals failed to reveal the positive force–frequency relationship that was observed in ERα agonist and E2 treated rats as well as in sham operated controls. ($n=10$ animals/group; one-way ANOVA; *sham vs. ovx<0.05, †ovx+ E2 vs. ovx<0.05, ‡ovx+ 16α-LE2 vs. ovx<0.05).

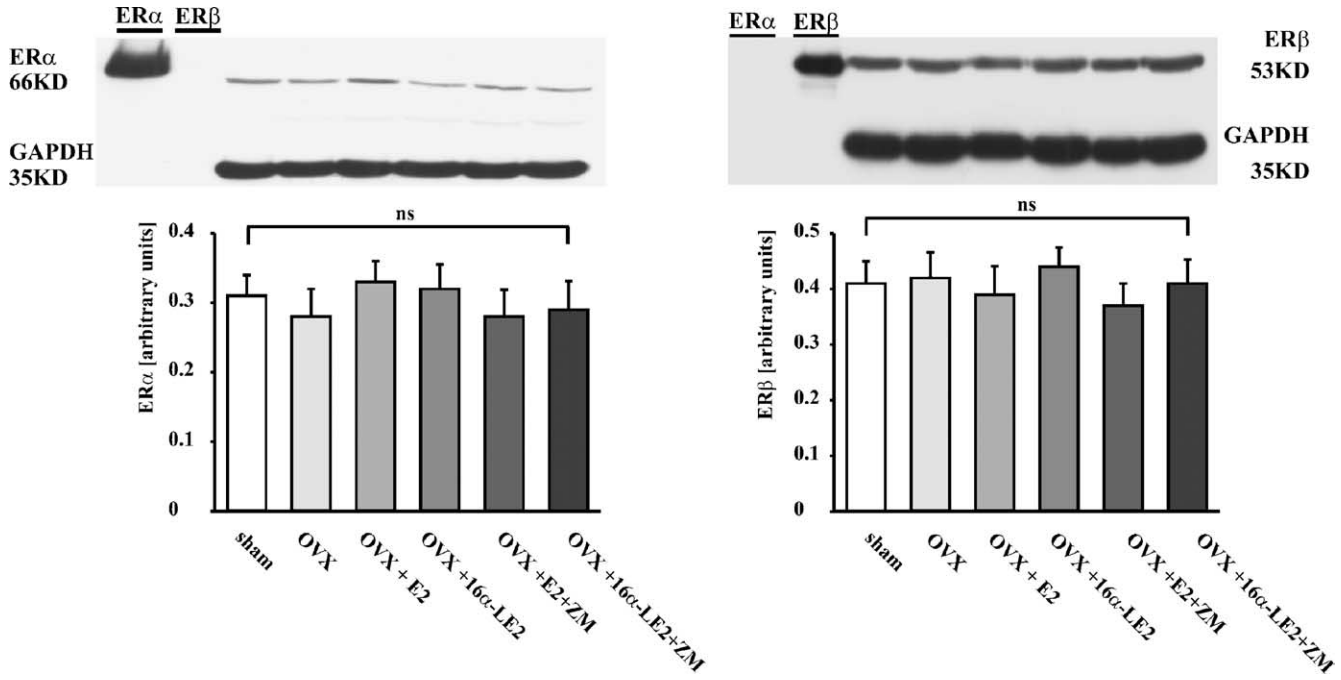


Fig. 2. Cardiac expression levels of ERα and ERβ. Western blot analysis of cardiac extracts for ERα and ERβ revealed comparable expression levels of ERα and ERβ in all groups. The ERα and ERβ antibodies detected their cognate protein at the expected molecular weight without cross reactivity as determined by blotting recombinant ERα and ERβ protein. ($n = 10$ animals/group; one-way ANOVA; $p > 0.05$).

182780 (Table 2). Ovariectomy, treatment with E2, 16α-LE2 or co-administration of ZM-182780 did not affect elevated mean arterial blood pressure, left ventricular contraction velocity and heart rate compared to sham operated rats (Tables 1 and 2).

3.4. Cardiac MRI

Cardiac mass determined by MRI was lower in 16α-LE2 treated SHR compared to ovariectomized rats (Table 3). Left ventricular end-diastolic volume was not statisti-

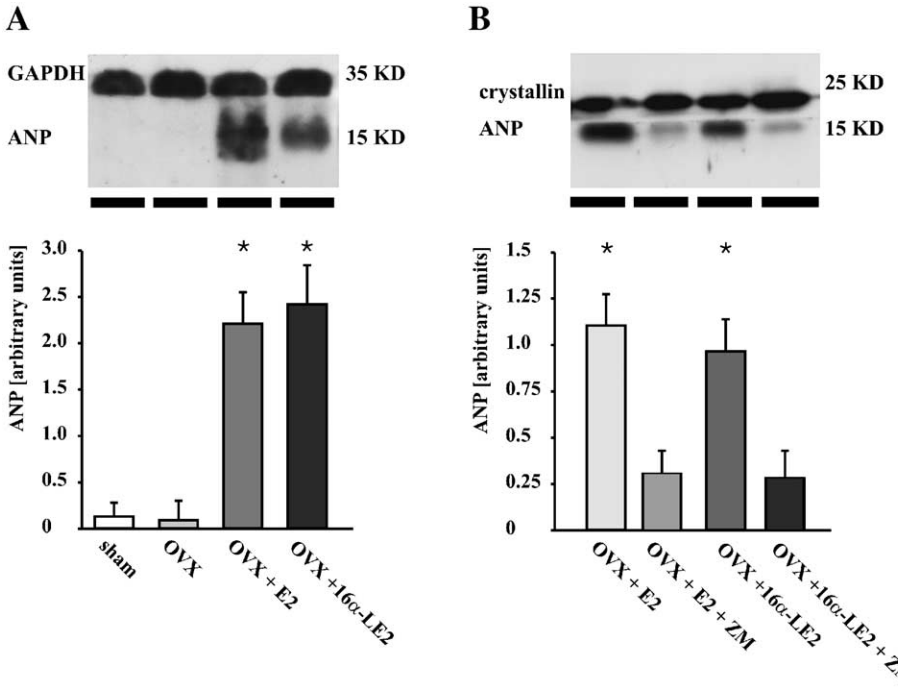


Fig. 3. (A) Cardiac ANP expression. ANP expression was elevated in left ventricular extracts ovx SHR treated with E2 or 16α-LE2 compared to sham operated and ovx SHR ($n = 10$ animals/group; one-way ANOVA; *$p < 0.05$ vs. ovx). (B) The ER antagonist ZM-182780 attenuated left ventricular ANP expression in E2 and 16α-LE2 treated SHR. ($n = 10$ animals / group; one-way ANOVA; *$p < 0.05$ vs. E2 + ZM or 16α-LE2 + ZM).

cally different among placebo and 16α-LE2 treated SHR but end-systolic volume was significantly lower and left ventricular ejection fraction was higher in the 16α-LE2 group compared to ovx rats receiving placebo. Right ventricular performance markers including end-systolic volume and ejection fraction improved to a comparable extent as left ventricular functional parameters upon 16α-LE2 treatment.

3.5. Isolated heart muscle preparations

Developed systolic force of heart muscle preparations was similar at basal stimulation rate in sham, ovx, 16α-LE2 and estradiol treated rats. At higher stimulation frequencies, force–frequency relationships were blunted in papillary muscle from ovx rats. Developed systolic force normalized for papillary muscle cross sectional area increased in 16α-LE2, sham operated and E2 treated animals (Fig. 1).

3.6. Cardiac estrogen receptor and ANP expression

Cardiac expression of ERα and ERβ protein was comparable among all treatment groups (Fig. 2). At the respective conditions, both ER antibodies specifically detected their cognate protein (ERα or ERβ) without cross-reactivity as verified by blotting of recombinant ERα and ERβ protein. Ventricular ANP expression was detected in E2 and 16α-LE2 treated SHR but not in ovx and sham operated rats (Fig. 3A). ZM-182780 attenuated LV ANP expression in SHR receiving E2 or 16α-LE2 (Fig. 3B).

3.7. Myosin heavy chain expression

The ratio of α-to β-isomyosin expression was shifted towards β-MHC in ovx SHR receiving placebo compared to sham operated control (Fig. 4A). Treatment of ovx rats with 16α-LE2 and E2 shifted the ratio of MHC protein

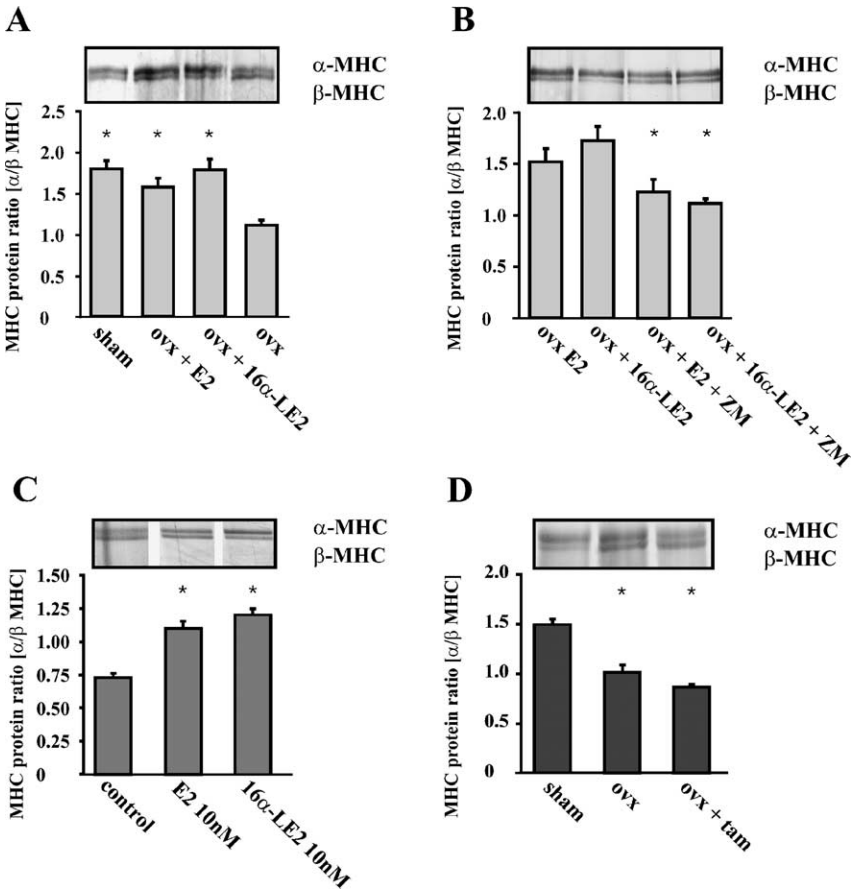


Fig. 4. Myosin heavy chain protein expression. (A) The ERα agonist 16α-LE2 and E2 shifted the decreased α/β-MHC protein ratio in ovx rats back towards predominant α-MHC expression ($n=10$ animals/group; one-way ANOVA; *$p<0.05$ vs. ovx). (B) The ER antagonist ZM-182780 shifted cardiac isomyosin expression towards predominant β-MHC expression in SHR receiving E2 or 16α-LE2 ($n=10$ animals/group; one-way ANOVA; *$p<0.05$ vs. ovx+E2 or vs. 16α-LE2). (C) Treatment of isolated cardiac myocytes with 16α-LE2 (10 nM) or E2 (10 nM) increased α-MHC relative to β-MHC expression compared to untreated control ($n=3–5$ independent experiments; one-way ANOVA; *$p<0.05$ vs. control). (D) Cardiac α-MHC expression was unaltered by tamoxifen treatment of ovx SHR ($n=10$ animals/group; one-way ANOVA; *$p<0.05$ vs. sham).

Table 4
Global and hemodynamic measurements in tamoxifen treated SHR

n	sham 10	ovx 10	ovx+tam 10
E2 serum level [pg/ml]	63±34	3±0.3	6±0.9
Uterus weight [mg]	362±34*	54±5	152±7*
Body weight [g]	197±5*	244±5	195±2*
Heart weight [mg]	861±17*	952±18	741±14*
Relative heart weight [mg/cm tibia length]	253±9*	299±9	211±4*
Cardiac output [ml/min]	60.6±3.5	58.4±1.8	51.9±2.2
Mean blood pressure [mm Hg]	163±4	157±6	164±4

Tamoxifen increased uterus weight and decreased relative heart weight and body weight in ovx SHR. Mean blood pressure, cardiac output and LV stroke volume were unaffected by tamoxifen treatment. ($n=10$ animals/group; one-way ANOVA; *$p<0.05$ vs. ovx).

expression towards α-MHC. Accordingly, MHC ratios were similar and statistically not different among sham operated controls and ovx SHR treated with 16α-LE2 and E2. Co-treatment with the ER antagonist ZM decreased α-MHC expression in SHR receiving E2 or 16α-LE2 (Fig. 4B). A shift towards predominant α-MHC expression was also observed in isolated cardiac myocytes incubated for 72 h with 10 nM E2 or 10 nM 16α-LE2 (Fig. 4C).

3.8. Cardiac hypertrophy, gene expression and LV function in tamoxifen treated SHR

Uterus weight increased significantly with tamoxifen treatment in ovx SHR but remained below the levels observed in intact rats (Table 4). Body weight was significantly lower in sham operated and in tamoxifen treated rats compared to ovariectomized animals receiving placebo treatment. Mean arterial blood pressure was comparably elevated in all groups but absolute and relative heart weights were significantly lower in intact and tamoxifen treated rats compared to the placebo group. In contrast to 17β-estradiol and 16α-LE2, tamoxifen did not improve cardiac output, cardiac index and stroke volume nor did tamoxifen increase myocardial α-MHC expression in ovx SHR (Fig. 4D).

4. Discussion

Cardiac mass is significantly attenuated by the non-selective ERα and ERβ agonist 17β-estradiol but it is currently unknown whether activation of a specific ER subtype might confer a similar protective effect in cardiac hypertrophy. The current study shows that the selective ERα agonist 16α-LE2 is sufficient to attenuate cardiac hypertrophy and to improve hemodynamic function in estrogen-deficient SHR, which is linked to differential expression patterns of cardiac myosin heavy chains.

Cardiac mass in SHR increases due to elevated cardiac afterload and inhibition of cardiac hypertrophy by 16α-LE2 and E2 might be due to lower blood pressure levels. As we have previously shown, endothelial dysfunction is improved by selective activation of ERα in ovx SHR but this does not affect blood pressure levels, which were comparably elevated among all treatment groups and do thus not explain the anti-hypertrophic effect of 16α-LE2 and E2 [9]. Instead, activation of ERα is more likely to modulate the signal transduction pathways that become activated in cardiac muscle growth. Estradiol has recently been shown to attenuate cardiac myocyte hypertrophy in vitro via induction of cardiac ANP expression [19]. In keeping with these observations, left ventricular ANP expression was increased in E2 and 16α-LE2 treated compared to ovx SHR. Therefore it appears conceivable that estrogen dependent ANP expression attenuates cardiac hypertrophy also under in vivo conditions. Co-treatment with the pure ER antagonist ZM-182780 lowered cardiac ANP expression and increased cardiac mass in 16α-LE2 and E2 treated SHR, which supports the interpretation that 16α-LE2 and E2 both act via bona fide ER mediated mechanisms [20]. Multiple signal transduction pathways have been implicated in the development of cardiac hypertrophy including the mitogen activated protein kinase system as reviewed recently [21]. Because raloxifen inhibits p38 MAP-kinase phosphorylation and cardiac myocyte hypertrophy in vitro, we also analyzed p38 activation (data not shown) but cardiac p38 expression and phosphorylation were comparable among all treatment groups [22]. Therefore, activation or inhibition of p38 MAP-kinases, which is has recently been recognized to confer very different effects on cardiac myocyte hypertrophy under in vitro or in vivo conditions, appears unlikely to contribute to the anti-hypertrophic effects of 16α-LE2 or E2 [23–25].

The biological effects of nuclear hormone receptors are regulated by receptor expression and ligand dependent receptor activation. Since both ER subtypes are co-expressed in cardiac myocytes it is conceivable that cardiac mass is affected specifically by ERα or ERβ. Alternatively, both ER subtypes could have a redundant or even a synergistic function to inhibit cardiac growth. The aim of this study was to evaluate the functional importance of endogenous ERα activation in cardiac hypertrophy by employing a selective ERα agonist, which binds and transactivates ERα far more efficiently as ERβ. ER isotype-selectivity of 16α-LE2 at the current dose has very recently been shown under in vivo and in vitro conditions [8,10]. These studies demonstrate that even a 100-fold higher dose of 16α-LE2 does not activate ERβ because ovarian weight remained unaffected and low in hypophysectomized rats receiving high doses of the ERα agonist [10]. The current observation of decreased absolute and relative heart weight in 16α-LE2 treated SHR therefore supports the hypothesis that activation of endogenous ERα is sufficient to attenuate cardiac hypertrophy in SHR. Although we cannot exclude a redundant function of ERβ activation, current results provide no evidence for func-

tional synergism between ERα and ERβ activation because 16α-LE2 and E2 attenuated cardiac hypertrophy to a similar extent despite robust cardiac expression levels of ERα *and* ERβ. Future studies using selective ERβ agonists such as 8β-VE2 should provide further insight into the role of ERβ in cardiac hypertrophy. Because estrogens may exert ER independent effects, although at very high ligand dosages that are beyond the current dose of E2 and 16α-LE2, we analyzed cardiac hypertrophy, gene expression and function also in SHR co-treated with the non-selective ER antagonist ZM-182780. The observation that ZM-182780 efficiently blocked the effects of E2 and 16α-LE2 indicates that both agonists regulate cardiac gene expression and function via estrogen receptors and not by an ER independent mechanism.

Left ventricular function is frequently impaired in cardiac hypertrophy and has been reported to incline with serum estradiol levels but the functional importance of ERα and ERβ activation in regulating myocardial contractility is currently unknown [26]. As reported here, activation of ERα by 16α-LE2 increases cardiac output, cardiac index and stroke volume in ovariectomized SHR. These observations together with improved left and right ventricular performance markers in 16α-LE2 treated SHR in cardiac MRI studies support the interpretation that activation of ERα augments myocardial contractility to measurable extent. The observation that simultaneous activation of ERα *and* ERβ by 17β-estradiol improved cardiac output, cardiac index and stroke volume to a lesser extent than 16α-LE2 raises the question whether activation of ERβ might confer an inhibitory effect on cardiac performance. Although previous studies, which analyzed interactions between isotype-selective ER agonists in non-cardiac tissues, do not support this hypothesis, further studies are required to determine the hemodynamic effects of ERβ selective ligands [8]. Measurements of cardiac performance are subject to systemic pro and counter regulatory factors under in vivo conditions that cannot be completely eliminated. Therefore we sought more direct evidence for increased myocardial contractility upon activation of ERα by analyzing the contractile properties of isolated papillary muscle specimens. Force–frequency relationships, which were blunted in papillary muscle from ovx compared to sham operated SHR, improved significantly upon treatment with 16α-LE2 or E2 and therefore support the interpretation that activation of ERα increases myocardial contractility.

Myocardial contractile performance critically depends on intracellular calcium homeostasis and the expression profile of contractile proteins [27,28]. The contractile properties of cardiac myocytes are co-regulated by differential α- and β-myosin heavy chain expression because α-MHC exhibits a higher ATPase activity than the β-MHC isoform [27]. Therefore it is conceivable that increased cardiac α-MHC expression and improved cardiac output in 16α-LE2 treated SHR might be functionally linked processes. This interpretation is also supported by a significant decrease of cardiac contractility and ATPase activity in transgenic mice with low level cardiac overexpression of the slower β-MHC isoform [29].

The observation of lower cardiac mass in 16α-LE2 treated SHR might eventually explain improved cardiac output upon treatment with the ERα agonist since cardiac hypertrophy might impair myocardial contractility. To test this hypothesis we sought to determine functional cardiac parameters upon treatment with an ER ligand that should not enhance cardiac α-MHC expression but yet retain the anti-hypertrophic properties of E2 and 16α-LE2. The observation that tamoxifen attenuated cardiac hypertrophy but failed to enhance cardiac α-MHC expression and cardiac output supports the hypothesis that myocardial contractility in SHR treated with different estrogen receptor ligands is not fully explained by a reduction of cardiac hypertrophy. Instead, myocardial contractility appeared to be linked more closely with cardiac α-MHC expression.

Further studies will be required to determine the role of ERβ selective ligands in heart muscle disease, which might confer either different or similar effects than 16α-LE2.

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