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Original article

The selective estrogen receptor modulators (SERMs) raloxifene and tamoxifen improve ANP levels and decrease nuclear translocation of NF-kB in estrogen-deficient rats

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ABSTRACT

Background: The selective estrogen receptor modulators (SERMs) raloxifene and tamoxifen are used for the treatment of osteoporosis and cancer, respectively, in women. The impairment of both the Atrial Natriuretic Peptide (ANP) cell signaling system and the translocation of nuclear factor-kappa B (NF-kB) to the cell nucleus are associated with detrimental cardiovascular effects and inflammation. The effects of SERMs on these parameters in the cardiac tissue of estrogen-deficient rats has not been reported.
Methods: We investigated the effects of raloxifene and tamoxifen on ANP signaling, p65 NF-kB nuclear translocation, cardiac histology and contractility. Female rats were divided into five groups: control (SHAM), ovariectomized (OVX), OVX-treated 17-β-estradiol (E), OVX-treated raloxifene (RLX) and OVX-treated tamoxifen (TAM). The treatments started 21 days after ovariectomy and continued for 14 days.
Results: Ovariectomy reduced ANP mRNA in the left atrium (LA), decreased the content of ANP protein in the LA and in plasma, and increased the level of p65 NF-kB nuclear translocation in the left ventricle. Both 17-β-estradiol and SERMs were able to reverse these alterations, which were induced by the estrogen deficient state. The hemodynamic and cardiac structural parameters analyzed in the present work were not modified by the interventions.
Conclusions: Our study demonstrates, for the first time, the additional benefits of raloxifene and tamoxifen in an estrogen-deficient state. These include the normalization of plasmatic and cardiac ANP levels and cardiac p65 NF-kB translocation. Therefore, these treatments promote cardiovascular protection and may contribute to the prevention of cardiac dysfunction observed long-term in postmenopausal women.

Introduction

Atrial natriuretic peptide (ANP) is a polypeptide hormone secreted by cardiac muscle cells and is a very important regulator of cardiac homeostasis. Impairment of ANP function is associated with cardiovascular disorders [1]. The biological effects of ANP are mediated by the binding of this peptide to natriuretic peptide receptors (NPRs). Three types of NPR are known: NPR-A, NPR-B and NPR-C. NPR-A has guanylate cyclase activity, and it regulates the majority of the biological effects of ANP [2]. In mammals, cardiac muscle cells of the heart atria produce and secrete the polypeptide hormone ANP in a regulated manner [3]. Atrial expression of both ANP and B-type natriuretic peptide (BNP) is up-regulated during chronic hemodynamic overload as a protective mechanism [4].

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Moreover, ANP can counter-regulate the functions of renin-angiotensin, vasopressin, and the sympathetic nervous system, all of which participate in the pathophysiology of cardiovascular diseases [5]. In fact, therapies that are capable of normalizing ANP levels may contribute to protection against cardiovascular diseases [6]. The neurohormonal response to the failing myocardium is directly or indirectly linked to inflammation [7].

Nuclear factor-kB (NF-kB) is a family of transcription factors that has been implicated in the regulation of expression of inflammatory genes, including tumor necrosis factor alpha (TNF-α) and other cytokines. The inflammatory response itself is usually dependent on the activity of NF-kB [8]. Prolonged activation of NF-kB appears to be involved in impaired cardiovascular function [9]. However, NF-kB can have either protective or detrimental effects due its signaling complexity. Its activation is generally transient and while the short activation might be beneficial, the long activation usually promotes chronic inflammation and organism injury [10]. NF-kB has a dual role in context-dependent control of apoptosis leading to either cell proliferation, differentiation, and survival or, in a different way, programed cell death [11]. Moreover, the crosstalk between estrogen receptors (ERs) and NF-kB pathway contributes to the development of endocrine therapy resistance in breast cancers [11].

Selective estrogen receptor modulators (SERMs) are compounds that interact with ERs in target tissues and can act as agonists or antagonists [12]. Regarding the cardiovascular effects, raloxifene and tamoxifen have been reported to not only have cardioprotective effects in specific groups, but also to contribute to the healthy endothelial function and vasorelaxation. Additionaly, raloxifene and tamoxifen can decrease inflammatory cytokines, serum homocysteine levels, and C-reactive protein [13,14]. However, despite several beneficial effects of SERMs, some side effects are reported. Tamoxifen can cause hot flashes, night sweats, depression, forgetfulness, sleep alterations, weight gain, vaginal and sexual disturbance and, in some cases, endometrial hyperplasia, endometrial cancer or venous thromboembolic disease [15]. Raloxifen has also been related to venous thromboembolism [16].

Interestingly, NF-kB also can mediate cardiac hypertrophy under disruption of guanylyl cyclase/NPRA signaling [17,18]. Furthermore, previously published reports have demonstrated an anti-inflammatory role for ANP by showing that ANP-induced cyclic GMP signaling could suppress the release TNF-α in vitro [19,20] and in vivo [21,22] through inhibition of NF-kB activity as well as ANP can inhibit the NF-kB in THP-1 cells [23]. On the other hand, estrogen has been shown to have a profound influence in the ANP system, even though only few studies have investigated the effects of hormone replacement therapy (HRT) on the ANP system [24,25]. To our knowledge, there are no data available regarding the chronic effects of raloxifene and tamoxifen on the natriuretic peptide system in animal models of estrogen-deficiency. This is the first study to examine the effects of these SERMs on both the ANP system and NF-kB function in the hearts of estrogen-deficient animals.

In clinical practice, raloxifene is used for the treatment and prevention of post-menopausal osteoporosis [26], while tamoxifen is the most widely used anti-estrogen for the management of breast cancer [27,28]. Because of their potential long-term usage by patients, it is important to identify the effects of SERMs on cardiac function. We hypothesize that treatment with SERMs may be able to reverse negative alterations in cardiac tissue induced by estrogen-deficiency. Accordingly, the aim of this study was to investigate the effects of raloxifene and tamoxifen on the ANP system and p65 NF-kB translocation in cardiac tissue in ovariectomized rats, along with relevant hemodynamic and morphometric parameters.

Materials and methods

Experimental animals

These investigations were conducted in accordance with the biomedical research guidelines for the care and use of laboratory animals and were approved by Ethics Committee of the university where the research was conducted (no. 012/2008). Eight-week-old female Wistar rats were housed in groups with a 12-h (light)–12-h (dark) cycle – 25 °C. Standard rat chow and tap water were available *ad libitum*. Five groups (n = 5–8/group) were studied: control (SHAM); ovariectomized (OVX); OVX treated with 17-β-estradiol (E: 0.5 μg/kg/day; Sigma Chemical Co., St. Louis, MO, USA); OVX treated with raloxifene (RLX: 2.0 mg/kg/day; Eli Lilly, Indianapolis, IN, USA); and OVX treated with tamoxifen (TAM; 1.0 mg/kg/day; Sandoz, Cambé, PR, Brazil). The Study design in graphical representation is shown in Fig. 1.

Ovariectomy and treatments

Female rats were anesthetized with an intraperitoneal injection (*ip*) of ketamine/xylazine (70/10 mg/kg) and underwent bilateral ovariectomy as previously described [24]. The female SHAM group only underwent an incision. Twenty-one days after surgery, the ovariectomized female rats were subcutaneously given 17β-estradiol diluted in peanut oil while the SERMs were pulverized, dissolved in water and administrated by gavage. The SHAM and ovariectomized groups received only vehicle. These treatments lasted 14 days.

Estrous cycle phase determination

Daily vaginal smears were obtained from each SHAM rat between 8:00 and 10:00 a.m. as previously described to confirm that their oestrous cycles. The rats with normal oestrous cycles were sacrificed during the proestrus phase [29].

Measurements hemodynamic

In the end of treatment period, rats were anesthetized with ketamine/xylazine (70/10 mg/kg, *ip*) and left ventricular (LV) function was assessed as previously described [30]. Right common carotid artery was catheterized with a polyethylene catheter (P50) and connected to a pressure transducer (TRI 21, Letica Scientific Instruments, Spain) and then to a digital system (Powerlab/4SP ML750, ADInstruments, Australia). Parameters were measured as LV +dP/dT, which is the maximum rate of ventricular pressure increase or the peak positive value of the first derivative of the LV pressure, as well as the rate of pressure decay (-dP/dT). The signal was expressed in mmHg/s. Following this procedure, the catheter was withdrawn from the LV and the arterial pressure was measured again and if a decrease in the diastolic blood pressure was observed the animal was not computed. Data were analyzed using the LabChart software 7.

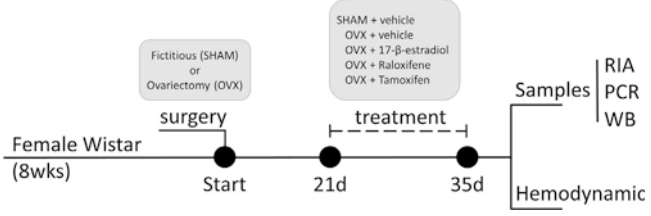

Fig. 1. Study design in graphical representation. The timeline shows the days (d) from the surgery procedure to final experiments, including the radioimmunoassay (RIA), polymerase chain reaction (PCR) and Western Blot (WB) analysis.

Collection of plasma and tissues

The rats were decapitated, and 5 mL of blood was collected in pre-chilled tubes containing heparin sulphate and protease inhibitors: 10^{-5} mol/L ethylenediaminetetraacetic acid (EDTA), 10^{-5} mol/L phenylmethylsulfonyl fluoride (PMSF), and 0.5×10^{-5} mol/L pepstatin A (Sigma, St. Louis, MO, USA). The blood samples were centrifuged and the plasma for ANP analysis was stored at −80 °C. The atrium and ventricle were removed, weighed, frozen in liquid nitrogen and stored at −80 °C.

Levels of ANP

The level of ANP was determined by performing double-antibody radioimmunoassay (RIA), as described by Gutkowska et al. [31], using a specific antibody that was donated.

The plasma was thawed and centrifuged. ANP was extracted using Sep-Pak C18 columns (Waters Associates, Milford, MA, USA). Absorbed ANP was eluted with 3 mL of 60% acetonitrile in 0.2% ammonium acetate, which was evaporated (Speed-Vac, Eppendorf, Hamburg, Germany) and stored at −20 °C for quantification by RIA.

In the atrial tissue, bothright (RA) and left atria (LA) were thawed and placed in a tube filled with 0.1 M acetic acid and protease inhibitors (10^{-5} M EDTA, 10^{-5} M PMSF and 0.5×10^{-5} M pepstatin A, all purchased from Sigma). The samples were then homogenised and centrifuged at 20,000*g* for 30 min at 4 °C, and the supernatant was diluted (final dilution: 1:2000) in phosphate buffer (0.01 mol/L sodium phosphate, 0.14 mmol/L bovine serum albumin, 0.1% Triton X-100, 0.1 mol/L NaCl and 0.01% sodium azide at pH 7.4). All of the samples were measured in the same assay, and the intra-assay coefficient of variation was <10%. The protein content of the tissue was determined using the Bradford method [32].

ANP mRNA expression

Total mRNA was extracted from the atria by a guanidine isothiocyanate method previously described by Chomczynski and Sacchi [33].

The mRNA expression level of ANP was determined by real-time PCR. cDNA was synthesized by the reverse transcription of mRNA. For this process, 1 μg of mRNA from each sample was mixed in plastic tubes with a solution containing the following compounds: diethyl pyrocarbonate water (DEPC), the reverse primer of the target gene (ANP) or the reverse primer of the housekeeping gene (ribosomal subunit s26), oligo dT, triphosphate deoxyribonucleotide (dNTP), dithiothreitol (DTT), specific buffer (10×) and a solution containing the Moloney murine leukemia virus (MMLV) reverse transcriptase enzyme, according to the manufacturer's guidelines (Invitrogen, CA, USA). After this process, the plastic tubes were heated to 40 °C for 60 min. After reaching room temperature, the tubes were stored at − 20 °C.

The samples were subjected to DNAse treatment prior to reverse transcription, performed by mixing 0.5 μg of total mRNA from the atria with 4 μl of water and 1 μl of buffer containing DNAse (1:1). This mixture was incubated for 15 min at room temperature. After the incubation period, EDTA was added to stop the reaction. The samples were then heated to 65 °C for 10 min.

After synthesizing the cDNA, PCR was performed to amplify the cDNA for ANP, using specific primers (ANP: 5′-GGA TTT CAA GAA CCT GCT AGA-3′ and 5′-CTT CAT CGG TCT GCT CGC TCA-3′). For this procedure, 2 μl of cDNA was added to wells of real-time PCR plates, followed by 1.5 μl of sense primer (1 pmol/μl), 1.5 μl of anti-sense primer (1 pmol/μl), 10 μl of Power SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) and 5 μl of DEPC water. Afterward, the plates were sealed and amplified in the thermocycler (ABI Prism 7000 SDS; Applied Biosystems, Warrington, UK).

Western blot analysis of left ventricle

The samples of cytoplasmic and nuclear extracts for measurement of the p65 NF-kB subunit were homogenised in lysis buffer containing: buffer A (10 mM HEPES (pH 7.9), 1.5 mM MgCl, 10 mM KCl, 1.0 mM DTT, 0.5 mM PMSF, 0,1% Triton-X, 10 μg/mL protease inhibitor cocktail) and buffer B (20 mM HEPES (pH 7.9), 25% glycerol, 1.5 mM $MgCl_2$, 420 mM NaCl, 0.2 mM EDTA, 1.0 mM DTT, 0.5 mM PMSF, 10 μg/mL protease inhibitor cocktail). The samples were gently triturated with buffer A. After 10 min incubation on ice, samples were centrifuged at 4 °C for 10 min at 4500 rpm. Supernatants were collected and represented the cytoplasmic protein extract and stored at −80 °C. The pellets were washed with a buffer A without the presence of Triton-X and centrifuged again. The supernatant was discarded and the pellets suspended in buffer B. The pellets were macerated and incubated on ice for 5 min. The suspension was centrifuged for 15 min at 16000*g* at 4 °C, and the supernatants containing the nuclear extracts stored at −80 °C until use [34]. Protein concentrations were determined using the method reported by Lowry [35]. The protein lysates, 60 μg for NF-kB (p65 subunit) were separated by 7.5%, using SDS-PAGE. The proteins were transferred to polyvinylidene difluoride (PVDF) membranes, which were incubated with mouse monoclonal antibodies for p65 nuclear factor kappa-B subunit (p65, 1:200, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). After washing, the membranes containing anti p65 NF-kB subunit were incubated with alkaline phosphatase-conjugated anti-rabbit IgG (1:7000, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The bands were visualised using an NBT/BCIP system (Invitrogen Corporation, CA, USA) and were quantified using *Image J* software. The results were calculated by the ratio of the density of specific bands of p65 NF-kB subunit in nuclear and cytoplasmic samples. The data are from five independent experiments.

Determination of histological analysis

At the end of the hemodynamic experiments, the animals were euthanized, the hearts were excised, and the hearts were fixed in 10% neutral buffered formalin solution for 24 h. Then, a mid-coronal section of the ventricle tissue was extracted and embedded in paraffin. A 5-μm-thick slice was stained with haematoxylin/eosin [36]. The tissue samples were examined using a light microscope. Photographs of the samples were obtained using an image acquisition system (Moticam Plus; Motic Inc., Canada). Morphometric analyses were performed in 400× magnification by counting the number of myocyte nuclei per high power field (10 fields/sample) and by measuring the area of the myocyte nuclei using the Moticam Plus histological analysis software [37].

Statistical analysis

Data are expressed as the mean ± standard error of mean (SEM). All statistical analysis was performed using one-way ANOVA, followed by Newman–Keuls test for ANP analysis, Fisher's LSD for NF-kB and Tukey for all other data. The software GraphPad Prism 7.02 was used. Data were considered statistically significant if $p < 0.05$.

Results

The effects of ovariectomy and treatments on body weight, uterine weight, hemodynamic parameters and morphology.

There were no differences in body weight among the groups at the beginning of the study. On the last day of treatment, the OVX group showed a significant body weight gain. RLX and

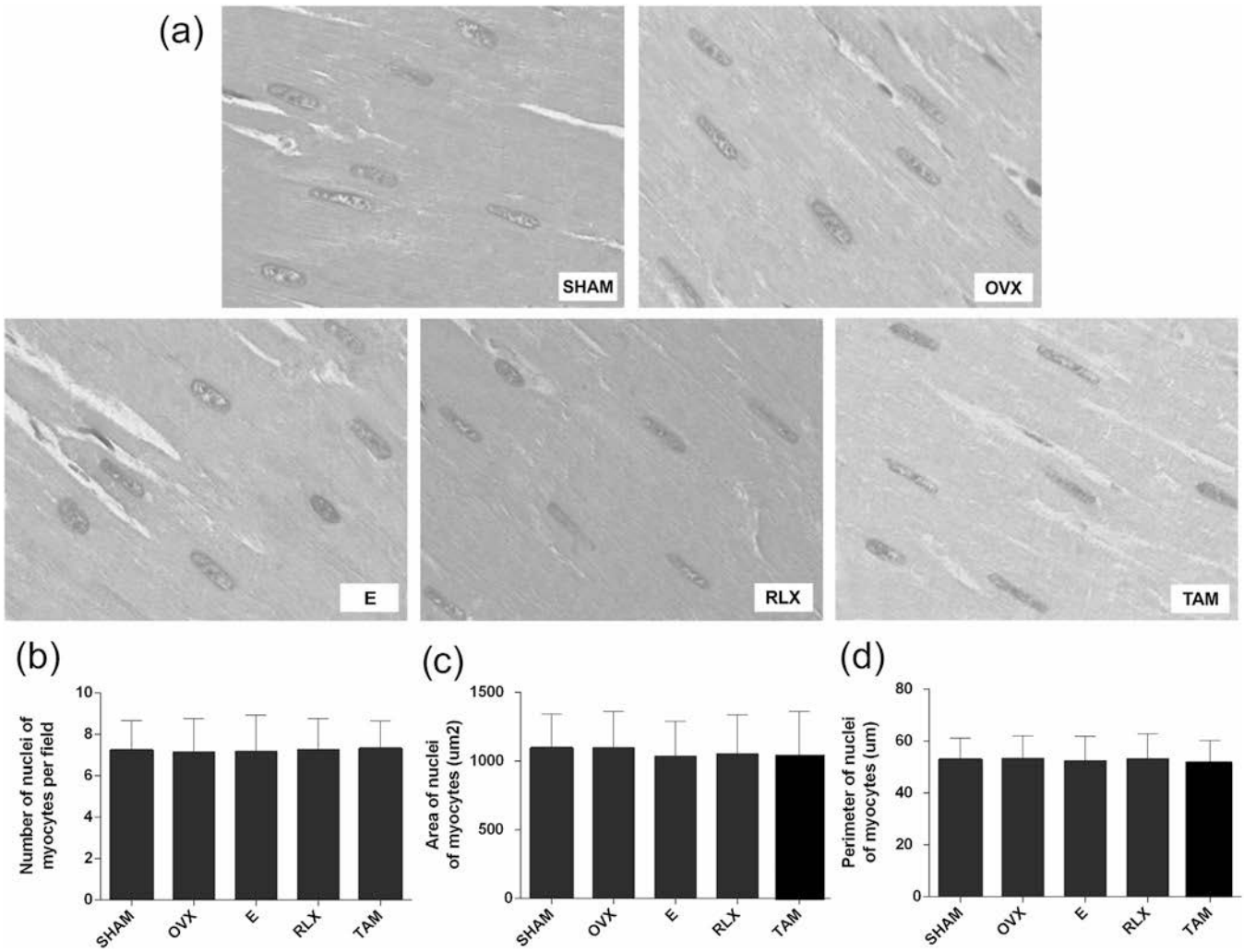

Fig. 2. Effects of ovariectomy and SERM treatments in the histological analysis of hearts. The column graphs show the number of myocyte nuclei (high-power field), perimeter of myocytes and area of myocyte nuclei in the left ventricular tissue of the control (SHAM), ovariectomized (OVX) and ovariectomized treated with 17-β-estradiol (E), raloxifene (RLX) and tamoxifen (TAM) groups. Samples were stained by the haematoxylin/eosin (H ± SEM).

17-β-estradiol treatments were able to prevent this gain in the OVX-treated animals. Interestingly, the TAM group showed a reduction in the final body weight compared to the SHAM group. As expected, we observed that ovariectomy significantly reduced the uterine weight compared with the SHAM group and 17-β-estradiol treatment restored this parameter. The RLX and TAM groups showed an increase in uterine weight compared with the OVX group, but these treatments did not restore the normal

Table 1
Effect of ovariectomy and/or 17β-estradiol (E), raloxifene (RLX), tamoxifen (TAM) treatments on ponderal data, hemodynamic parameters and histological data.

	SHAM (n = 5)	OVX (n = 5)	E (n = 5)	RLX (n = 5)	TAM (n = 5)
Ponderal data					
Initial body weight (g)	201 ± 2	203 ± 4	202 ± 3	203 ± 5	202 ± 6
Final body weight (g)	251.2 ± 3	295.9 ± 3**	257.9 ± 3	252.7 ± 2	215.6 ± 4†
Uterine weight (g)	0.38 ± 0.03	0.07 ± 0.03**	0.36 ± 0.05	0.29 ± 0.02‡	0.28 ± 0.04‡
Hemodynamic parameters					
Positive first time derivatives (+dP/dt) (mmHg/s)	5811.23 ± 86.88	6060.19 ± 93.35	5900.96 ± 99.18	6160.69 ± 63.28	5987.27 ± 75.15
Negative (-dP/dt) (mmHg/s)	−3856.87 ± 78.93	−3634.07 ± 117.33	−3879.64 ± 54.40	−3988.33 ± 129.94	−3600.31 ± 128.14
Systolic pressure left ventricule (mmHg)	106 ± 5	102 ± 10	106 ± 4	101 ± 9	104 ± 15
Histological data					
Number of nucleus per field	7.233 ± 1.23	7.166 ±1.09	7.200 ± 1.53	7.266 ± 1.34	7.300 ± 1.32
Perimeter of myocytes (μm)	529.20 ± 82.34	533.40 ± 89.23	524.95 ± 98.36	532.64 ± 91.23	516.16 ± 80.39
Area of myocytes nuclei (μm^2)	10931.43 ± 3456	10991.21 ± 3467	10362.30 ± 2657	10542.44 ± 3789	10315.43 ± 3987

The results are the means ± SEM. Statistical significance is indicated by $^{**}p < 0.01$ *vs.* the SHAM group; $^{\dagger}p < 0.05$ *vs.* the SHAM, OVX, E and RLX; $^{\ddagger}p < 0.01$ *vs.* SHAM and E group.

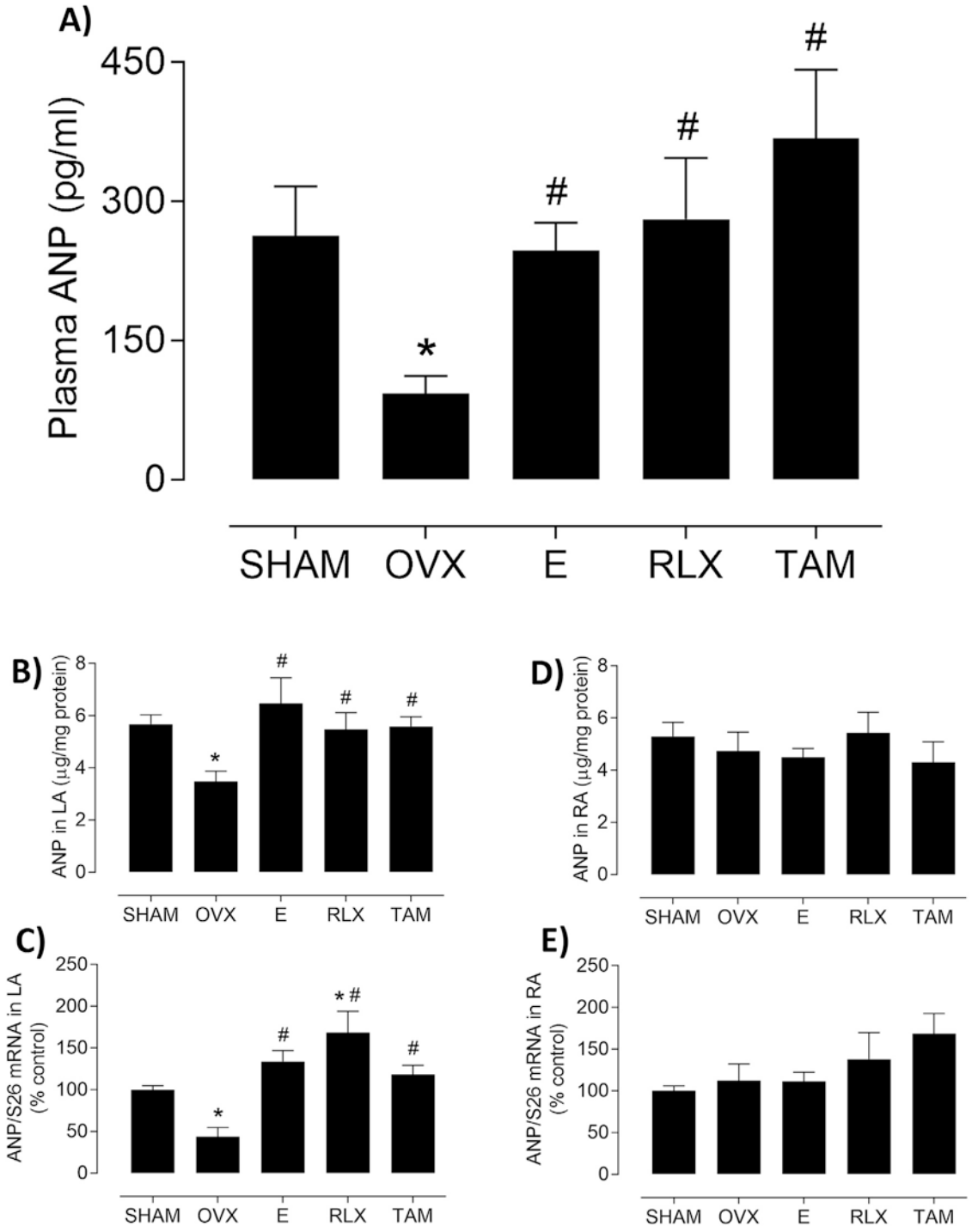

Fig. 3. Effects of ovariectomy and SERM treatments on ANP levels. Plasma ANP levels (2A); ANP concentration (2B) and mRNA expression (2C) in the left atrium, as well as ANP concentration (2D) and mRNA expression (2E) in the right atrium from the control (SHAM), ovariectomized (OVX) and ovariectomized treated with 17-β-estradiol (E), raloxifene (RLX) and tamoxifen (TAM) groups. Data are expressed as the mean ± SEM. $^{*}p < 0.05$ *vs.* SHAM; $^{\#}p < 0.05$ *vs.* OVX.

values observed in the SHAM group. These data are shown in Table 1.

There were no observed differences in hemodynamic and histologic parameters (number, perimeter and area of myocyte nuclei in left ventricular tissue). These data are shown in Fig. 2 and Table 1.

Levels of plasma ANP

As shown in Fig. 3A, the plasma ANP concentrations were lower in the OVX group than in the SHAM group. Treatment with 17-β-estradiol, RLX and TAM increased the plasma levels of ANP in OVX rats (SHAM: 263 ± 53; OVX: 93 ± 20*; E: 247 ± 30#; RLX:

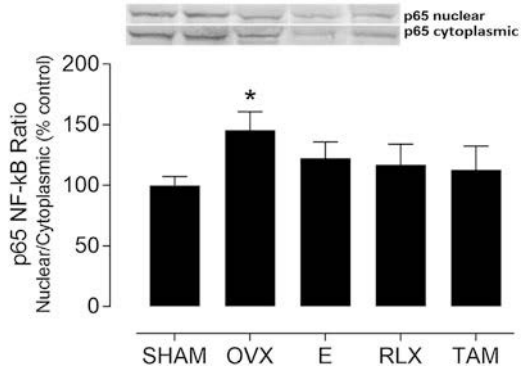

Fig. 4. Effects of ovariectomy and SERM treatments on the nuclear translocation of p65 NF-kB in left ventricle. Western blot analysis of nuclear and cytoplasmic p65 NF-kB (upper panels, representative blots) in treated rats. The column graphs refer to the nuclear/cytoplasmic ratio of p65 NF-kB. The data are from five independent experiments. Images from western blots were cropped (from the same gel) and arranged to improve the clarity and conciseness of the presentation. The groups are control (SHAM), ovariectomized (OVX) and ovariectomized treated with 17-β-estradiol (E), raloxifene (RLX) and tamoxifen (TAM). Data are expressed as the mean ± SEM. $^{*}p < 0.05$ *vs.* SHAM.

$281 \pm 66^{\#}$; TAM: $369 \pm 74^{\#}$ [pg/ml]; $^{*}p < 0.05$ *vs.* SHAM; $^{\#}p < 0.05$ *vs.* OVX).

Levels of cardiac ANP

Radioimmunoassay revealed that ovariectomy decreased ANP concentrations in the left atrium (LA), whereas all the treatments promoted an increase in ANP (Fig. 3B). The ANP concentration in the LA decreased from 5.68 ± 0.35 μg/mg protein in the SHAM group to 3.48 ± 0.40 μg/mg protein in the OVX group ($p < 0.05$). However, in the 17-β estradiol, RAL and TAM groups, the ANP levels in the LA increased to values similar to the SHAM group (E: 6.48 ± 0.97; RLX: 5.47 ± 0.64; TAM: 5.59 ± 0.37 μg/mg protein $p < 0.05$ *vs.* OVX).

Reinforcing these results, Fig. 3C shows ANP mRNA expression in the LA of the studied groups. The OVX group had lower ANP mRNA levels in the LA compared with the SHAM group. The 17-β-estradiol, RAL and TAM treatments led to an increase in ANP mRNA expression in the LA of OVX rats (SHAM: 100 ± 5; OVX: $44 \pm 11^{*}$; E: $134 \pm 13^{\#}$; RLX: $169 \pm 25^{*\#}$; TAM: $119 \pm 11^{\#}$ [% control]; $^{*}p < 0.05$ *vs.* SHAM; $^{\#}p < 0.05$ *vs.* OVX).

We did not observe any differences in ANP protein or mRNA levels in the right atrium (RA) among the groups (Fig. 3D and E).

NF-kB translocation

Fig. 4 shows the nuclear/cytoplasmic ratio of p65 NF-kB in left ventricular tissue from rats of all groups, and it serves as a measure of NF-kB nuclear translocation and activation. Ovariectomy caused a significant elevation in the translocation of p65 compared to the SHAM group. Treatments with 17-β-estradiol, RAL and TAM were able to normalize the p65 nuclear/cytoplasmic ratio in the left ventricular tissue of the OVX rats.

Discussion

The present study demonstrates that treatments with 17-β-estradiol and SERMs reversed both the cardiac impairment of the ANP system and the increased nuclear translocation of p65 NF-kB induced by ovariectomy. Despite the negative changes in the OVX group, the hemodynamic and structural parameters analyzed in the left ventricular tissue were not altered in these animals during the study period. One of the main findings of our study was that raloxifene and tamoxifen can increase ANP concentrations and mRNA expression in the LA as well as the plasma levels of ANP peptide, which are both reduced in OVX rats. The reduction of ANP levels in OVX rats described in our study agrees with previously published data showing that estrogen-deficiency can result in depletion of ANP [38].

It is well known that cardiac natriuretic hormones regulate several important physiological functions and that they can promote beneficial effects in cardiovascular disorders [6,39]. We show that decreased levels of ANP induced by estrogen deficiency occur together with an increase in NF-kB translocation, which is independent of hemodynamic alterations (left ventricular contractility or relaxation) or cardiac hypertrophy since the estrogenic deficiency did not alter these parameters. It remains possible that if the interval following ovariectomy were longer, changes in these parameters might have appeared. A role for estrogen in regulating cardiac contractility has been previously identified [40,41]. For instance, Ribeiro et al. [42] observed cardiac contractile dysfunction in response to calcium and β-adrenergic receptor stimulation in OVX rats; however, their interval following ovariectomy was 60 days. Another study from the same group demonstrated that myocardial contractility was preserved in the early phase of ovariectomy [43]. In our study, neither ovariectomy nor treatments with SERMs promoted alterations in left ventricular contractility or cardiac hypertrophy 35 days after ovariectomy.

Cardiovascular diseases are correlated with elevated markers of systemic inflammation, such as C-reactive protein, IL-6, and TNF-α [44,45]. Inflammatory cytokines can activate NF-kB, which can stimulate the production of reactive oxygen species and other cytokines [46,47], thus promoting more inflammation. It has been demonstrated that ovariectomy causes an increase in plasma pro-inflammatory cytokines associated with reactive oxygen species and vascular dysfunction [14]. Here, we show that an estrogen-deficient state can also increase p65 NF-kB translocation in left ventricular tissue, which could be related to the down regulation of ANP levels observed in cardiac tissue. In fact, the interplay between ANP and NF-kB has been reported by Ladetzki-Baehs et al. who demonstrated that the cardiovascular peptide ANP is a potent regulator of NF-kB activation and downstream TNF-α production in mouse tissue [21]. Similar to our findings, other groups investigating inflammation in estrogen-deficient animal models also found an increase in TNF-α plasma levels [24] that could be associated with increased levels of p65 NF-kB in left ventricular tissue. It is noteworthy that elevated TNF-α levels have been reported to be directly correlated with functional heart failure [48,49].

Interestingly, while estrogen has been reported to regulate negatively NF-kB, it is also true that this factor can modulate the activity of ERs [50]. For instance, NF-kB modulations of ERs include down-regulation of ERs expression, enhanced ERs recruitment to DNA, and increased transcriptional activity of both liganded and unliganded ERs [50]. These data point to the possibility that estrogen reduction can lead to ERs downregulation through NF-kB pathway. Indeed, it was reported a decrease in atrial ERs by ovariectomy [51], and SERMs are known to act via these receptors [12]. However, in our study, the treatments with 17-β-estradiol, raloxifene and tamoxifen were able to normalize the translocation of p65 NF-kB. Recently, Jamdade et al. demonstrated that raloxifene inhibits the NF-kB pathway in mammary tumors via a direct anti-inflammatory mechanism [52]. Therefore, despite the structural differences between raloxifene and tamoxifen and their differing clinical indications [53], these drugs can both act to inhibit detrimental cardiac alterations resulting from estrogen deficiency.

Conclusion

In conclusion, our study demonstrated, for the first time, the influence of the SERMs raloxifene and tamoxifen on the ANP peptide signaling system and the translocation of NF-kB, thus showing additional benefits for these drugs clinically used for osteoporosis and breast cancer, respectively. Estrogen-deficiency in female rats leads to impairment in the ANP signaling system as well as increased nuclear translocation of p65 NF-kB, which is an important mediator of inflammation in the heart. We observed that these negative alterations could be ameliorated by raloxifene and tamoxifen in ovariectomized rats. Therefore, these treatments resulted in cardiovascular protection and may have value as therapeutic agents for the prevention of cardiac dysfunction observed long-term in postmenopausal women.

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