

Significant role of female sex hormones in cardiac myofilament activation in angiotensin II-mediated hypertensive rats

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Abstract Ovariectomy leads to suppression of cardiac myofilament activation in healthy rats implicating the physiological essence of female sex hormones on myocardial contraction. However, the possible function of these hormones during pathologically induced myofilament adaptation is not known. In this study, sham-operated and ovariectomized female rats were chronically exposed to angiotensin II (AII), which has been shown to cause myocardial adaptation. In the shams, AII induced cardiac adaptation by increasing myofilament Ca^{2+} sensitivity. Interestingly, this hypersensitivity was further enhanced in AII-infused ovariectomized rats. Ovariectomy increased the phosphorylation levels of cardiac tropomyosin, which may underlie the mechanism of hypersensitivity. On the other hand, AII infusion did not alter maximal tension that was suppressed after ovariectomy. This finding coincided with a comparable increase in β -isoform of myosin heavy chains in both ovariectomized groups. Together, it is conceivable that female sex hormones serve as predominant factors that regulate cardiac myofilament activation. Furthermore, they may prevent stress-induced myofilament maladaptation.

Keywords Myocardial stiffness · Tropomyosin ·
Regulatory light chain · Myosin heavy chain

Introduction

Similar adaptation of cardiac myofilament Ca^{2+} activation seen in both female sex hormone deficiency and heart failure strongly calls for more studies on the regulatory role of sex hormones in heart function. In 10-week post-ovariectomized rats, suppression of the maximum contractile activity was found by using biochemical measurement of myofilament ATPase activity and mechanical measurement of force contraction [1, 2]. Furthermore, both techniques clearly showed that the increased myofilament Ca^{2+} responsiveness in ovariectomized rat hearts resembled that in human failure hearts [1–4]. Lack of female sex hormones also shifted the expression of cardiac myosin heavy chain isoform from predominant α -isoform toward more β -isoform. In addition, supplementation of estrogen consistently abolished all these changes. These previous evidences revealed the significance of female sex hormones in regulating the cellular and molecular function of cardiac myocytes. However, whether these cardiac changes found in the ovariectomized model lead to heart disease development that might happen in postmenopausal patients is still a question. Generally, the ovariectomized model has been used for evaluating the physiological function of female sex hormones without pathological complications. But in reality, the postmenopausal patient commonly has one or more heart disease risks in combination [5]. Based on this fact, it is therefore possible that lack of female sex hormones would induce an exponential development of myocardial maladaptation in a woman who already has a heart disease risk condition. Consequently, the use of hormone replacement therapy might help reduce severe development of a cardiac problem in this patient. To prove this possibility, cardiac contractile activity in the presence and absence of female sex hormones under pathological complication needs to be compared.

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In the present study, we tested whether female sex hormones played any mechanistic role in the adaptive response of cardiac contractile machinery chronically exposed to hypertension induced by an angiotensin II (AII) infusion. We compared the contractile force of the left ventricular skinned papillary fibers from rats 10 weeks after ovariectomy or sham operation with/without 4 weeks of continuous angiotensin II infusion. A possible regulatory role of sex hormones on length-dependent modifications of cardiac myofilaments was evaluated by measuring the active force at two sarcomere lengths. Myofilament phosphorylation was also analyzed to demonstrate potential adaptive interactions among the myofilament proteins. Results indicate that the role of female sex hormones in regulating cardiac contractile activation under physiological conditions still has a beneficial impact under the pathological condition of AII-mediated hypertension.

Materials and methods

Materials

All chemicals were obtained from Sigma Chemical (St. Louis, MO) and USB Corporation (Cleveland, OH); electrophoresis reagents were from Bio-Rad (Hercules, CA) and Amersham Pharmacia Biotech (Buckinghamshire, UK); Pro-Q diamond stain was from Invitrogen (Eugene, OR); polyclonal antibodies of angiotensin II receptor type I and β -actin were obtained from Lifespan Biosciences (Seattle, WA) and AVIVA Bioscience (San Diego, CA), respectively; and horseradish peroxidase-conjugated goat anti-rabbit IgG (H+L) (ZyMax grade) was from Zymed (San Francisco, CA); ECL was from Pierce (Thermo Scientific).

Animal preparation

Female Sprague–Dawley rats were sham operated (SHAM) or ovariectomized (OVX) at 8 weeks of age as previously described [2]. Rats were individually housed in standard animal cages and provided with rat chow and water ad libitum. Six weeks after surgery, both SHAM and OVX rats were subcutaneously implanted for 4 weeks with a mini-osmotic pump (Alzet Model 2004) containing angiotensin II in normal saline with 2 % acetic acid, or normal saline with 2 % acetic acid as vehicle control. The rate of angiotensin II release was 0.7 mg/kg body weight (BW) per day [6]. Increased blood pressure or cardiac hypertrophy is an indication of successful chronic infusion. Animal protocols were approved by the Experimental Animal Committee, Faculty of Science, Mahidol University, in accordance with the guidelines of the National Laboratory Animal Centre, Thailand.

Measurements of sarcomere length—passive tension and pCa-active tension

Ten weeks after surgery, rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg BW) followed by heparin (1,000 units/kg BW). Blood pressure was measured for 15 min from the right femoral artery using a polyethylene tube (PE-50) connected to a blood pressure transducer (MLT0380/D, ADInstrument), equipped with an offline recorder (MacLab/4e, ADInstrument). The heart was then rapidly removed and perfused with a low-calcium Krebs-Henseleit solution containing 2,3-butanedione monoxime using a Langendorff perfusion system as previously described [1]. Both non-branched trabeculae from the right ventricular free wall and the left ventricular papillary muscle were dissected, and the

Table 1 Characteristics of sham-operated (SHAM) and ovariectomized (OVX) rats with and without angiotensin II (AII) infusion

| | Non AII Infusion | | AII Infusion | |
|----------------------------|---------------------|----------------------|-----------------------------------|-------------------------------------|
| | SHAM (No.) | OVX (No.) | SHAM (No.) | OVX (No.) |
| Body weight (g) | 298 ± 3 (14) | 353 ± 7* (12) | 294 ± 3 [#] (13) | 354 ± 3* [†] (14) |
| Heart weight (g) | 1.47 ± 0.02 (14) | 1.53 ± 0.04 (12) | 1.56 ± 0.03* (13) | 1.77 ± 0.03* ^{#,†} (14) |
| Heart/body weight | 0.47 ± 0.01 (14) | 0.42 ± 0.01* (12) | 0.52 ± 0.01* [#] (13) | 0.49 ± 0.01 ^{#,†} (14) |
| Uterine weight (g) | 0.54 ± 0.02 (14) | 0.14 ± 0.01* (12) | 0.54 ± 0.03 [#] (13) | 0.13 ± 0.03* [†] (14) |
| Systolic pressure (mm Hg) | 123 ± 2 (5) | 121 ± 3 (6) | 161 ± 9* [#] (5) | 162 ± 4* [#] (6) |
| Diastolic pressure (mm Hg) | 82 ± 3 (5) | 81 ± 4 (6) | 106 ± 6* [#] (5) | 101 ± 4* [#] (6) |

Data are mean ± SE. *[#] $P < 0.05$, significantly different from SHAM and OVX, respectively, using Student–Newman–Keuls test after ANOVA. [†] $P < 0.05$, significantly different from SHAM + AII using unpaired t -test

remaining ventricular tissue was rapidly frozen and kept at -80°C until analyzed. Papillary muscle was cut longitudinally into small fiber bundles (150–250 μm in diameter) in ice-cold high relaxing (HR) buffer containing 20 mM EGTA, 6.76 mM MgCl_2 , 6.25 mM MgATP^{2-} , 40 mM potassium propionate, 12 mM creatine phosphate, 40 mM BES, 0.1 mM DTT, pH 7.0, ionic strength 0.15 M, 2.5 $\mu\text{g}/\text{ml}$ pepstatin A, 1 $\mu\text{g}/\text{ml}$ leupeptin, and 50 μM phenylmethylsulfonyl fluoride (PMSF). To determine myofibrillar Ca^{2+} activation, the left ventricular stripped papillary fibers were skinned by incubating for 1 h at 25°C in HR buffer containing 1 % Triton X-100. Then the skinned fiber bundle was attached using aluminum T-clips at one end to a displacement generator and at the other end to a force transducer (KG-7). Active tension was measured at two fixed sarcomere lengths of 2.0 and 2.3 μm at 20°C in the activating solution containing 20 mM EGTA, 12.4 mM MgCl_2 , 6.25 mM MgATP^{2-} , 0.52–40 mM potassium propionate (in association with $[\text{Ca}^{2+}]$), 12 mM creatine phosphate, 40 mM BES, 0.1 mM DTT, pH 7.0, ionic strength 0.15 M, 2.5 $\mu\text{g}/\text{ml}$ pepstatin A, 1 $\mu\text{g}/\text{ml}$ leupeptin, and 50 μM PMSF at various $[\text{Ca}^{2+}]$ ranging in pCa between 7.0–4.5. For the measurement of myocardial stiffness, passive force was measured by stretching and holding the skinned right ventricular trabeculae (prepared as described above but skinned at 4°C overnight and placed in HR buffer at 20°C) at sarcomere lengths ranging from 1.9 to 2.5 μm as determined by laser diffraction pattern. Cross-sectional area of fiber bundles was calculated based on an elliptical model.

Detection of myosin heavy chain (MHC) isoforms

Frozen left ventricular muscle was mixed and homogenized in extraction sample buffer (50 mM Tris pH 6.8, 2.5 % SDS, 10 % glycerol, 1 mM DTT, 1 mM PMSF, 1 $\mu\text{g}/\text{ml}$ leupeptin, 1 $\mu\text{g}/\text{ml}$ pepstatin A, 10 $\mu\text{g}/\text{ml}$ aprotinin) [7], and the homogenate was electrophoresed in 7 % SDS-PAGE for 17 h at 4°C . MHC bands on the gel were detected by Coomassie stain. Band density was analyzed using Image Master Labscan version 3.01 and Image Master TotalLab version 1.0 (Amersham Pharmacia Biotech). Protein concentration was determined using a biconchonic acid assay (Pierce Thermo Scientific).

Determination of phosphorylation level of myofibrillar proteins

Frozen left ventricular muscle was homogenized (50 mg per ml) in a buffer solution (60 mM KCl, 30 mM imidazole, 2.5 mM MgCl_2 , and a cocktail of protease and phosphatase inhibitors, Sigma) at 4°C , and then centrifuged at 12,000g, 4°C for 10 min. The pellet was re-suspended in 1 ml of the

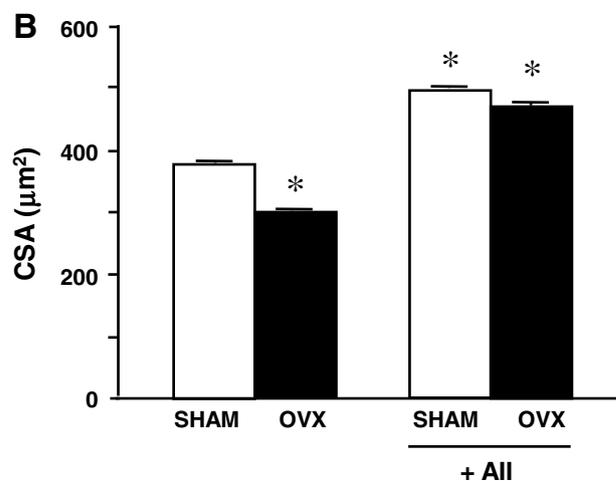
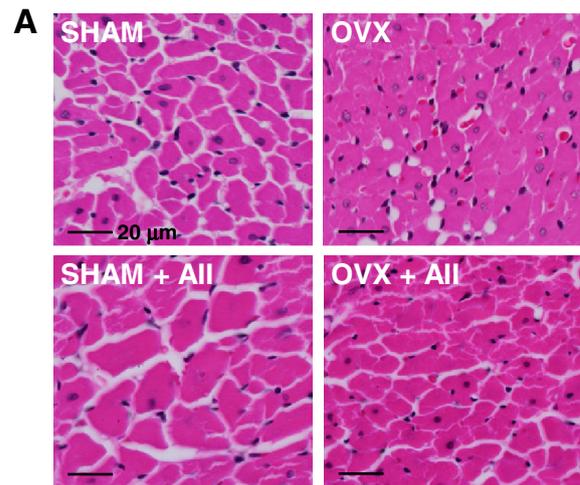


Fig. 1 Effect of ovariectomy and angiotensin II (AII)-induced hypertension on histological cardiomyocyte cross-sectional area (CSA). **a** Histological images of representative left ventricular muscle section from sham control (SHAM) and ovariectomized (OVX) rats, with/without AII infusion, stained with hematoxylin and eosin. **b** Comparison of the CSA among the four experimental groups. Data are mean \pm SE from 5 to 6 hearts of each group (average of 100 myocytes per heart). *Significantly different ($P < 0.05$) from SHAM using Student–Newman–Keuls test after ANOVA

above buffer solution containing 1 % Triton-X100, homogenized and centrifuged at 3,000g, at 4°C for 10 min. The pellet was again homogenized in 1 ml of buffer solution without Triton and centrifuged as described above. The final pellet was mixed with RIPA buffer (50 mM Tris, 0.15 M NaCl, 1 % Triton-X100, 1 % SDS, 1 mM EDTA) containing protease and phosphatase inhibitors, and myofibrillar proteins were separated using 12.5 % SDS-PAGE. The gel was then soaked in 100 ml of 50 % methanol and 10 % acetic acid. To detect phosphorylation, the gel was stained in 60 ml of Pro-Q diamond according to the manufacturer's protocol (Molecular Probes; Invitrogen P3 3300) and phosphorylated (phospho)proteins were detected using a

Fig. 2 Effect of ovariectomy and angiotensin II (AII)-induced hypertension on cardiac myofilament Ca^{2+} activation. **a** Relations of active tension-pCa ($-\log[\text{Ca}^{2+}]$) of skinned papillary muscle from SHAM and OVX rats, with/without AII, at two sarcomere lengths of 2.0 and 2.3 μm . **b** Comparison of the maximum active tension among the four experimental groups at the two sarcomere lengths. Data are mean \pm SE from 12 to 13 fibers of 7–8 hearts in each group. * \dagger Significantly different ($P < 0.05$) from SHAM and SHAM + AII group at the same sarcomere length, respectively, using Student–Newman–Keuls test after ANOVA

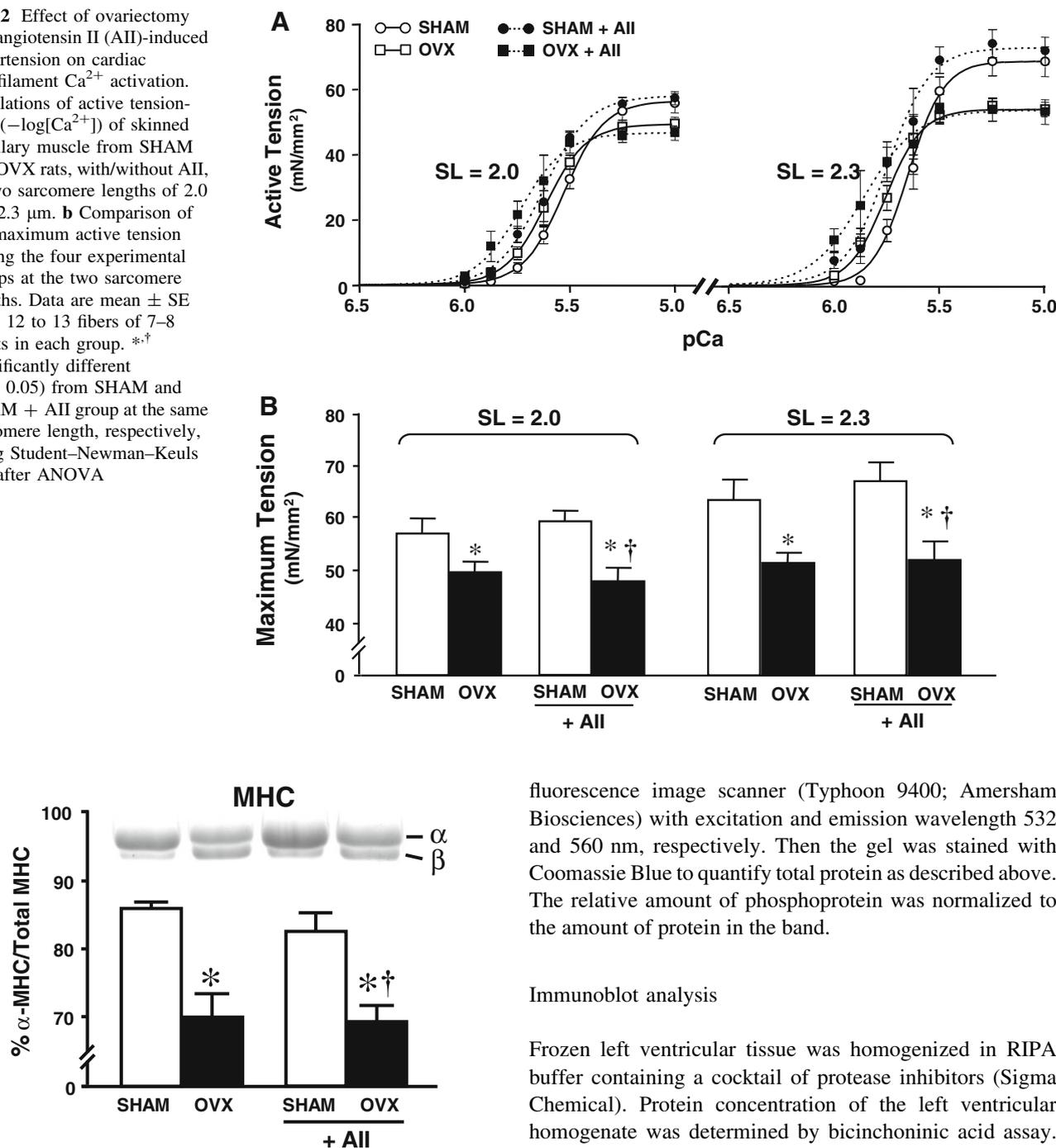


Fig. 3 Effect of ovariectomy and angiotensin II (AII)-induced hypertension on the expression of myosin heavy chain (MHC) isoforms in rat ventricular muscle. *Top* representative electrophoresis analysis of cardiac MHC (upper: α -MHC, lower: β -MHC) from left ventricular homogenate. *Bottom* percentage of α -MHC per total MHC of the heart from SHAM and OVX rats, with/without AII. Data are mean \pm SE from 5 to 7 hearts of each group. * \dagger Significantly different ($P < 0.05$) from SHAM and SHAM + AII group, respectively, using Student–Newman–Keuls test after ANOVA

fluorescence image scanner (Typhoon 9400; Amersham Biosciences) with excitation and emission wavelength 532 and 560 nm, respectively. Then the gel was stained with Coomassie Blue to quantify total protein as described above. The relative amount of phosphoprotein was normalized to the amount of protein in the band.

Immunoblot analysis

Frozen left ventricular tissue was homogenized in RIPA buffer containing a cocktail of protease inhibitors (Sigma Chemical). Protein concentration of the left ventricular homogenate was determined by bicinchoninic acid assay. Polyclonal antibody against AT1R (1:5,000 dilution) was used for analyzing the protein contents of AT1R, in 100 μg of tissue homogenate. The amount of protein was determined and represented per the amount of β -actin detected using polyclonal antibody against β -actin (1:10,000 dilution) in the same blot. Band density was analyzed using Image Master Labscan version 3.01 and Image Master Totalab version 1.0 (Amersham Pharmacia Biotech).

Data and statistical analysis

The relationship of pCa and developed tension was fitted to the Hill equation using nonlinear least squares regression analysis (GraphPad Prism version 4.0) in order to obtain pCa₅₀ (half-maximal activating Ca²⁺ concentration). Passive tension-sarcomere length relation was fitted to an exponential growth equation $Y = \text{Start} \cdot \text{Exp}^{(K \cdot X)}$ (GraphPad Prism version 4.0), where K is myocardial passive stiffness. Data are presented as mean ± SE and were analyzed by one-way ANOVA, followed by a Student–Newman–Keuls test for multiple comparisons, or by an unpaired *t*-test between two groups of interest. A *P* value < 0.05 is considered significantly different.

Results

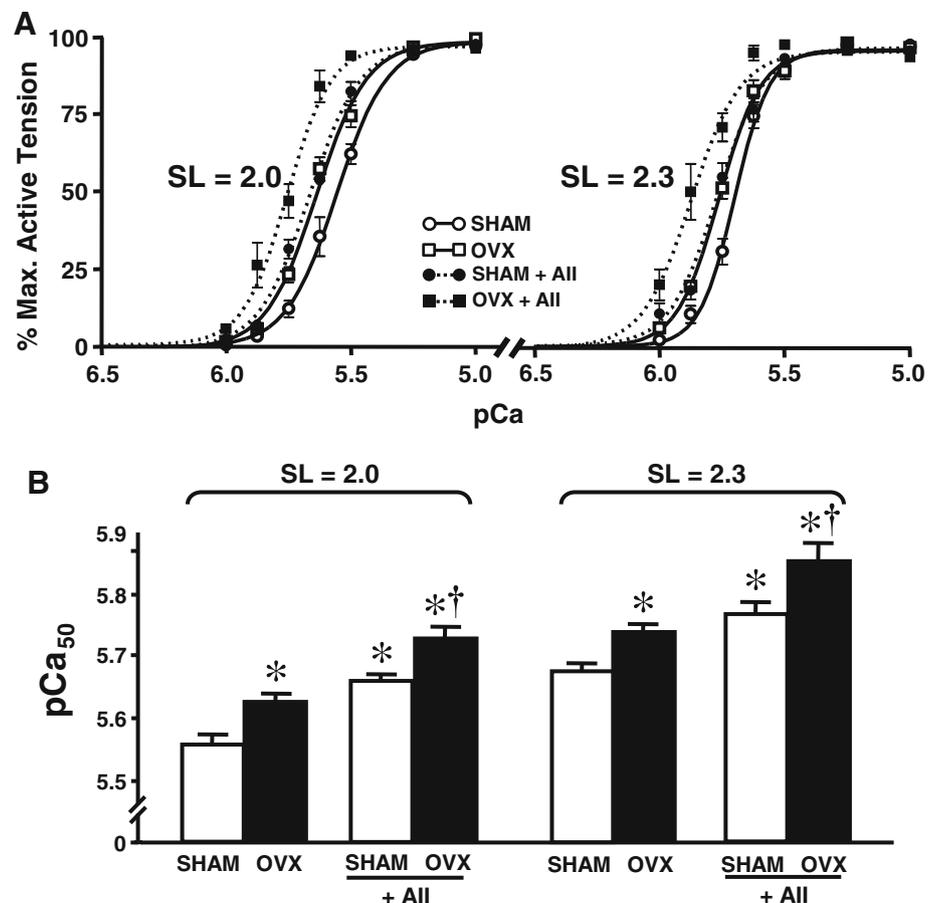
Similar to our previous study [8], deficiency of female sex hormones after ovariectomy induced an increase in body weight but decreases in uterine weight, and heart weight/body weight, as compared to those of sham controls. On the other hand, angiotensin II infusion induced increased

heart weight in sham-operated and higher in ovariectomized rats (Table 1); however, heart weight/body weight was not different between them. The effect of angiotensin II infusion also showed a larger myocyte cross-sectional area (Fig. 1). While ovariectomy had no effect on blood pressure, hypertension, i.e., increased systolic and diastolic pressures, was presented in AII-infused sham and ovariectomized rats at the same degree.

Using the left ventricular skinned fiber preparation, the pCa-tension relationship (Fig. 2) demonstrated no significant difference in maximum developed tension between AII-infused and vehicle-infused sham groups. The only effect of female sex hormone deficiency was that it reduced the maximum tension by the same degree in both AII-infused and vehicle-infused groups when compared to sham. Changes in the maximum tension of isometric contraction were similar whether at sarcomere lengths of 2.0 or 2.3 μm.

A shift of MHC isoforms from the predominant α- to β-isoform induced by either a lack of ovarian sex hormones or chronic angiotensin II infusion has previously been reported [3, 9]. As shown in Fig. 3, a significant decrease in the α-MHC/total MHC ratio in hearts of ovariectomized rats compared to sham was observed, regardless of

Fig. 4 Effect of ovariectomy and angiotensin II (AII)-induced hypertension on cardiac myofilament Ca²⁺ sensitivity. **a** Relations of percentage of maximum active tension-pCa of skinned papillary muscle from SHAM and OVX rats, with/without AII, at two sarcomere lengths of 2.0 and 2.3 μm. **b** Comparison of the pCa₅₀ (pCa at the half maximum tension) among the four experimental groups at the two sarcomere lengths. Data are mean ± SE from 12 to 13 fibers of 7–8 hearts in each group. *†Significantly different (*P* < 0.05) from SHAM and SHAM + AII group at the same sarcomere length, respectively, using Student–Newman–Keuls test after ANOVA



angiotensin II-induced hypertension. This result confirmed a significant regulatory role of female sex hormones in the regulation of MHC isoform expression over the angiotensin II effect.

Myofilament Ca^{2+} sensitivity of skinned left ventricular stripped papillary fibers from vehicle-infused ovariectomized rats was significantly increased compared to the sham control ($\text{pCa}_{50} = 5.64 \pm 0.01$ and 5.57 ± 0.02 , respectively), as expected (Fig. 4). Calcium hypersensitivity of myofilaments was also detected in the heart muscle fibers from AII-infused sham rats ($\text{pCa}_{50} = 5.67 \pm 0.01$). Interestingly, skinned fiber bundles from hearts of AII-infused ovariectomized rats was significantly more sensitive to $[\text{Ca}^{2+}]$ ($\text{pCa}_{50} = 5.75 \pm 0.02$) when compared with either vehicle-infused ovariectomized rats or AII-infused sham rats. This pattern of change occurred similarly in two sarcomeric length detections with no significant difference in ΔpCa_{50} among four experimental groups. Thus, although cardiac overload by angiotensin II-induced hypertension per se induced cardiac myofilament hypersensitivity, the effect of female sex hormones on cardiac myofilament Ca^{2+} sensitivity was not disrupted.

In order to identify possible mechanisms underlying the change in myofilament Ca^{2+} sensitivity, phosphorylation of cardiac myofilament proteins was determined. While there were no significant differences in the total amount of myofilament proteins among the four experimental groups, the phospho-tropomyosin content was significantly higher in both AII-infused and vehicle-infused ovariectomized groups compared to sham (ranging about 105–127 % higher than vehicle-infused sham) (Fig. 5). There was no effect of angiotensin II on the level of phospho-tropomyosin in the heart from sham animals. A significant decrease in phospho-regulatory light chain was detected only in AII-infused ovariectomized rats (73 ± 5 % of the level in sham). The levels of phospho-myosin-binding protein C, phospho-troponin T, and phospho-troponin I remained unchanged in the four groups. In order to determine if female sex hormones were involved in the activation of angiotensin II, expression levels of cardiac AT1R in the four experimental groups were measured by Western blotting. As shown in Fig. 6, results demonstrated no change in the cardiac AT1R levels in the four groups.

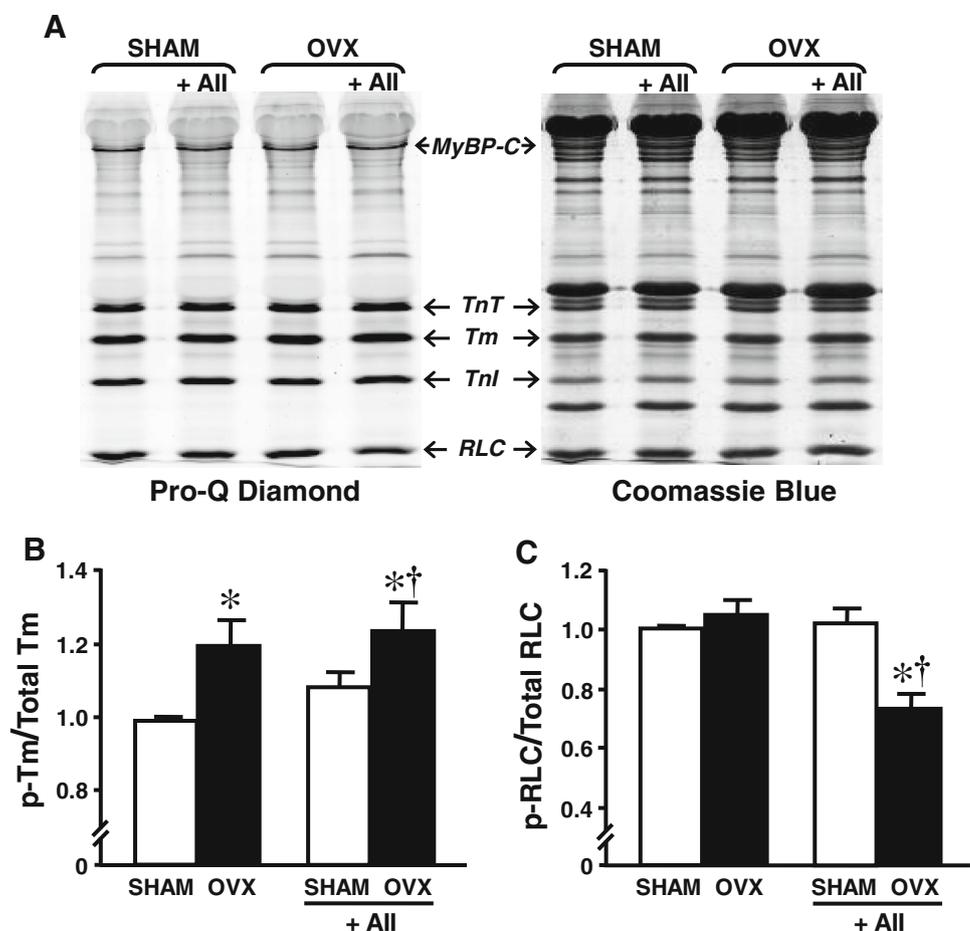
Finally, we tested the passive stiffness of the heart in order to find the possible mechanism underlying the

Fig. 5 Effect of ovariectomy and angiotensin II (AII)-induced hypertension on cardiac myofilament protein phosphorylation.

a Representative of myofilament protein phosphorylation stained with Pro-Q diamond and total protein expression stained with Coomassie dye of ventricular samples from SHAM and OVX rats, with/without AII.

b Relative amount of phospho-tropomyosin per total tropomyosin. **c** Relative amount of phospho-regulatory light chain per total regulatory light chain. Data are mean \pm SE from 6 to 9 hearts of each group.

*†Significantly different ($P < 0.05$) from SHAM and SHAM + AII group at the same sarcomere length, respectively, using Student–Newman–Keuls test after ANOVA. *MyBP-C* myosin binding protein C, *TnT* troponin T, *Tm* tropomyosin, *TnI* troponin I, *RLC* regulatory light chain



changes in myofilament Ca^{2+} sensitivity based on the Frank–Starling mechanism. There was no significant difference in the myocardial stiffness as assessed by the exponential constant between the sham and ovariectomized groups without angiotensin II (Fig. 7). In contrast, angiotensin II treatment consistently increased the exponential constant for myocardial stiffness in both sham and ovariectomized rats at the same degree (K constant = 13.5 ± 0.8 in AII-infused sham; 12.1 ± 0.6 in AII-infused OVX).

Discussion

The use of hormone replacement therapy in preventing/treating cardiovascular disease in menopausal women has been recently reassessed. This study sought to test the mechanistic role of female sex hormones in the adaptive response of cardiac contractile machinery under pathological stress. Results from this study demonstrated no significant effect of ovarian sex hormones on cardiac hypertrophy or hypertension induced by angiotensin II infusion. On the other hand, the effect of female sex hormone on cardiac myofilament activation was still persistent under angiotensin II-mediated hypertension, especially on the myofilament Ca^{2+} sensitivity. The profound effect of female sex hormones exerted on the expression of myosin heavy chain isoforms also correlated with the effect on

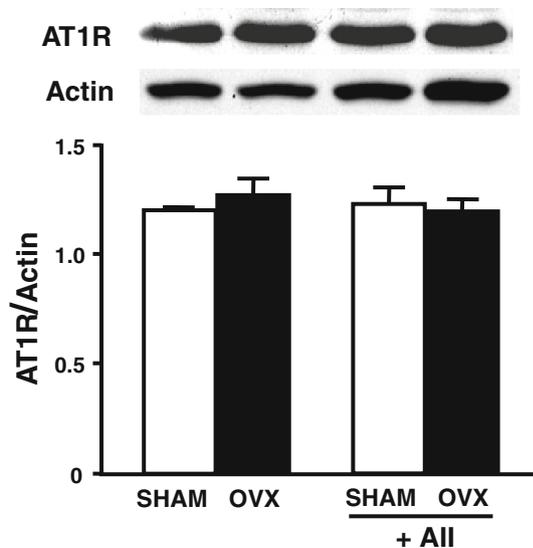


Fig. 6 Effect of ovariectomy and angiotensin II (AII)-induced hypertension on the expression of cardiac angiotensin II receptor type 1 (AT1R). *Top* representative electrophoresis analysis of cardiac AT1R and actin from left ventricular homogenate. *Bottom* ratio of AT1R to actin of the heart from SHAM and OVX rats, with/without AII. Data are mean \pm SE from 9 hearts of each group. No significant difference among the four experimental groups by ANOVA

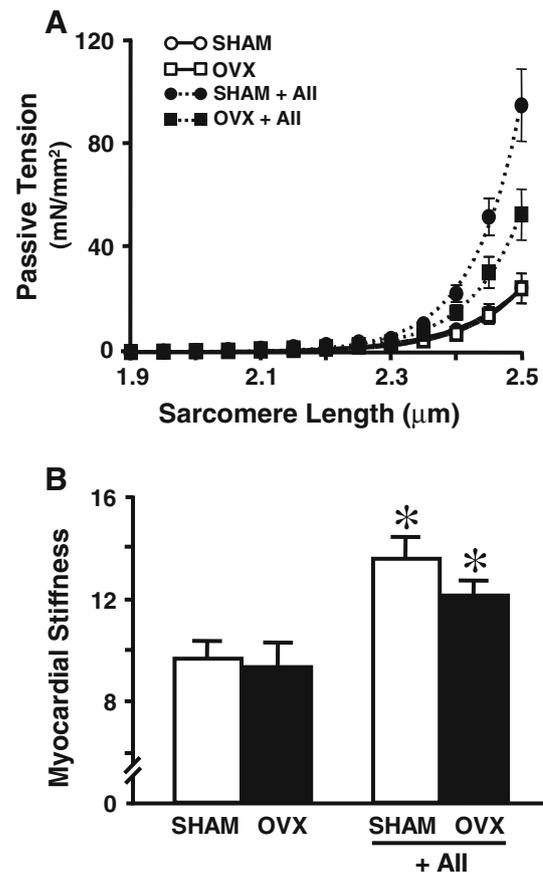


Fig. 7 Effect of ovariectomy and angiotensin II (AII)-induced hypertension on myocardial stiffness. **a** Comparison of passive tension at various sarcomere lengths ranging from 1.9 to 2.5 μ m of right ventricular trabeculae from SHAM and OVX rats, with/without AII. **b** Myocardial stiffness derived from exponential constant of passive tension-sarcomere length relation of the four experimental groups. Data are mean \pm SE from 9 to 10 fibers from 5 hearts of each group. *Significantly different ($P < 0.05$) from SHAM using Student–Newman–Keuls test after ANOVA

maximum active tension. Increases in the levels of phospho-tropomyosin in ovariectomized rats might be the underlying cause of cardiac myofilament Ca^{2+} hypersensitivity following ovarian sex hormone deprivation. However, an increase in myofilament Ca^{2+} sensitivity of the heart from AII-infused sham-operated rats without change in the phosphorylation levels of cardiac myofilament proteins suggests that female sex hormones and angiotensin II exerted their actions on myofilament Ca^{2+} activation via different pathways.

Female sex hormones and cardiac hypertrophy

Information concerning the role of female sex hormones, especially estrogen, on cardiac hypertrophy is still inconclusive. Our finding of smaller cross-sectional areas of myocytes in OVX compared to sham, despite a similar

heart weight is supported by a recent publication also showing a smaller cross-sectional area of cardiomyocytes from OVX mice than OVX mice with estrogen supplementation [10]. They demonstrated a significant role of estrogen in regulating cardiac muscle growth. Whether the length of the cardiomyocytes between sham and OVX is different or not is not yet answered.

Previous *in vitro* and *in vivo* studies have shown that female sex hormones could partially protect against cardiac hypertrophy induced by angiotensin II [9, 11]. Estrogen supplement could reverse the hypertrophic effect of angiotensin II by 50 %. The partial anti-hypertrophic action of the female sex hormones has also been shown in the model of transverse aortic constriction-induced cardiac hypertrophy [12]. Notably, in these studies, the animals were treated with either angiotensin II infusion or aortic banding for only 2 weeks. In contrast, the present study demonstrated no significant protective effect of female sex hormones on the induction of cardiac hypertrophy by 4-week angiotensin II infusion. It was possible that the different results were due to the duration of induced hypertension. Therefore, instead of prevention, female sex hormones might just delay the onset of cardiac hypertrophy.

Female sex hormones and myofilament Ca^{2+} sensitivity

As in previous reports, female sex hormone deprivation induced decreases in maximum myofilament activity and increases in Ca^{2+} sensitivity [2, 3]. Since the vasodilation effect of estrogen is well established, change in cardiac contractile function after menopause has been proposed as a secondary effect due to vascular dysfunction. The present results, however, confirmed that female sex hormones on their own directly regulate the maximum cardiac contractile activity partly through the expression of myosin heavy chain, independent of vascular hypertension. However, whether the effects of female sex hormones on cardiac myofilament Ca^{2+} sensitivity is a direct action or an adaptation is still not clear. Increased myofilament Ca^{2+} sensitivity of the heart in ovariectomized rats is possibly an adaptation to compensate the suppression in maximum tension in order to cope a normal force generation at physiological intracellular Ca^{2+} concentration ($\text{pCa} < 5.75$) (Fig. 2a). On the other hand, angiotensin II-induced myofilament Ca^{2+} hypersensitivity induced a higher force contraction at all physiological Ca^{2+} ranges. It is not known at this point whether increased myofilament Ca^{2+} sensitivity by chronic angiotensin II infusion is an adaptation of the ventricle to cope with an increase in afterload.

Moreover, the underlying mechanism of myofilament Ca^{2+} hypersensitivity demonstrated in either case was consistently found at longer sarcomere length (Fig. 4), and therefore the mechanism could be related or similar to the

“Starling” mechanism. Since many investigations have hypothesized that changes in myocardial stiffness probably account for the length-dependent change in myofilament Ca^{2+} sensitivity [1, 13–15], myofilament Ca^{2+} hypersensitivity due to angiotensin II induction might be associated with enhanced myocardial stiffness.

It has previously been documented that a mutation of Tm, which was strongly associated with familial dilated cardiomyopathy, affected myofilament Ca^{2+} sensitivity [16]. The serine-238 of Tm could be phosphorylated by p38 MAPK [17], and skinned cardiac fibers containing the phosphorylated α -Tm also had higher myofilament Ca^{2+} sensitivity than fibers containing the non-phosphorylated form [18]. Phospho-p38 MAPK has been found to be slightly increased in ovariectomized rat heart [19], and was reduced by 17β -estradiol supplement [20]. In addition, the pressure overload-induced increase in phospho-p38 MAPK could be attenuated by estrogen [20]. Another piece of evidence which supports estrogen modulating p38 MAPK was a recent report that inhibition of p38 MAPK prevents the estrogen-induced decrease in myofilament Ca^{2+} sensitivity [21]. In addition to the p38 MAPK pathway, phosphorylation of Tm in cardiomyocytes has been shown to be enhanced by PKC-zeta activation [22]. However, neither estrogen nor progesterone supplementation affected the expression of PKC-zeta in the ventricular muscle of ovariectomized rats [23]. Thus, cardiac myofilament hypersensitivity to Ca^{2+} activation in ovariectomized rats was likely to be due to p38 MAPK phosphorylation of Tm.

Angiotensin II-induced hypertension and myofilament Ca^{2+} activation

The mechanism underlying the increase in myofilament Ca^{2+} sensitivity in the heart of angiotensin II-induced hypertensive rats remains poorly understood. Angiotensin II was reported to directly enhance the myosin light chain kinase activity in cultured cardiac myocytes, leading to an increase in phosphorylation of the regulatory light chain (RLC) [24], which in turn enhanced Ca^{2+} sensitivity of the cardiac myofilament [25, 26]. However, while we detected enhanced myofilament Ca^{2+} sensitivity in both angiotensin II-infused sham and ovariectomized rats, RLC phosphorylation was not changed in the former group but decreased in the latter group. Thus, the mechanistic response of myofilament Ca^{2+} hypersensitivity in the present finding should not involve RLC phosphorylation.

In conclusion, we have shown in this study that female sex hormones exert a persistent regulatory effect on cardiac myofilament Ca^{2+} activation even under stress from direct angiotensin II activation and hypertension. Although the presence of female sex hormones could not normalize cardiac myofilament Ca^{2+} hypersensitivity due to

angiotensin II induction, without them further increases in sensitivity might lead to severe myocardial problems. This data provides support regarding the benefits of female sex hormones in heart disease patients.

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Conflict of interest The authors declare that they have no conflict of interest.

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