Life Sciences 219 (2019) 209–218

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Role of cardiac mast cells in exercise training-mediated cardiac remodeling in angiotensin II-infused ovariectomized rats

Rerknapat Jitmana[a], Sulaksana Raksapharm[a], Anusak Kijtawornrat[b], Vitoon Saengsirisuwan[a], Tepmanas Bupha-Intr[a,*]

[a] Department of Physiology, Faculty of Science, Mahidol University, 272 Rama 6 Road, Bangkok 10400, Thailand
[b] Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Henri-Dunant Road., Pathumwan, Bangkok 10330, Thailand

ARTICLE INFO

Keywords:
Hypertrophy
Collagen content
Chymase
TGF-β1

ABSTRACT

Aims: Regular exercise is recommended in postmenopausal women to prevent the development of heart disease, but mechanism underlying the protection is not completely understood. Many studies have suggested that exercise training notably mediated whole body immune and inflammatory functions. Whether exercise training prevents cardiac dysfunction after deprivation of female sex hormones by inhibiting cardiac immune activation is therefore interesting.

Main methods: Nine-week treadmill running program was introduced in sham-operated and ovariectomized rats. In addition, chronic angiotensin II infusion was further challenged to activate pathological cardiac remodeling. Cardiac remodeling in associated with the density and degranulation of cardiac mast cells was then evaluated.

Key findings: With exogenous angiotensin II-induced hypertension, cardiac hypertrophy with myocardial fibrosis was shown similarly in both sham-operated controls and ovariectomized rats. Although exercise training did not prevent cardiac hypertrophy, myocardial fibrosis was abolished by exercise. While ovariectomy increased both cardiac mast cell density and degranulation percentage, angiotensin II infusion only enhanced mast cell density. Exercise training could not decrease the density of mast cells, but it did normalize the percentage of degranulation in all groups. Correlation analysis suggested that cardiac mast cell activation is inversely associated with cardiomyocyte hypertrophy due to exercise training but is directly correlated to cardiac hypertrophy by angiotensin II infusion.

Significance: Exercise training could attenuate cardiac mast cell hyperactivation induced by either deprivation of female sex hormones or excessive angiotensin II. Additionally, cardiac mast cells could be a solution in the distinction between physiological and pathological hypertrophic development.

1. Introduction

Regular aerobic exercise has been recommended to prevent cardiovascular diseases in high-risk population. Upon aging, deprivation of female sex hormones is well-accepted as a cause of high heart disease prevalence [1]. Although regular exercise can lower the incidence of heart problems in postmenopausal women [2], the mechanism of protection is incompletely understood.

Anti-inflammation has been proposed as one protective mechanism of exercise training in preventing cardiac abnormality. In systemic circulation, exercise training suppressed several pro-inflammatory cytokines and stimulated anti-inflammatory products [3]. Recently, we reported that exercise training decreased mast cell hyperactivation in the heart of ovariectomized rats similar to estrogen supplementation [4]. As innate immune cells in the heart, cardiac mast cells are involved in cardiac remodeling under various pathological conditions [5,6]. Inhibition of cardiac mast cell activation impressively attenuated adverse cardiac remodeling [5,7,8]. Interestingly, cardiac mast cell activity is effectively regulated by female sex hormones [4–6,9]. Estrogen could attenuate an increase in cardiac mast cell activity induced by transverse aortic constriction [6] or volume overload [9]. Therefore, the inhibition of cardiac mast cell degranulation by exercise training might be a major signal in preventing cardiac abnormality after female sex hormone deprivation [4]. According to various complications are usually occurred after menopause such as hypertension and insulin resistance, whether exercise training effectively attenuates cardiac mast cell activation with pathological complication is interesting.

In postmenopausal women, hypertension in another significant

* Corresponding author.
E-mail address: tepmanas.bup@mahidol.ac.th (T. Bupha-Intr).

https://doi.org/10.1016/j.lfs.2019.01.018
Received 24 September 2018; Received in revised form 3 January 2019; Accepted 13 January 2019
Available online 15 January 2019
0024-3205/

Table 1
General characteristics of sham-operated and ovariectomized rats with/without AII infusion or exercise training.

	Saline				Angiotensin II			
	SHAM		OVX		SHAM		OVX	
	Sedentary	Exercise	Sedentary	Exercise	Sedentary	Exercise	Sedentary	Exercise
n	8	8	8	9	8	7	8	8
BW, g	299 ± 3	317 ± 5*	353 ± 7*	352 ± 4*	294 ± 3	301 ± 3	354 ± 2*	342 ± 5*
UW, g	0.56 ± 0.02	0.55 ± 0.02	0.14 ± 0.01*	0.11 ± 0.01	0.57 ± 0.03	0.53 ± 0.03	0.13 ± 0.01*	0.13 ± 0.01*
SBP, mm Hg	123 ± 3	119 ± 4	121 ± 2	123 ± 4	165 ± 6*	159 ± 5*	162 ± 4*	164 ± 5*
DBP, mm Hg	80 ± 2	80 ± 3	80 ± 3	81 ± 3	105 ± 8*	105 ± 4*	100 ± 3*	104 ± 3*
HW, g	1.45 ± 0.02	1.76 ± 0.02*	1.52 ± 0.04	1.61 ± 0.02*	1.56 ± 0.04	1.65 ± 0.05*	1.77 ± 0.03*	1.83 ± 0.06*
SW, g	0.14 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
HW/BW	0.47 ± 0.01	0.54 ± 0.01*	0.42 ± 0.01*	0.47 ± 0.01#	0.52 ± 0.01*	0.56 ± 0.01*	0.49 ± 0.01#	0.52 ± 0.01#
CSA#, $(\mu m)^2$	185 ± 2	224 ± 3*	150 ± 2*	187 ± 2#	247 ± 3*	225 ± 2*†	224 ± 2*	206 ± 3#†
CS activity	84 ± 7	128 ± 4*	88 ± 3	127 ± 8*	86 ± 8	123 ± 3*	100 ± 6	120 ± 4*

Data are means ± SEM from 7 to 9 rats each group (# Cardiomyocyte cross-sectional area, n = 120 cells from 5 hearts per group). *SHAM*, sham-operated control; *OVX*, ovariectomized rats; *BW*, body weight; *UW*, uterine weight; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *HW*, heart weight; *SW*, soleus weight; *HW/BW*, heart per body weight; *CSA*, cardiomyocyte cross-sectional area; *CS activity*, citrate synthase activity. *$P < 0.05$, #$P < 0.05$, †$P < 0.05$, significantly different from sedentary SHAM, sedentary OVX, and from sedentary group with angiotensin II in the same hormonal condition, respectively, using Student Newman-Keuls test after three-way ANOVA.

cardiovascular complication leading to heart disease [10]. Among various causes inducing high blood pressure, the upregulation of renin-angiotensin-aldosterone system has been attended [11]. It has been demonstrated that angiotensin II, a potent vasoconstrictor, significantly activates systemic inflammation leading to vascular injury [12]. However, the activation of systemic angiotensin II on cardiac inflammation is still unclear. While cardiac mast cells are well-known in inducing pathological cardiac remodeling, partly by activating angiotensin II production [13], it has also been reported that exogenous angiotensin II activated the degranulation of isolated cardiac mast cells [14]. Angiotensin II-mediated activation of mast cells may constitute a positive feedback mechanism contributing to elevated tissue levels of angiotensin II. Therefore, it is interesting to understand whether the elevation of plasma angiotensin II accelerates, in vivo, the activation of cardiac mast cells.

In the present study, we aimed to answer three specific questions: *1*) does chronic angiotensin II administration stimulate cardiac mast cell activation in vivo, *2*) does the lack of female sex hormones enhance angiotensin II-induced pathological cardiac remodeling, in which cardiac mast cell activation is intensified, and *3*) could exercise training at moderate intensity attenuate angiotensin II-induced pathological cardiac remodeling by suppressing cardiac mast cell activity? To answer these questions, experiments in which exogenous angiotensin II was administered to both sham-operated and ovariectomized rats subjected to a sedentary lifestyle or exercise training were performed. Four weeks of continuous angiotensin II infusion was applied to the rats during the last phase of a nine-week treadmill running program. Hypertrophy of cardiomyocytes and the degree of collagen deposition were determined as remodeling indices.

2. Materials and methods

2.1. Materials

All chemicals were obtained from Sigma Chemical (St. Louis, MO) and USB Corporation (Cleveland, OH); electrophoresis reagents were obtained from Bio-Rad (Hercules, CA) and Amersham Pharmacia Biotech (Buckinghamshire, UK); polyclonal anti-chymase (ab186417), monoclonal anti-interleukin 6 (ab9324), polyclonal anti-interleukin 10 (ab192271) and polyclonal anti-TGF-beta1 (ab179695) antibodies were obtained from Abcam (Cambridge, MA); Polyclonal anti-β-actin (ACTB) was obtained from AVIVA Bioscience (ARP40173_P050); horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (H + L) (ZyMax grade) was obtained from Zymed (San Francisco, CA); HRP-conjugated donkey anti-mouse IgG (43R-ID041HRP) was obtained from Fitzgerald (Acton, MA); and enhanced chemiluminescence reagents were obtained from Pierce (Thermo Scientific).

2.2. Ethical approval

The animal protocols were approved by the Experimental Animal Committee, Faculty of Science, Mahidol University (AAALAC international accreditation), and they were performed in accordance with Guide for the care and use of laboratory animals, eighth edition (National Research Council, USA).

2.3. Animal procedure

Female Sprague-Dawley rats were randomized into two groups: one group underwent a sham operation and the other rats were ovariectomized (OVX) at 8 weeks of age. Rats were paired and housed in standard cages, with rat chow and water provided ad libitum. One week after surgery, rats from each group were randomly divided into sedentary and exercise-trained groups. A 9-week running program on a motor-driven treadmill for 5 days/wk was implemented in the exercised groups. During the first week of the exercise program, rats were pretrained at a fixed speed of 21 m/min with 0% grade, with the running time varying from 2 × 5 min with a 10-min resting interval on day 1 to 2 × 25 min on day 5. From the second week and until the end of the program, the rats ran for 2 × 30 min at a fixed speed of 21 m/min, with 7.5% and 5.5% grades for the sham and OVX groups, respectively. The exercise intensities were calculated for a work rate of 65–75% maximum oxygen consumption based on body weight, as previously described [15]. An air blower was used to stimulate continuous running instead of electric shock. Five rats were discarded from the exercise groups after 2 weeks of training because they were unable to maintain the running speed. Increased plantaris citrate synthase activity indicated success of the aerobic exercise training (Table 1).

Six weeks after surgery, the sedentary and exercised rats from both the sham and OVX groups were subcutaneously implanted with a mini-osmotic pump (Alzet model 2004, California, USA) containing angiotensin II in normal saline with 2% acetic acid for 4 weeks. The rate of angiotensin II release was 0.7 mg/kg body weight (BW) per day [16]. For the non-angiotensin II-treated groups, rats were implanted with a mini-osmotic pump containing normal saline with 2% acetic acid without angiotensin II for 4 weeks instead. In the exercise groups, angiotensin II implantation was performed 24 h after the last exercise bout. After implantation, the rats had 4 days of rest before continuing

the exercise program. Blood pressure was measured using the CODA tail-cuff system (Kent Scientific Corporation, CT) 24 h before the rats were sacrificed. Increased blood pressure was an indication of successful chronic angiotensin II infusion. Three angiotensin II-infused rats were discarded from the study due to them having low blood pressure (< 140 mm Hg). The rats were anesthetized with 50 mg/kg pentobarbital sodium, and they underwent echocardiography before being sacrificed. Echocardiography using 2D, M-mode (Mindray M-9 echocardiography machine) was performed on the chest wall.

2.4. Measurement of cardiomyocyte size, collagen content and mast call activation

The heart was transversely excised at the middle and immediately placed in 10% neutral-buffered formalin at room temperature and incubated for 24 h before being embedded in paraffin. Samples were sectioned at a thickness of 5 μm and stained with hematoxylin and eosin. The cardiomyocyte cross-sectional area was calculated using a digital microscope (×400 magnification) with ImageJ (version 1.51K) software. Only cardiomyocytes with a nucleus, a clear cell boundary, and a round or rectangular shape (length:width < 1.5) were analyzed. The cardiomyocyte cross-sectional area of 6 cells per image field was counted, and 5 random fields were included for each heart (30 cells per rat heart).

Separated sections were stained with Picrosirius red dye (Direct red; Sigma) for 1 h as previously described [17]. Histological sections were examined under a light microscope (×400 magnification), and the images were recorded using a high-resolution digital camera. The red-colored area in the ventricular tissue represented collagen content. The ratio of collagen content to ventricular myocytes was analyzed based on a color histogram using ImageJ. All histological samples were double-blind to the examiners.

For the cardiac mast cells, the rehydrated tissue section was stained with 1 mg/mL toluidine blue for 3 min, and the dye was washed out with distilled water twice before dehydration. The cell counting was blinded in whole sections at a magnification of ×100 [4]. The density of mast cells was expressed as the mean number of mast cells per mm^2 of the tissue section. Degranulated mast cells were identified by extruded granules and the appearance of ruptured cell membranes or irregular border as granules were released from the cytoplasm. To reduce personal bias, cardiac mast cell density and degranulation percentage were examined independently by two blinded pathological investigators.

2.5. Immunoblot analysis

Frozen left ventricular tissue was homogenized in RIPA buffer containing a cocktail of protease inhibitors (Sigma Chemical). The protein concentration of the left ventricular homogenate was determined by bicinchoninic acid assay. Antibodies against chymase (1:1000 dilution) and TGF-β1 (1:5000) were used for analyzing protein contents of 100 μg tissue homogenate, while 150 μg tissue homogenate were used for antibodies against interleukin 10 (1:2500) and interleukin 6 (1:2000). The amount of protein was determined and represented per amount of β-actin, which was 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 Totallab version 1.0 (Amersham Pharmacia Biotech).

2.6. Data and statistical analysis

The data are presented as the mean ± SE, and they were analyzed by using 3-way ANOVA, followed by the Student-Newman-Keuls test for multiple comparisons (SPSS Statistics version 18.0). A P value < 0.05 was considered to indicate a significant difference.

3. Results

3.1. General characteristics

To evaluate the possible mechanisms of aerobic exercise training in preventing pathological cardiac remodeling, the cardiac mast cell density and degranulation percentage were studied in angiotensin II-infused rats in the presence and absence of female sex hormones. The general characteristics of the rats are reported in Table 1. The body weight of the rats was increased by the lack of female sex hormones or exercise training but not by chronic angiotensin II infusion. As expected, angiotensin II infusion induced significant increases in systolic and diastolic blood pressure to a similar degree in the sham-operated control and ovariectomized rats, in which exercise training could not suppress the hypertensive effect of angiotensin II infusion. Plantaris citrate synthase activity was increased to the same degree in all exercise-trained groups, indicating a similar level of training.

Based on the heart weight-to-body weight ratio, the lack of female sex hormones decreased the hypertrophic index, whereas both angiotensin II infusion and exercise training induced hypertrophy of the heart. However, no additive effect of angiotensin II infusion and exercise training on cardiac hypertrophy was observed in either the sham-operated or ovariectomized rats. This result was consistent with the cardiomyocyte cross-sectional area, in which ovariectomized rats had a decreased cell cross-sectional area, but exercise training activated cardiomyocyte hypertrophy. Chronic angiotensin II increased the cardiomyocyte cross-sectional area in both the sham-operated and ovariectomized rats (33% and 49% from non-angiotensin II groups, respectively). No synergistic hypertrophy existed between angiotensin II activation and exercise training, but exercise training significantly reduced the cell cross-sectional area in both the angiotensin II-infused sham-operated and ovariectomized rats.

Echocardiography also confirmed the effect of exercise training and chronic angiotensin II infusion (Table 2). Exercise training increased the left ventricular mass index without affecting the relative wall thickness in both the sham-operated and ovariectomized groups. Chronic angiotensin II infusion, on the other hand, increased the relative wall thickness, suggesting concentric hypertrophy. Unfortunately, exercise training did not attenuate angiotensin II-induced concentric hypertrophy. Interestingly, chronic angiotensin II infusion significantly increased the ejection fraction in all treated groups, even under an anesthetic condition, while exercise training had no effect on this factor.

3.2. Myocardial collagen content

To investigate the protective effect of exercise training on angiotensin II-induced collagen deposition, a histochemical technique was performed (Fig. 1). The lack of ovarian sex hormones had no effect on myocardial collagen content, as previously reported [18], but collagen deposition was significantly increased by chronic angiotensin II infusion in both the sedentary sham and sedentary ovariectomized rats (15.6 ± 2.1% in angiotensin II-infused sham versus 7.3 ± 0.9% in vehicle-treated sham control, and 12.9 ± 1.3% in angiotensin II-infused OVX versus 7.3 ± 0.7% in vehicle-treated OVX rats). Interestingly, exercise training completely eliminated the effect of angiotensin II on increased collagen content.

3.3. Cardiac mast cell activation

This result showed that ovarian sex hormone deprivation increased cardiac mast cell number per tissue area (increased by 28.5% from sham-control), which was expected (Fig. 2B). Interestingly, the four-week angiotensin II infusion increased mast cell density in the hearts of the sham rats (increased by 33.6% from sham-control), but no additive action was shown in the ovariectomized rats. Unfortunately, regular

Table 2
Echocardiographic parameters in sham-operated and ovariectomized rats with/without AII infusion or exercise training.

	Saline				Angiotensin II			
	SHAM		OVX		SHAM		OVX	
	Sedentary	Exercise	Sedentary	Exercise	Sedentary	Exercise	Sedentary	Exercise
n	8	8	8	9	8	7	8	8
Heart rate	503 ± 11	497 ± 16	480 ± 14	483 ± 19	451 ± 12*	448 ± 12*	472 ± 18	508 ± 20
IVSd	0.155 ± 0.004	0.166 ± 0.006*	0.183 ± 0.010*	0.173 ± 0.010*	0.204 ± 0.018*	0.223 ± 0.007*	0.209 ± 0.005*	0.228 ± 0.017*
IVSs	0.246 ± 0.011	0.264 ± 0.009	0.268 ± 0.015	0.271 ± 0.010	0.294 ± 0.013	0.334 ± 0.011*#	0.336 ± 0.011*#	0.356 ± 0.016*#
LVIDd	0.586 ± 0.011	0.628 ± 0.014*	0.625 ± 0.016*	0.623 ± 0.011*	0.553 ± 0.025#	0.510 ± 0.027#	0.549 ± 0.022#	0.568 ± 0.016
LVIDs	0.356 ± 0.016	0.344 ± 0.020	0.410 ± 0.023*	0.384 ± 0.019	0.290 ± 0.021	0.267 ± 0.012*#	0.269 ± 0.010*#	0.261 ± 0.010*#
LVPWd	0.160 ± 0.005	0.168 ± 0.010	0.164 ± 0.007	0.174 ± 0.004*	0.169 ± 0.005	0.216 ± 0.010*	0.206 ± 0.008*#	0.205 ± 0.007*#
LVPWs	0.241 ± 0.004	0.260 ± 0.010	0.245 ± 0.009	0.268 ± 0.008*#	0.280 ± 0.012*	0.317 ± 0.016*#	0.303 ± 0.008*#	0.330 ± 0.009*#
%EF	38.9 ± 2.5	44.3 ± 1.8	35.8 ± 2.3	37.9 ± 2.4	48.6 ± 2.7*#	48.1 ± 2.5*#	51.6 ± 2.3*#	54.3 ± 1.8*#
LVmass index	1.62 ± 0.06	2.02 ± 0.08*	1.69 ± 0.08	1.94 ± 0.09*#	2.32 ± 0.06*#	2.66 ± 0.09*#	2.33 ± 0.09*#	2.53 ± 0.15*
RWT	0.539 ± 0.019	0.551 ± 0.032	0.556 ± 0.028	0.566 ± 0.034	0.740 ± 0.054*	0.847 ± 0.055*#	0.762 ± 0.027*#	0.776 ± 0.045*#

Data are means ± SEM from each group (n = 7–9 rats/group). *SHAM*, sham-operated control; *OVX*, ovariectomized rats; Data includes systolic (*s*) and diastolic parameters (*d*) of interventricular septum, *IVS*; left ventricular posterior wall, *LVPW*; left ventricular internal diameter, *LVID*; ejection fraction, *EF*; relative wall thickness, *RWT*; and left ventricular mass index. $^{*}P < 0.05$, $^{\#}P < 0.05$, †$P < 0.05$, significantly different from sedentary SHAM, sedentary OVX, and from sedentary group with angiotensin II in the same hormonal condition, respectively, using Student Newman-Keuls test after three-way ANOVA.

aerobic exercise did not prevent the increase in cardiac mast cell density induced by either ovariectomy or angiotensin II infusion. Three-way ANOVA analysis indicated that there was an interaction between female sex hormone condition and regular aerobic exercise but not between the presence of angiotensin II and cardiac mast cell density ($P = 0.0427$). In addition, our results demonstrate an increase in the percentage of mast cell degranulation in the hearts of ovariectomized rats (increased by 11.2% from sham-control), in which regular aerobic exercise attenuated this change (Fig. 2C). Four-week angiotensin II infusion did not induce mast cell degranulation in sham-operated sedentary rats and had no additional effect on this change in the hearts of sedentary ovariectomized rats (increased by 11.7% from sham-control). As hypothesized, regular aerobic exercise attenuated an increase in mast cell degranulation in the hearts of angiotensin II-infused ovariectomized rats. Surprisingly, it had no association between changes in the cardiac mast cell density and degranulation to chymase protein expression in the heart (Fig. 3A). Increased chymase expression was observed only in angiotensin II-infused ovariectomized rat hearts, and this was prevented by regular exercise. Three-way ANOVA indicated no interaction among 3 independent factors with the percentage of cardiac mast cell degranulation and chymase expression. These findings suggested that 1) exogenous angiotensin II stimulates cardiac mast cell proliferation and chymase production, 2) the presence of female sex hormones attenuated cardiac mast cell degranulation and chymase production, and 3) regular aerobic exercise also exerts an inhibitory effect on cardiac mast cell degranulation and chymase production.

Other inflammatory factors were also examined in this study. No significant difference in the expression of interleukin 6, a pro-inflammatory factor, and interleukin 10, an anti-inflammatory factor, were observed in the heart among all experimental groups (Fig. 3B & C). These findings indicate no effect of female sex hormones, angiotensin II, or regular exercise on the cardiac inflammatory factor. Nevertheless, angiotensin II infusion induced a significant upregulation of TGF-β1 in the heart of both sham-operated and ovariectomized rats (Fig. 3D). Unfortunately, exercise training could not attenuate this action. It is interesting whether exercise training diminish angiotensin II-increased collagen deposition by further interfering downstream signaling of TGF–β1.

Next, the role of cardiac mast cells in myocardial remodeling was indirectly determined by determining the relationship among mast cell activation, cardiomyocyte hypertrophy and myocardial collagen deposition. Fig. 4A is representative images of cardiomyocyte cross-section of left ventricle from eight experimental groups, in which data was summarized in Table 1. Fig. 4B and D demonstrate the relationship of the cross-sectional area of cardiomyocytes to total density of MCs and degranulation density of MCs, respectively, among the sedentary and exercise groups without angiotensin II infusion. Both graphs suggest that hypertrophy of cardiomyocytes due to exercise training is inversely associated with cardiac mast cell activity, with a strong correlation shown for both ($r^2 = 0.6669$, $P < 0.0001$; $r^2 = 0.7261$, $P < 0.0001$, respectively). On the other hand, the relationships of cardiomyocyte hypertrophy due to angiotensin II infusion and cardiac mast cell activation (Fig. 4C and E) were direct but less correlated ($r^2 = 0.1745$, $P = 0.0423$ in total density; $r^2 = 0.1478$, $P = 0.0636$ in degranulation density). These results distinguished the opposite role of cardiac mast cells in physiological- and pathological-induced cardiac hypertrophy.

In addition, the relationship between cardiac mast cell activation on collagen content was evaluated between the sham-operated and ovariectomized groups (Fig. 5). The results demonstrated that both cardiac mast cell density and degranulation were directly correlated with the percentage of myocardial collagen content ($r^2 = 0.1817$, $P = 0.0266$ in total density; $r^2 = 0.1957$, $P = 0.0209$ in degranulation density). However, the correlation was not shown in the ovariectomized groups ($r^2 = 0.04486$, $P = 0.3094$ in total density; $r^2 = 0.118$, $P = 0.1023$ in degranulation density). The relationship between cardiac mast cell activity and fibrosis due to angiotensin II induction seems to be unconvincing.

4. Discussion

The present study is the first to report the effect of chronic angiotensin II infusion on in vivo cardiac mast cell activation that possibly involves in cardiac remodeling process. We also compared the protective effect of female sex hormones and exercise training in modulating cardiac mast cell activity under chronic angiotensin II infusion. Both chronic angiotensin II infusion and exercise training induced cardiac hypertrophy independent of female sex hormone condition, but they did so in different ways. Cardiac mast cell activation extent was also different between chronic angiotensin II infusion and exercise training. Hypertrophy in the heart due to exercise training showed a lower level of cardiac mast cell activity, whereas cardiac hypertrophy due to angiotensin II infusion exhibited cardiac mast cell hyperactivation. Exercise training also attenuated chronic angiotensin II-induced cardiac fibrosis in both sham-operated and ovariectomized rats. The weak correlation between cardiac collagen deposition and cardiac mast cells activation suggests that the mechanism of angiotensin II that induced cardiac fibrosis might not apply to cardiac mast cells. Increased chymase expression in the hearts of angiotensin II-infused ovariectomized

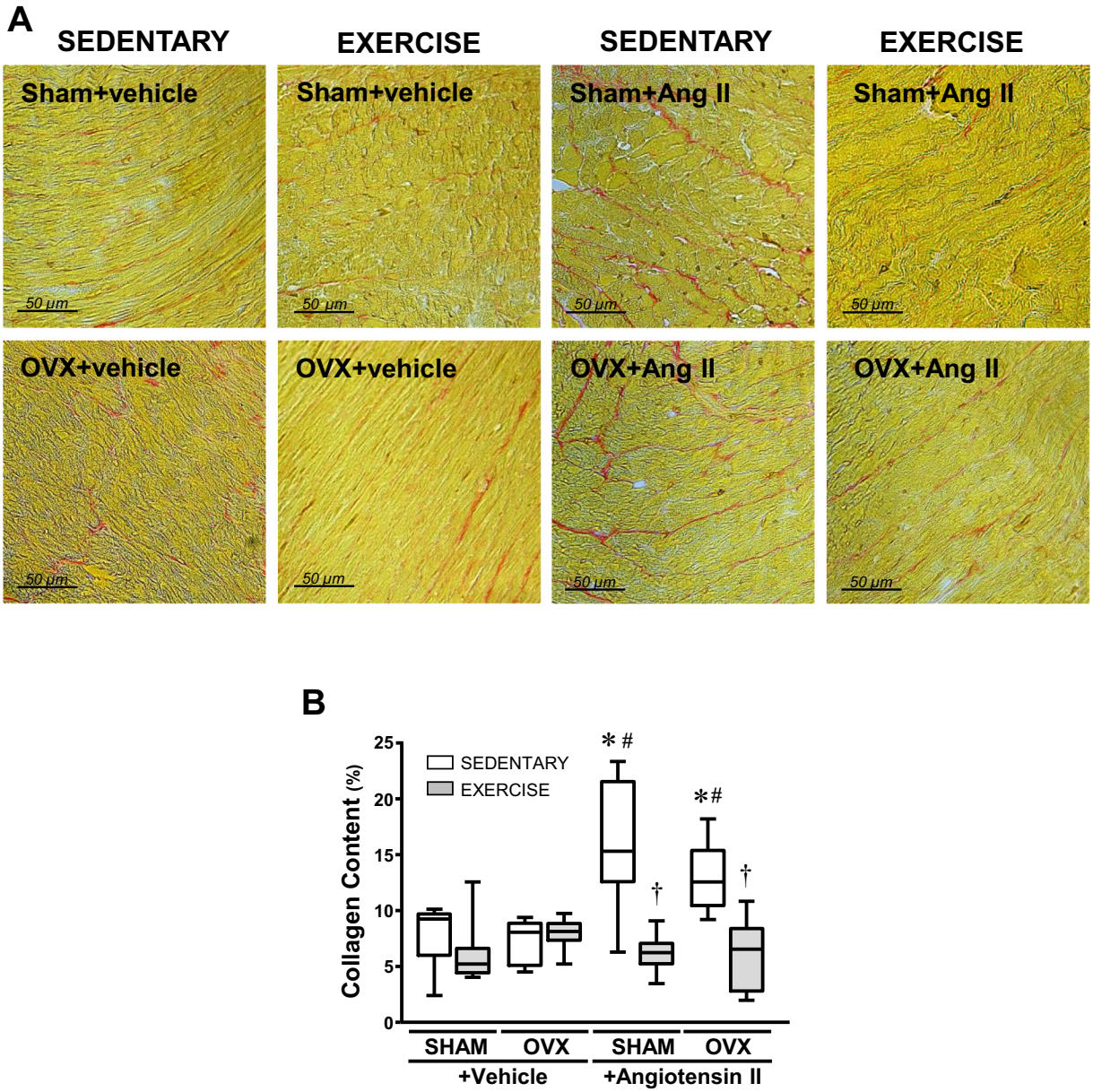

Fig. 1. Effect of exercise training and 4-week angiotensin II (AII)-infusion on myocardial collagen deposition. A) Representative images of myocardial collagen content stained with Picrosirius Red from the heart of sedentary and exercise-trained sham and OVX rats with/without angiotensin II infusion. B) Boxplot comparing the percentage of collagen content among the sedentary and exercise-trained experimental groups. Data are expressed as the mean ± SE from 150 image fields of 5 hearts in each group. *, #, † Significantly different ($P < 0.05$) from sedentary sham, sedentary OVX, and sedentary groups with the same treatment, respectively, using the Student–Newman–Keuls test after 3-way ANOVA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

rats could imply that angiotensin II activates chymase production, while female sex hormones and exercise training attenuated this effect. On the other hand, upregulation of TGF-β1 in the hearts of angiotensin II-infused rats could not be prevented by a presence of female sex hormones or exercise training.

4.1. Angiotensin II and cardiac remodeling

The significant role of angiotensin II in the development of heart failure has been extensively recognized. Chronic angiotensin II stress induces cardiac remodeling by directly activating cardiac cells and indirectly increasing afterload to the heart [19,20]. Additionally, angiotensin II-induced cardiac inflammation has been proposed as a significant mechanism in hypertrophic development [21,22]. Cardiac hypertrophy induced by angiotensin II infusion was significantly attenuated in mice with a deletion of tumor necrosis factor-α [21]. The inhibition of chemokine monocyte chemoattractant protein 1 or inflammasome signaling could also prevent cardiac fibrosis induced by angiotensin II infusion [22]. Although evidence indicates the importance of inflammation on the pathological cardiac hypertrophic process, the mechanism of angiotensin II-induced cardiac inflammation is still unknown. One possible mechanism through which angiotensin II activates the transmission of proinflammation from cardiomyocytes to macrophages was also proposed [23]. However, no change in interleukin 6 expression in the present finding further support a role of cardiac mast cell activation as another signaling of cardiac inflammation by chronic angiotensin II infusion. It has been previously reported that isolated cardiac mast cells expressing angiotensin II receptor types

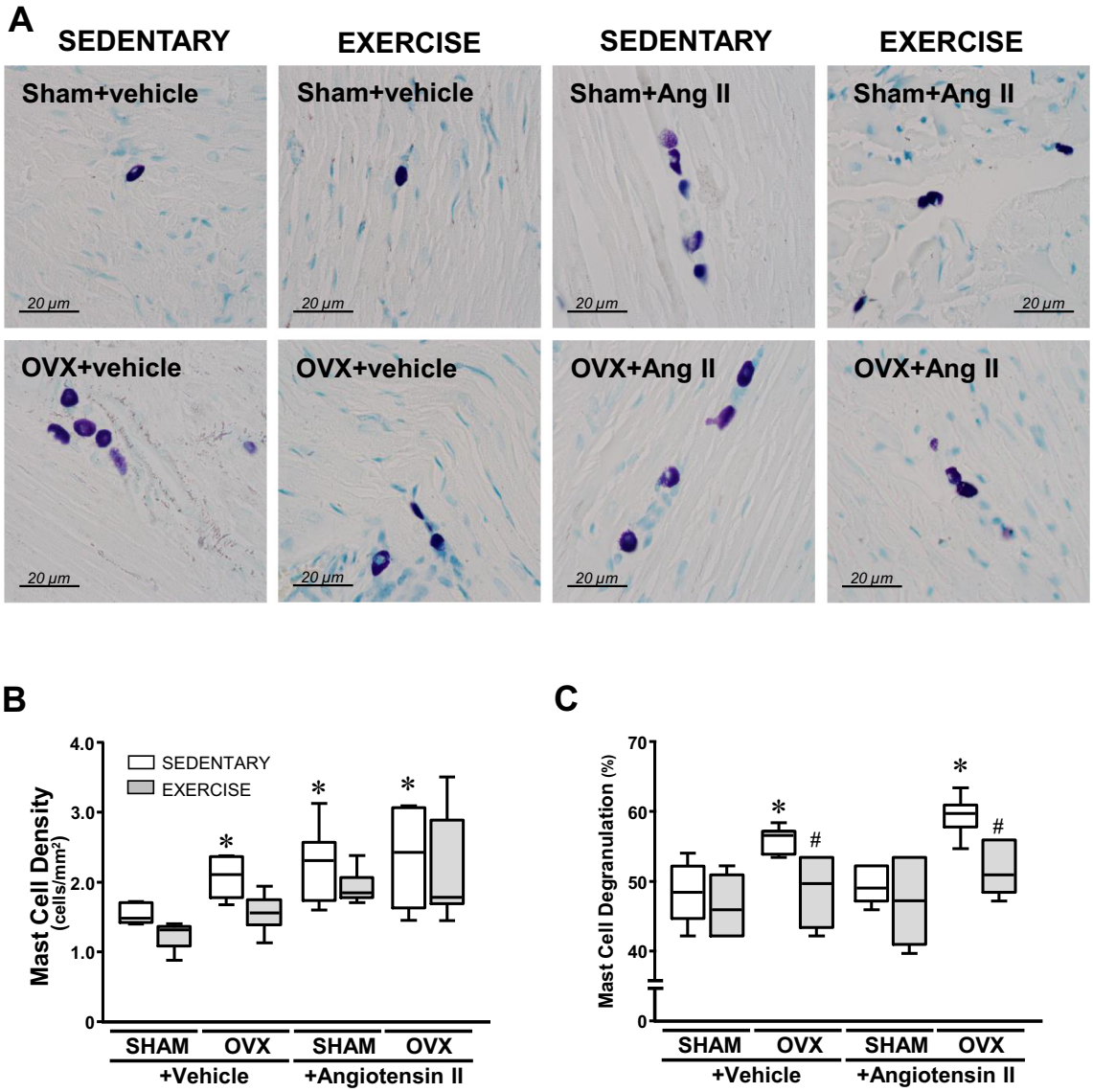

Fig. 2. Effect of exercise training and 4-week angiotensin II (AII)-infusion on cardiac mast cell activation. A) Representative images (×1000) of toluidine blue-stained cardiac mast cells from sections of each heart from sedentary and exercise-trained sham and OVX rats with/without angiotensin II infusion. B) Cardiac mast cell density and C) Percentage of cardiac mast cell degranulation were shown in a boxplot. Data are expressed as the mean ± SE from 8 to 9 hearts in each group. *, # Significantly different ($P < 0.05$) from sedentary sham and sedentary OVX, using the Student–Newman–Keuls test after 3-way ANOVA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

I and II could be degranulated by exogenous angiotensin II [14]. The increased collagen deposition in the hearts of chronic angiotensin II infusion rats is a result that is consistent with the results from a previous report demonstrating that chymase released from mast cells induced a profibrotic response in rat cardiac fibroblasts [24]. This activation can work together with an upregulation of TGF-β1 due to the direct stimulation of angiotensin II. However, a previous study demonstrated that the chymase enzyme could be synthesized by other types of cells [25] and that the production of chymase in mast cells is varied depending on different stimulations [26]. Therefore, angiotensin II could be the one that activates chymase production, while female sex hormones and exercise training suppresses chymase production. Further study on the specific inhibition of cardiac mast cell activation in angiotensin II-induced cardiac remodeling model is needed. Another point of interest is the mechanism regarding the effect of angiotensin II on cardiac mast cell proliferation. A previous study demonstrated that an increased cardiac mast cell density is mainly due to the maturation and differentiation of immature resident cardiac mast cells [27]. Whether angiotensin II directly activates immature resident cardiac mast cells or indirectly stimulates other inflammatory cells via chemotaxis [28] is interesting.

4.2. Protective effect of female sex hormones

Although many investigations have confirmed the cardiovascular protective effect of female sex hormones, no attenuated effect of sex steroids against the effect of angiotensin II on cardiac remodeling, in the form of both hypertrophy and fibrosis, was observed in the present study. Previous studies demonstrated that estrogen, via the estrogen receptor beta, directly inhibited angiotensin II-induced hypertrophy in cardiomyocyte culture [29] and attenuated collagen production of cultured cardiac fibroblasts [30]. Estrogen supplementation in angiotensin II-infused ovariectomized mice could significantly lower blood pressure and consequently attenuate cardiac hypertrophy [31]. However, the high level of exogenous angiotensin II used in our study might overrule the antihypertensive effect of female sex hormones. Without

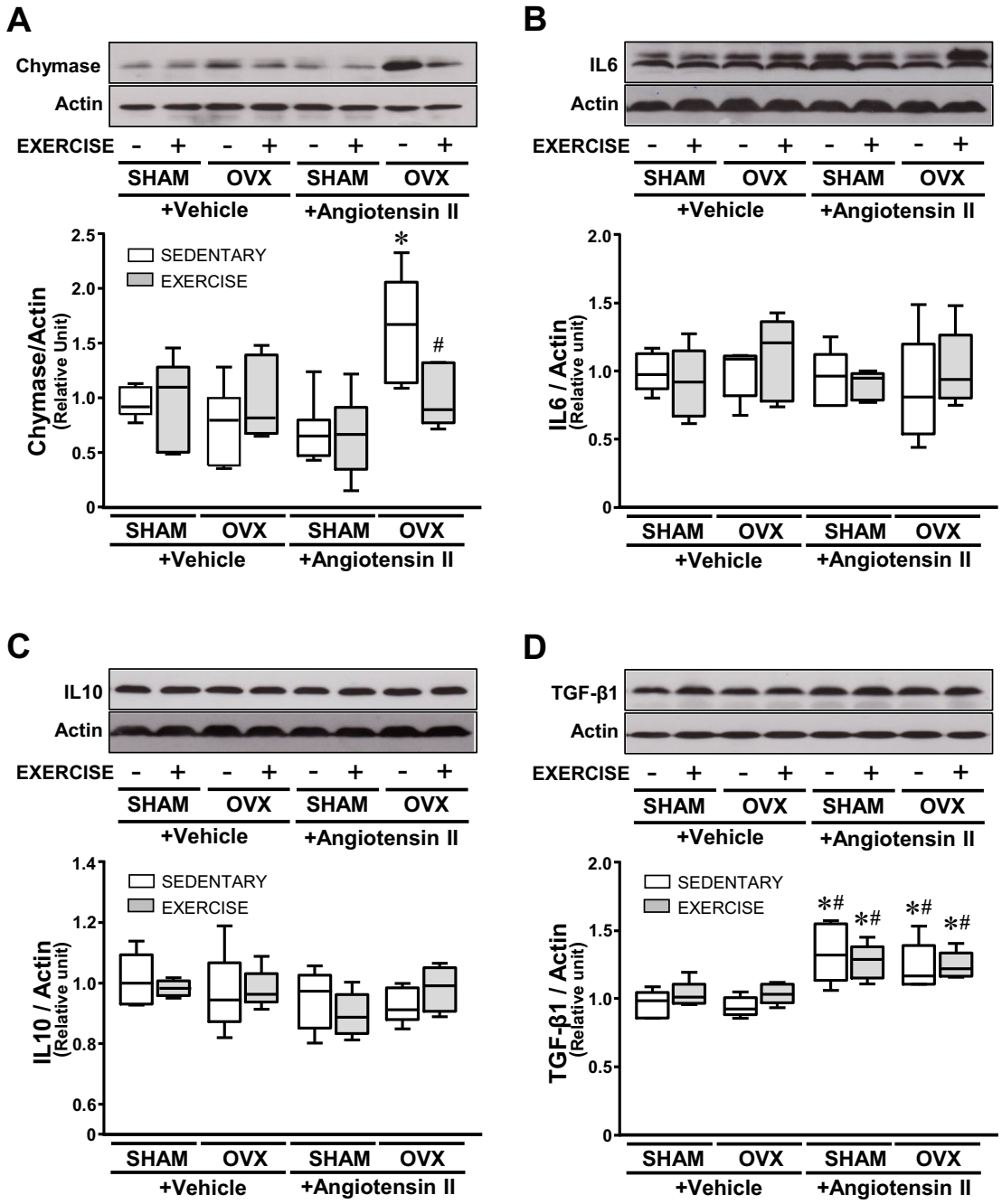

Fig. 3. Effect of exercise training and 4-week angiotensin II-infusion on inflammatory factor expressions. Immunoblot analysis demonstrating A) chymase, B) IL6, C) IL10, and D) TGF-β1 expression in which relative amounts of protein per actin. Data are expressed as the mean ± SE from 5 hearts in each group. *, # Significantly different ($P < 0.05$) from sedentary sham and sedentary OVX, using the Student–Newman–Keuls test after 3-way ANOVA.

the protection against angiotensin II-induced hypertension, we observed no protective effect of female sex hormones on changes in both cardiac structure and function as well as the upregulation of TGF-β1. Whether physical overload due to hypertension is an overriding factor in myocardial remodeling process is interesting, and another point of interest is the inhibitory effect of female sex hormones on cardiac mast cell activation. Many previous studies demonstrated that the presence of estrogen attenuated cardiac mast cell density and degranulation under pathological stressors, such as pressure and volume overload [6,9]. However, under chronic angiotensin II infusion, the presence of female sex hormones did not inhibit cardiac mast cell hyperactivation. Since estrogen supplementation can significantly reduce angiotensin II production [32], whether those suppressive effects of estrogen on cardiac mast cell activation are a result of decreased cardiac angiotensin II activation is questioned.

4.3. Exercise training and cardiac remodeling

Although aerobic exercise training did not reduce cardiac hypertrophy in a concentric fashion in angiotensin II-infused rats, exercise training reduced angiotensin II-increased cardiomyocyte hypertrophy and collagen deposition. These results suggest that exercise training overcomes the effect of chronic angiotensin II induction. It has been previously demonstrated that low-to-moderate intensity aerobic exercise can decrease the cardiac collagen content in spontaneous hypertensive rats [33]. In that model, exercise training also significantly

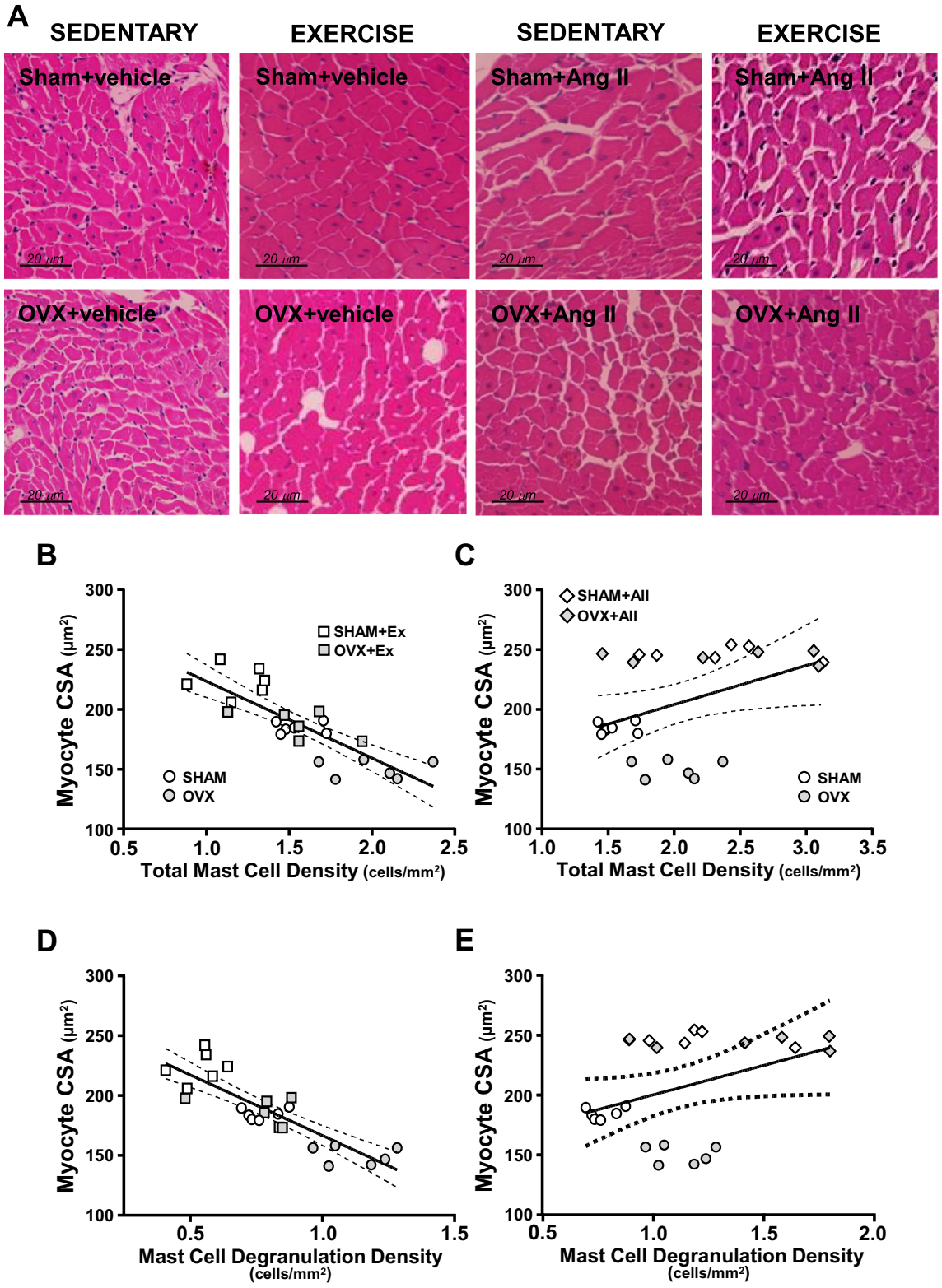

Fig. 4. Relationship between total cardiac MC density and degranulated MC density in the cardiomyocyte cross-sectional area. A) Representative images of histological section from the left ventricle of sedentary and exercise-trained sham and OVX rats with/without angiotensin II infusion. B) and D) indicate four experimental groups of sedentary and exercise-trained sham and OVX rats. C) and E) indicate four experimental groups of sedentary sham and OVX rats with and without angiotensin II infusion. intervals. The solid line is the line of best fit on linear regression, and the dotted lines represent the 95% confidence. Linear regression analysis indicated an opposite relationship between the cardiomyocyte cross-sectional area and cardiac mast cell activation between exercise-training and angiotensin II treatment-induced cardiac hypertrophy.

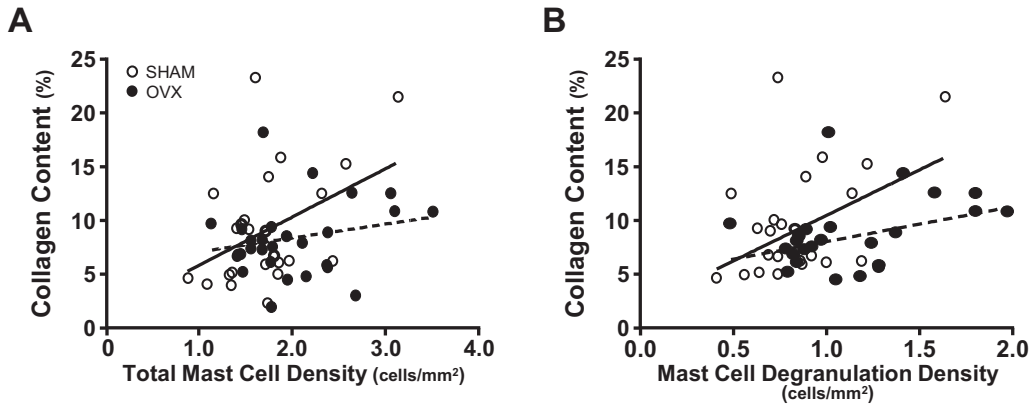

Fig. 5. Relationship between total cardiac MC density (A) and degranulated MC density (B) to the percentage of collagen content in the heart of all experimental sham rats (○, opened circle) and of all experimental OVX rats (●, closed circle). Solid line represents a best fit of linear regression in sham-operated groups, while dotted line a best fit of linear regression in ovariectomized groups. Linear regression is presented by solid line Linear regression analysis indicated the direct relationship of the percentage of collagen content cardiac to total mast cell density and degranulated mast cell density in the sham-operated group ($r^2 = 0.1817$ and $r^2 = 0.1957$, respectively) but no correlation in the ovariectomized groups ($r^2 = 0.04486$ and $r^2 = 0.118$, respectively).

reduced blood pressure. It is clear from our present finding that the antifibrotic effect of exercise training is independent of physical overload. However, mechanisms underlying the antifibrotic activation of exercise training are controversial. It is well established that a major fibrotic signaling factor in the heart is transforming growth factor-beta (TGF-β). Chronic angiotensin II infusion increased TGF-β in the cardiac tissue, which then activated fibroblast activity [34]. Similar to the present finding, no change in TGF-β expression were mostly reported in the heart after exercise training [35,36]. However, exercise training did significantly attenuate the increase in TGF-β in the heart of rats with sustained β-adrenergic hyperactivity [36]. Interestingly, our present study demonstrated no protective effect of exercise training on angiotensin II-increased TGF-β expression, though exercise training could attenuate angiotensin II-induced cardiac fibrosis. It is therefore interesting whether the protective action of exercise training is targeting at downstream signaling beyond TGF-β stimulation.

Anti-mast cell activation of exercise training could be another mechanistic form of activation for reducing cardiomyocyte hypertrophy and myocardial fibrosis. Among the many chemokines released by degranulation of cardiac mast cells, chymase has been proposed as having a multifunctional role in cardiac hypertrophy and fibrosis because it activates local angiotensin II synthesis and, consequently, TGF-β expression [37]. A possible effect of regular aerobic exercise on cardiac mast cell degranulation could be the suppression of the intracellular Ca^{2+}-mediated signaling pathway. An increase in intracellular Ca^{2+} activates the SNARE complex and then induces granule membrane fusion, leading to the release of all secretory factors [38]. It has been demonstrated that long-term treatment with a β_2 adrenergic agonist inhibited the Ca^{2+}-activated K^+ channel, leading to hyperpolarization and then to the inhibition of Ca^{2+} influx and degranulation of human mast cells [39]. Further study regarding the effect of regular aerobic exercise and sympathetic signaling on intracellular signal regulating mast cell degranulation needs to be investigated. Moreover, different chymase levels between angiotensin II-infused sham and angiotensin II-infused ovariectomized rats leads to another concern regarding the regulatory role of female sex hormones on chymase production and on the degranulation process of cardiac mast cells.

4.4. Limitations

Unlike what occurs in hypertensive patients and hypertensive rat models, the infusion of angiotensin II by using mini-osmotic pumps suddenly increased and sustained blood pressure throughout a perfusion period. Thus, the results from the present study lacked an antihypertensive effect of exercise training that was found in hypertension patients who underwent regular exercise. Another limitation is that the role of cardiac mast cells in cardiac contraction could not be identified because angiotensin II directly increased the left ventricular ejection fraction in all experimental groups. This increase is not surprising since increases in intracellular Ca^{2+} transients and myofilament Ca^{2+} sensitivity by angiotensin II have been reported [40,41].

5. Conclusion

In the present study, our questions are fulfilled that systemic angiotensin II can stimulate cardiac mast cell proliferation in vivo in independent of female sex hormones. Exercise training suppresses cardiac mast cell hyperactivation by mainly inhibiting degranulation. Another interested finding is that cardiac mast cells could be key in the distinction between physiological and pathological hypertrophic development.

Acknowledgments

We would like to thank Dr. Chutima Rattanasopa and Ms. Sukanya Phungphong for assisting in the technical aspects of the histopathological and immunoblot analyses.

This work was supported by the Thailand Research Fund (RSA6080064 to Bupha-Intr), and the Faculty of Science of Mahidol University provided additional financial support for this research project.

Competing interests

The authors declare that they have no conflicts of interest.

References

[1] G. Schillaci, P. Verdecchia, C. Borgioni, A. Ciucci, C. Porcellati, Early cardiac changes after menopause, Hypertension 32 (1998) 764–769 (DOI).
[2] N.M. Grindler, N.F. Santoro, Menopause and exercise, Menopause 22 (2015) 1351–1358, https://doi.org/10.1097/GME.0000000000000536.
[3] A.M. Petersen, B.K. Pedersen, The anti-inflammatory effect of exercise, J. Appl. Physiol. (1985) 98 (2005) 1154–1162, https://doi.org/10.1152/japplphysiol.00164.2004.
[4] S. Phungphong, A. Kijtawornrat, J. Wattanapermpool, T. Bupha-Intr, Regular exercise modulates cardiac mast cell activation in ovariectomized rats, J. Physiol. Sci. 66 (2016) 165–173, https://doi.org/10.1007/s12576-015-0409-0.
[5] H. Wang, J. da Silva, A. Alencar, G. Zapata-Sudo, M.R. Lin, X. Sun, S. Ahmad, C.M. Ferrario, L. Groban, Mast cell inhibition attenuates cardiac remodeling and diastolic dysfunction in middle-aged, ovariectomized Fischer 344 x brown Norway rats, J. Cardiovasc. Pharmacol. 68 (2016) 49–57, https://doi.org/10.1097/FJC.0000000000000385.
[6] J. Li, S. Jubair, J.S. Janicki, Estrogen inhibits mast cell chymase release to prevent

pressure overload-induced adverse cardiac remodeling, Hypertension 65 (2015) 328–334, https://doi.org/10.1161/HYPERTENSIONAHA.114.04238.
[7] S.P. Levick, J.D. Gardner, M. Holland, M. Hauer-Jensen, J.S. Janicki, G.L. Brower, Protection from adverse myocardial remodeling secondary to chronic volume overload in mast cell deficient rats, J. Mol. Cell. Cardiol. 45 (2008) 56–61, https://doi.org/10.1016/j.yjmcc.2008.04.010.
[8] G.L. Brower, J.S. Janicki, Pharmacologic inhibition of mast cell degranulation prevents left ventricular remodeling induced by chronic volume overload in rats, J. Card. Fail. 11 (2005) 548–556, https://doi.org/10.1016/j.cardfail.2005.05.005.
[9] H. Lu, G.C. Melendez, S.P. Levick, J.S. Janicki, Prevention of adverse cardiac remodeling to volume overload in female rats is the result of an estrogen-altered mast cell phenotype, Am. J. Physiol. Heart Circ. Physiol. 302 (2012) H811–H817, https://doi.org/10.1152/ajpheart.00980.2011.
[10] R. Lima, M. Wofford, J.F. Reckelhoff, Hypertension in postmenopausal women, Curr. Hypertens. Rep. 14 (2012) 254–260, https://doi.org/10.1007/s11906-012-0260-0.
[11] H. Schunkert, A.H. Danser, H.W. Hense, F.H. Derkx, S. Kurzinger, G.A. Riegger, Effects of estrogen replacement therapy on the renin-angiotensin system in postmenopausal women, Circulation 95 (1997) 39–45 (DOI).
[12] M. Abu-Taha, C. Rius, C. Hermenegildo, I. Noguera, J.M. Cerda-Nicolas, A.C. Issekutz, P.J. Jose, J. Cortijo, E.J. Morcillo, M.J. Sanz, Menopause and ovariectomy cause a low grade of systemic inflammation that may be prevented by chronic treatment with low doses of estrogen or losartan, J. Immunol. 183 (2009) 1393–1402, https://doi.org/10.4049/jimmunol.0803157.
[13] C.J. Mackins, S. Kano, N. Seyedi, U. Schafer, A.C. Reid, T. Machida, R.B. Silver, R. Levi, Cardiac mast cell-derived renin promotes local angiotensin formation, norepinephrine release, and arrhythmias in ischemia/reperfusion, J. Clin. Invest. 116 (2006) 1063–1070, https://doi.org/10.1172/JCI25713.
[14] S.M. Biscotte, S.P. Levick, M.L. Bertling, L.G. Morgan, J.S. Janicki, G.L. Brower, Angiotensin II mediated activation of cardiac mast cells, FASEB J. 21 (2007) A1253 (DOI).
[15] T.G. Bedford, C.M. Tipton, N.C. Wilson, R.A. Oppliger, C.V. Gisolfi, Maximum oxygen consumption of rats and its changes with various experimental procedures, J. Appl. Physiol. Respir. Environ. Exerc. Physiol. 47 (1979) 1278–1283, https://doi.org/10.1152/jappl.1979.47.6.1278.
[16] N. Ishizaka, K. Saito, H. Mitani, I. Yamazaki, M. Sata, S. Usui, I. Mori, M. Ohno, R. Nagai, Iron overload augments angiotensin II-induced cardiac fibrosis and promotes neointima formation, Circulation 106 (2002) 1840–1846 (DOI).
[17] M.A. Silver, R. Pick, C.G. Brilla, J.E. Jalil, J.S. Janicki, K.T. Weber, Reactive and reparative fibrillar collagen remodelling in the hypertrophied rat left ventricle: two experimental models of myocardial fibrosis, Cardiovasc. Res. 24 (1990) 741–747, https://doi.org/10.1093/cvr/24.9.741.
[18] T. Bupha-Intr, Y.W. Oo, J. Wattanapermpool, Increased myocardial stiffness with maintenance of length-dependent calcium activation by female sex hormones in diabetic rats, Am. J. Physiol. Heart Circ. Physiol. 300 (2011) H1661–H1668, https://doi.org/10.1152/ajpheart.00411.2010.
[19] Y. Lu, S. Yang, Angiotensin II induces cardiomyocyte hypertrophy probably through histone deacetylases, Tohoku J. Exp. Med. 219 (2009) 17–23 (DOI).
[20] A.W. Norris, T.M. Bahr, T.D. Scholz, E.S. Peterson, K.A. Volk, J.L. Segar, Angiotensin ii induced cardiovascular load regulates cardiac remodeling and related gene expression in late gestation fetal sheep, Pediatr. Res. 75 (2014) 689–696, https://doi.org/10.1038/pr.2014.37.
[21] S. Sriramula, M. Haque, D.S. Majid, J. Francis, Involvement of tumor necrosis factor-alpha in angiotensin II-mediated effects on salt appetite, hypertension, and cardiac hypertrophy, Hypertension 51 (2008) 1345–1351, https://doi.org/10.1161/HYPERTENSIONAHA.107.102152.
[22] A. Willeford, T. Suetomi, A. Nickle, H.M. Hoffman, S. Miyamoto, J. Heller Brown, CaMKIIdelta-mediated inflammatory gene expression and inflammasome activation in cardiomyocytes initiate inflammation and induce fibrosis, JCI Insight 3 (2018), https://doi.org/10.1172/jci.insight.97054.
[23] L. Yang, L. Ni, Q. Duan, X. Wang, C. Chen, S. Chen, S. Chaugai, D.C. Zeldin, J.R. Tang, D.W. Wang, CYP epoxygenase 2J2 prevents cardiac fibrosis by suppression of transmission of pro-inflammation from cardiomyocytes to macrophages, Prostaglandins Other Lipid Mediat. 116–117 (2015) 64–75, https://doi.org/10.1016/j.prostaglandins.2015.01.004.
[24] X.Y. Zhao, L.Y. Zhao, Q.S. Zheng, J.L. Su, H. Guan, F.J. Shang, X.L. Niu, Y.P. He, X.L. Lu, Chymase induces profibrotic response via transforming growth factor-beta 1/Smad activation in rat cardiac fibroblasts, Mol. Cell. Biochem. 310 (2008) 159–166, https://doi.org/10.1007/s11010-007-9676-2.
[25] L. Fu, C.C. Wei, P.C. Powell, W.E. Bradley, S. Ahmad, C.M. Ferrario, J.F. Collawn, L.J. Dell'Italia, Increased fibroblast chymase production mediates procollagen autophagic digestion in volume overload, J. Mol. Cell. Cardiol. 92 (2016) 1–9, https://doi.org/10.1016/j.yjmcc.2016.01.019.
[26] K. Ahn, S. Takai, R. Pawankar, A. Kuramasu, H. Ohtsu, D. Kempuraj, H. Tomita, M. Iida, K. Matsumoto, A. Akasawa, M. Miyazaki, H. Saito, Regulation of chymase production in human mast cell progenitors, J. Allergy Clin. Immunol. 106 (2000) 321–328, https://doi.org/10.1067/mai.2000.108107.
[27] M.F. Forman, G.L. Brower, J.S. Janicki, Rat cardiac mast cell maturation and differentiation following acute ventricular volume overload, Inflamm. Res. 55 (2006) 408–415, https://doi.org/10.1007/s00011-006-6016-z.
[28] M. Jurewicz, D.H. McDermott, J.M. Sechler, K. Tinckam, A. Takakura, C.B. Carpenter, E. Milford, R. Abdi, Human T and natural killer cells possess a functional renin-angiotensin system: further mechanisms of angiotensin II-induced inflammation, J. Am. Soc. Nephrol. 18 (2007) 1093–1102, https://doi.org/10.1681/ASN.2006070707.
[29] N. Hoa, L. Ge, K.S. Korach, E.R. Levin, Estrogen receptor beta maintains expression of KLF15 to prevent cardiac myocyte hypertrophy in female rodents, Mol. Cell. Endocrinol. (2017), https://doi.org/10.1016/j.mce.2017.11.004.
[30] A. Pedram, M. Razandi, F. O'Mahony, D. Lubahn, E.R. Levin, Estrogen receptor-beta prevents cardiac fibrosis, Mol. Endocrinol. 24 (2010) 2152–2165, https://doi.org/10.1210/me.2010-0154.
[31] A. Pedram, M. Razandi, R. Narayanan, J.T. Dalton, T.A. McKinsey, E.R. Levin, Estrogen regulates histone deacetylases to prevent cardiac hypertrophy, Mol. Biol. Cell 24 (2013) 3805–3818, https://doi.org/10.1091/mbc.E13-08-0444.
[32] P.E. Gallagher, P. Li, J.R. Lenhart, M.C. Chappell, K.B. Brosnihan, Estrogen regulation of angiotensin-converting enzyme mRNA, Hypertension 33 (1999) 323–328 (DOI).
[33] L.V. Rossoni, R.A. Oliveira, R.R. Caffaro, M. Miana, D. Sanz-Rosa, M.K. Koike, S.L. Do Amaral, L.C. Michelini, V. Lahera, V. Cachofeiro, Cardiac benefits of exercise training in aging spontaneously hypertensive rats, J. Hypertens. 29 (2011) 2349–2358, https://doi.org/10.1097/HJH.0b013e32834d2532.
[34] H. Zhang, J. Wu, H. Dong, S.A. Khan, M.L. Chu, T. Tsuda, Fibulin-2 deficiency attenuates angiotensin II-induced cardiac hypertrophy by reducing transforming growth factor-beta signalling, Clin. Sci. (Lond.) 126 (2014) 275–288, https://doi.org/10.1042/CS20120636.
[35] A. Calderone, R.J. Murphy, J. Lavoie, F. Colombo, L. Beliveau, TGF-beta(1) and prepro-ANP mRNAs are differentially regulated in exercise-induced cardiac hypertrophy, J. Appl. Physiol. (1985) 91 (2001) 771–776, https://doi.org/10.1152/jappl.2001.91.2.771.
[36] A.J. Serra, M.H. Santos, D.S. Bocalini, E.L. Antonio, R.F. Levy, A.A. Santos, M.L. Higuchi, J.A. Silva, F.C. Magalhaes, V.G. Barauna, J.E. Krieger, P.J. Tucci, Exercise training inhibits inflammatory cytokines and more than prevents myocardial dysfunction in rats with sustained beta-adrenergic hyperactivity, J. Physiol. 588 (2010) 2431–2442, https://doi.org/10.1113/jphysiol.2010.187310.
[37] L.J. Dell'Italia, J.F. Collawn, C.M. Ferrario, Multifunctional role of chymase in acute and chronic tissue injury and remodeling, Circ. Res. 122 (2018) 319–336, https://doi.org/10.1161/circresaha.117.310978.
[38] S. Wernersson, G. Pejler, Mast cell secretory granules: armed for battle, Nat. Rev. Immunol. 14 (2014) 478–494, https://doi.org/10.1038/nri3690.
[39] S.M. Duffy, G. Cruse, W.J. Lawley, P. Bradding, β2-Adrenoceptor regulation of the K + channel iKCa1 in human mast cells, FASEB J. 19 (2005) 1006–1008, https://doi.org/10.1096/fj.04-3439fje.
[40] S. Pandit, W. Woranush, J. Wattanapermpool, T. Bupha-Intr, Significant role of female sex hormones in cardiac myofilament activation in angiotensin II-mediated hypertensive rats, J. Physiol. Sci. 64 (2014) 269–277, https://doi.org/10.1007/s12576-014-0316-9.
[41] R.M. Touyz, J. Fareh, G. Thibault, E.L. Schiffrin, Intracellular Ca2+ modulation by angiotensin II and endothelin-1 in cardiomyocytes and fibroblasts from hypertrophied hearts of spontaneously hypertensive rats, Hypertension 28 (1996) 797–805 (DOI).