Chinese Journal of Physiology 51(3): 146-151, 2008

The Influence of Gender on Cardiac Fibrosis Induced by Sympathetic Stimulation

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Abstract

We examined the influence of sex steroids on cardiac effects of sympathetic agents in mice. The mice were divided into four groups: males, neutered males (N-males), females, and neutered females (N-females). Dobutamine (DOB; 2.5, 5.0, 10 μg/kg/min) or isoproterenol (ISO; 0.01, 0.02, 0.04 μg/kg/min) were given intravenously to compare the fractional shortening (FS). These mice received isoproterenol twice daily at a dose of 7.5 μg/g/day for 3 weeks. The rate of cardiac fibrosis was evaluated pathologically with Azan stain after 3 weeks of ISO. DOB and ISO significantly increased the FS in the male group, compared with other groups. There was no significant difference in FS between the female and N-female groups. Cardiac fibrosis significantly increased in the male group, compared with the N-male group. The female and N-female groups had increased cardiac fibrosis, compared with the male and N-male groups. These findings suggest that testosterone is one of the factors of modulation of the response to the sympathetic nervous system. Further study is needed to clarify the relationships between female sex steroids and cardiac fibrosis.

Key Words: cardiac contractility, fibrosis, gender difference, sympathetic agent

Introduction

Men are at a greater risk of cardiovascular disease than women at every age (4, 14). However, the incidence of cardiovascular disease in women increases in the postmenopausal period (9). Furthermore, the Beta-Blocker Evaluation of Survival Trial (BEAT) revealed that coronary artery disease confers an increased risk of mortality in women compared to men (6). In basic studies, an antihypertensive effect of estrogen mediated by the angiotensin type-1 (AT-1) receptor in vascular endothelial cells has been reported (19, 20). Estrogen promotes the production of nitric oxide and prostacyclin, and down-regulates the expression of the AT-1 receptor (2, 11). Furthermore, testosterone influences the cardiovascular system by activating the renin-angiotensin-aldosterone (RAA) system (3, 23, 27). Testosterone modulates the expression of L-type Ca^{2+} and Na^{+}-Ca^{2+} channels in myocytes (7, 8), myocyte hypertrophy, and the promotion of apoptosis (1, 12,

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Received: April 13, 2007; Revised: August 13, 2007; Accepted: August 23, 2007.
 ISSN : 0304-4920. http://www.cps.org.tw

17). These reports suggest that sex steroids participate in cardiovascular function and tissue remodeling, although the effects of sex steroids on the cardiovascular system remained controversial. This study evaluated the effect of gender on cardiac function in response to dobutamine (DOB) and isoproterenol (ISO), and investigated the cardiac remodeling induced by the chronic administration of isoproterenol in mice.

Materials and Methods

Animals

Age-matched 4-week-old CD-1 mice[a] (both sexes) were housed individually in an air-conditioned room on a 12-h dark-light cycle and were fed a standard diet with unrestricted access to tap water. The mice were divided into four groups: males, neutered males (N-males), females, and neutered females (N-females). The mice were randomly subjected to bilateral ovariectomy or castration at 6 weeks of age as N-males or N-females. The operations were performed using sterile techniques. For surgical procedures, the mice were anesthetized with a mixture of ketamine (0.065 mg/g, i.p.), xylazine (0.013 mg/g, i.p.), and acepromazine (0.002 mg/g, i.p.). After 2 weeks, the 8-week-old mice were subjected to this study. This study was approved by the Institutional Laboratory Animal Care and Use Committee of the School of Veterinary Medicine and Animal Science of Kitasato University, Japan.

Administration of Sympathetic Agents

The mice were assigned to four different subgroups: males (n = 7), N-males (n = 6), females (n = 9), and N-females (n = 8). A catheter (Natsume Production Place, SP10, Tokyo, Japan) made of polyethylene was implanted in the jugular vein of the mice and used to infuse the sympathetic agents. Under anesthesia, the right jugular vein was exposed and catheterized using sterile saline. The catheter was exteriorized between the scapulas. The mice were allowed to recover for 3 days. Following anesthesia, the mice were stabilized for at least 15 min and placed in the prone position above a SONA gel (Toshiba, Tokyo, Japan). DOB (Sigma, St. Louis, MO, USA) was administered at 2.5, 5, or 10 μg/kg/min for 5 min *via* the catheter. Similarly, ISO (Sigma, St. Louis, MO, USA) was infused at 0.01, 0.02, or 0.04 μg/kg/min for 5 min (13). Each examination was conducted at three-day intervals for recovery. The heart rate was monitored using electrocardiography. All measurements were performed before and after administering the sympathetic agents. The rate of change in fractional shortening (FS) was determined in comparison with baseline.

Echocardiography Examination

As an index of left ventricular contractility, FS was measured by echocardiography using a 12-MHz probe (SONOS 5500, S12, Hewlett Packard, Littleton, MA, USA). M-mode measurements of left ventricular internal diameter (LVID) were made from short axis view. Left ventricular diastolic internal dimensions ($LVID_d$) were measured at the onset of the QRS complex on an electrocardiography recording. Left ventricular systolic internal dimensions (LVIDs) were measured at the peak of the T-wave on an electrocardiography recording. Left ventricular FS was calculated as follows; $FS = (LVID_d - LVID_s) / LVID_d \times 100$. The average of three cardiac cycles was calculated. Data were stored digitally and analyzed off-line by a single observer.

Chronic Isoproterenol Administration

The ISO treatment mice were assigned to four different subgroups: males (n = 9), N-males (n = 13), females (n = 11), and N-females (n = 11). The control group was similarly divided into four subgroups (n = 5). The control subgroups were given sterile saline, and the ISO subgroups received isoproterenol twice daily at a dose of 7.5 μg/g/day. The treatment and control were given subcutaneously in a volume of 0.5 ml/mouse between the scapulas for 3 weeks.

After the 3-week treatment, the mice were anesthetized with diethylether, and their hearts were removed after euthanasia. The hearts were cut into coronal sections at the level of the papillary muscles, fixed with 10% formalin, and embedded in paraffin. Transverse sections (4 μm) were cut and stained using routine methods with Azan stain to detect collagen. The sections were deparaffinized in xylene, dehydrated, rehydrated in distilled water, immersed in 5% potassium dichromate solution for 30 min, and stained with Azocarmine G for 30 min. The sections were then immersed in a 3% tungsten (IV) phosphoric acid n-hydrate solution for 1 h and stained with aniline blue-orange G for 20 min. The images, the cross-sectional area of the LV sections, were imported into the NIH-Image program (National Institutes of Health), which was used for quantitative analysis of the fibrosis region that stained with the Azan stain, which modified the methods reported by Shimamura *et al*. (21). The percentage of cardiac fibrosis was calculated as follows; the area of fibrosis was divided by the cross-sectional area of the heart.

Statistical Analysis

The results were expressed as the mean ± SD.

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Table 1. The changes of the fractional shortening with dobutamine.

		Dobutamine		
	Baseline	2.5 μg/kg/min	5.0 μg/kg/min	10 μg/kg/min
Male	28.0 ± 5.2	31.1 ± 6.2	38.2 ± 4.0†	47.6 ± 9.2†
N-male	31.7 ± 6.0	35.4 ± 5.4	40.2 ± 5.9†	43.6 ± 7.6†
Female	29.8 ± 4.6	29.4 ± 10.7	32.5 ± 10.8†	39.4 ± 12.2†
N-female	29.1 ± 2.2	29.7 ± 10.5	31.8 ± 11.0*	39.5 ± 12.7†

The data are given as the mean ± SD. *$P < 0.05$ *vs*. baseline. †$P < 0.01$ *vs*. baseline.

Table 2. Changes in the heart rate with dobutamine.

		Dobutamine		
	Baseline	2.5 μg/kg/min	5.0 μg/kg/min	10 μg/kg/min
Male	183 ± 11	156 ± 10	157 ± 15	160 ± 10
N-male	183 ± 21	161 ± 10	154 ± 10	164 ± 12
Female	198 ± 7	158 ± 8	165 ± 9	166 ± 10
N-female	185 ± 8	158 ± 10	158 ± 8	170 ± 9

No significant differences were observed among the groups.

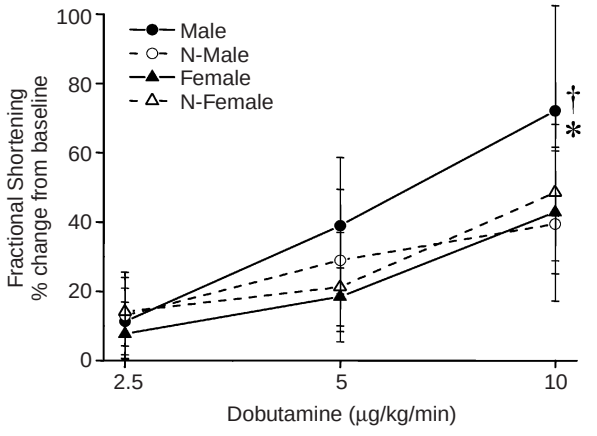

Fig. 1. The changes rate of the fractional shortening with dobutamine. *$P < 0.05$ *vs*. N-male and N-female, $P < 0.01$ *vs*. Female. Male group was significantly increased the FS%.

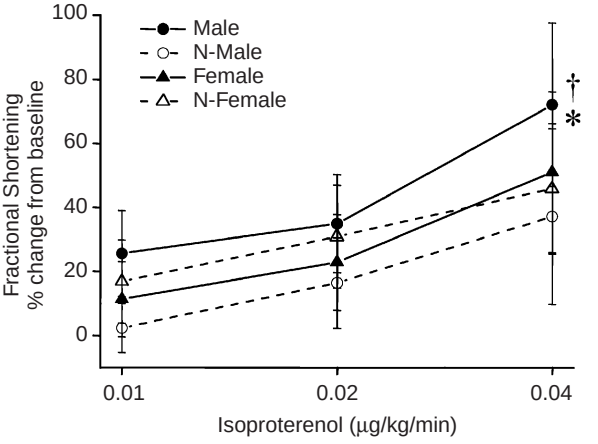

Fig. 2. The change rate of the fractional shortening with isoproterenol. The data are described as mean ± SD. *$P < 0.05$ *vs*. N-male and N-female, $P < 0.01$ *vs*. N-male and Female. The fractional shortening in the male group increased significantly.

The echocardiography data were analyzed using two-way analysis of variance (ANOVA). The histological data were analyzed using one-way ANOVA. Individual values were compared using the post hoc Tukey Studentized range method. A difference was considered statistically significant when $P < 0.05$.

Results

Acute Dobutamine Administration

Dobutamine administration significantly increased the FS from baseline in each group (Table 1). In the males, the rate of change in FS significantly increased with DOB in a dose-dependent manner from baseline (14 ± 4, 39 ± 8, and 72 ± 12% for 2.5, 5, or 10 μg/kg/min, respectively; all $P < 0.05$) (Fig. 1). These responses were significantly elevated compared to those in the other groups ($P < 0.05$). In the females and N-females, the FS values were elevated, but did not differ significantly between the two groups (Fig. 1). The heart rate did not differ significantly among groups (Table 2).

Table 3. The changes of the fractional shortening with isoproterenol.

		Isoproterenol		
	Baseline	2.5 μg/kg/min	5.0 μg/kg/min	10 μg/kg/min
Male	29.5 ± 6.0	35.6 ± 7.0†	37.5 ± 6.5†	48.9 ± 12.2†
N-male	30.0 ± 5.9	30.4 ± 6.1	33.8 ± 5.5*	38.7 ± 5.7†
Female	29.9 ± 6.8	32.8 ± 5.1	36.5 ± 7.4†	44.0 ± 5.3†
N-female	30.5 ± 4.7	35.4 ± 4.8†	39.4 ± 4.1†	43.8 ± 4.9†

The data are given as the mean ± SD. $^{*}P < 0.05$ *vs*. baseline. $^{\dagger}P < 0.01$ *vs*. baseline.

Table 4. Changes in the heart rate with isoproterenol.

		Isoproterenol		
	Baseline	2.5 μg/kg/min	5.0 μg/kg/min	10 μg/kg/min
Male	180 ± 9	166 ± 8	163 ± 9	171 ± 11
N-male	185 ± 10	160 ± 10	160 ± 11	167 ± 12
Female	220 ± 7	177 ± 9	167 ± 9	172 ± 13
N-female	187 ± 10	163 ± 12	169 ± 15	175 ± 16

No significant differences were observed among the groups.

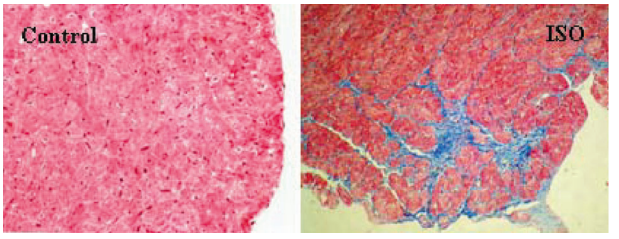

Fig. 3. The pathophysiological findings with chronic isoproterenol administration.

Acute Isoproterenol Administration

Isoproterenol administration significantly increased the FS from baseline in each group (Table 3). In the males, the rate of change in FS was markedly increased with ISO in a dose-dependent manner from baseline (26 ± 5, 38 ± 4, and 72 ± 9% for 0.01, 0.02, or 0.04 μg/kg/min, respectively; $P < 0.05$) (Fig. 2). These responses were significantly elevated, compared to those in the other groups ($P < 0.05$). In the females and N-female groups, the FS values were elevated but did not differ significantly between the two groups (Fig. 2). The heart rate did not change significantly (Table 4).

Chronic Isoproterenol Administration

The area of fibrosis significantly increased in the ISO-treated subjects, compared to the controls

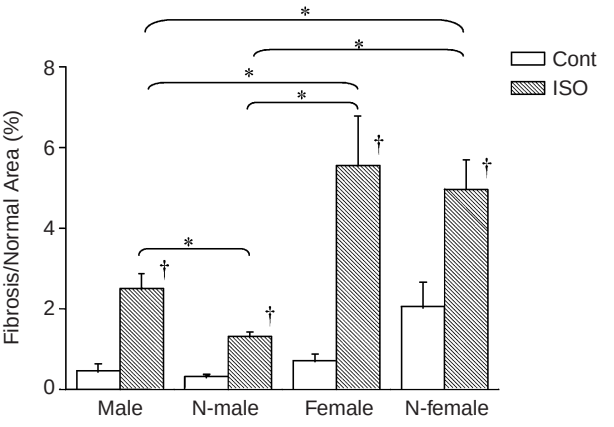

Fig. 4. Comparison of cardiac fibrosis (fibrosis/normal area). Open bar; control mice (Cont). Closed bar; isoproterenol administered mice (ISO). $^{\dagger}P < 0.05$: control mice *vs*. isoproterenol administered; $^{*}P < 0.05$: Male *vs*. N-male, Female *vs*. Male or N-male, N-female *vs*. Male or N-male.

(Fig. 3). Cardiac fibrosis was located mainly in the left ventricular subendocardial area. The ISO-induced fibrous region significantly increased in the males compared to the N-males (2.47 ± 0.41 *vs*. 1.28 ± 0.15%; $P < 0.05$). Whereas no significant difference was observed between the female and N-female groups (5.52 ± 1.26 *vs*. 4.96 ± 0.75%), the fibrous regions in the females and N-females were significantly greater than those in the males and N-males ($P < 0.05$; Fig. 4).

Discussion

Isoproterenol and dobutamine markedly increased the FS in all groups. In particular, the rate of change for FS was significantly higher in the males than in other groups. A previous report showed that castration decreases serum testosterone levels (5, 8). Functional androgen receptors have recently been found on cardiac myocytes in humans, dogs, and rats (18), and testosterone is known to modulate the gene expression of Na^+-Ca^{2+} channels in myocytes (7, 8). Moreover, testosterone treatment increased β-adrenoceptor and L-type Ca^{2+} channel mRNA levels in cultured cardiac myocytes (7). Vicencio *et al*. reported that testosterone induced increase in intracellular Ca^{2+} in cultured myocyte, which is through activation of a plasma membrane receptor associated with G protein-phospholipase C/inositol 1,4,5-trisphosphate signaling pathway (26). Therefore, our results indicate that testosterone modulates the expression of Na^+-Ca^{2+} channels, β-adrenoceptor, and L-type Ca^{2+} channel, and the cardiac contractility *via* the adrenergic receptor.

Isoproterenol increased the FS in females and N-females, although the FS did not differ between the females and N-females in this study. 17β-estradiol significantly decreased the stimulatory actions of isoproterenol on the heart rate and systolic and diastolic pressure in isolated perfused rat heart (16). In addition, β_1-adrenoceptors were up-regulated in the ovariectomized rat heart, and ovarian sex hormones, such as estrogen and progesterone, prevented the up-regulation of β_1-adrenoceptors (22). However, the echocardiographic findings, FS and $LVID_d$, did not differ in a transgenic strain of female mice that overexpressed the β_2-adrenergic receptor with or without gonadectomy (5). This discrepancy may be explained by the methodological differences between *in vivo* and *in vitro*. In the present study, FS did not differ in females with or without gonadectomy, although previous reports indicated a relationship between female sex steroids and the cardiac function. Therefore, further studies are needed to elucidate the net effects of female sex hormones on the cardiac function.

The cardiac fibrosis was more extensive in the males than in the N-males. Recent studies have shown that testosterone can induce the apoptosis of vascular endothelial cells, and an androgen receptor antagonist abolished this effect (17). Chen *et al*. (3) showed that renal and hepatic renin and angiotensinogen gene expression are also testosterone-dependent in spontaneously hypertensive rats. They demonstrated that the blood pressure and plasma renin activity were higher in intact males than in females. Furthermore, castration retarded the development of hypertension and lowered plasma renin levels (3). In addition, anti-androgen treatment decreased the activity of the RAA system in kidney and attenuated systolic blood pressure, fibrosis, and accumulation of collagen in hypertensive transgenic rats (1). Thus, these reports suggested that testosterone does have an effect on the control of systemic blood pressure and fibrosis *via* the RAA system in kidney and liver. Because castration attenuated the cardiac fibrosis, compared with intact males in present study, it indicated the possibility of testosterone is modulating the fibrosis through the sympathetic system.

Fibrosis was not significantly different between the females and N-females, although it significantly increased in the females and N-females, compared to the males and N-males. Recent studies have reported that estrogen abolished increasing cardiac weights, rates of collagen I/III, and AT-1 receptor expression in neutered female rats (28). A selective estrogen receptor modulator, raloxifene, prevents the decrease in FS and the increase in the left ventricular mass in pressure-overloaded mice (20). van Eickels *et al*. (24) reported that 17β-estradiol reduced infarct size and apoptosis in myocytes with myocardial infarcted mice, although 17β-estradiol increased ventricular hypertrophy. This discrepancy may be caused by the net effects of female sex steroids. The estrogen metabolites estrone and 2-hydroxyestrone stimulated fibroblast proliferation, and an ACE inhibitor inhibited this effect of estrone (10, 11). Also progesterone stimulates the proliferation of vascular endothelial cells (25). These reports indicate that estrous cycles influence the prognosis in female with cardiovascular disease. In present study, the relationship between female sex steroids and cardiac fibrosis are still unclear.

In this study, we focused on the gender-related differences in response to catecholamines in rats. Therefore, it is necessary to note that the cardiovascular and fibrotic response in our model may differ from other heart disease models. In addition, we evaluated the cardiac fibrosis by histologically. Recent reports have demonstrated that the RAA system in the heart or matrix metalloprotease activity participate in cardiac fibrosis, *i.e.*, collagen accumulation (15, 27). Further study will be required to clarify the relationship between sex steroids and collagen accumulation.

In conclusion, male mice significantly increased the FS and cardiac fibrosis compared with castrated males in response to adrenergic agents. Although the FS and cardiac fibrosis were not different in the females with or without gonadectomy, cardiac fibrosis was increased in the females and N-females compared with the males and N-males. These findings suggest that testosterone may influence to the cardiovascular system and fibrosis in male mice. However, further study is needed to elucidate the effects of female sex steroids on cardiac remodeling.

Acknowledgments

This study was supported in part by a Grant-in-Aid for General Scientific Research (C-16580268) from the Japanese Ministry of Education, and a Grant for Encouraging Young Scientists (no. 2939) in Priority Areas from Kitasato University School of Veterinary Medicine and Animal Sciences, Japan.

References

1. Baltatu, O., Cayla, C., Iliescu, R., Andreev, D. and Bader, M. Abolition of end-organ damage by anti-androgen treatment in female hypertensive transgenic rats. *Hypertension* 41: 830-833, 2003.
2. Caulin, G.T., Garcia, C.G., Sarrel, P., Sessa, W.C. and Bender, J.R. 17β-Estradiol regulation of human endothelial cell basal nitric oxide release. *Circ. Res.* 81: 885-892, 1997.
3. Chen, Y.F., Naftilan, A.J. and Oparil, S. Androgen-dependent angiotensinogen and renin messenger RNA expression in hypertensive rats. *Hypertension* 19: 456-463, 1992.
4. Fodor, J.G., Tzerovska, R., Dorner, T. and Rieder, A. Do we diagnose and treat coronary heart disease differently in men and women? *Wien. Med. Wochenschr*. 154: 423-425, 2004.
5. Gao, X.M., Agrotis, A., Autelitano, D.J., Percy, E., Woodcock, E.A., Jennings, G.L., Dart, A.M. and Du, X.J. Sex hormones and cardiomyopathic phenotype induced by cardiac beta 2-adrenergic receptor overexpression. *Endocrinology* 144: 4097-4105, 2003.
6. Ghali, J.K., Krause-Steinrauf, H.J., Adams, K.F., Khan, S.S., Rosenberg, Y.D., Yancy, C.W., Young, J.B., Goldman, S., Peberdy, M.A. and Lindenfeld, J. Gender differences in advanced heart failure: insights from the BEST study. *J. Am. Coll. Cardiol*. 42: 2128-2134, 2003.
7. Golden, K.L., Marsh, J.D. and Jiang, Y. Testosterone regulates mRNA levels of calcium regulatory proteins in cardiac myocytes. *Horm. Metab. Res*. 36: 197-202, 2004.
8. Golden, K.L., Marsh, J.D., Jiang, Y., Brown, T. and Moulden, J. Gonadectomy of adult male rats reduces contractility of isolated cardiac myocytes. *Am. J. Physiol. Endocrinol. Metab*. 285: 449-453, 2003.
9. Grodstein, F., Manson, J.E., Colditz, G.A., Willett, W.C., Speizer, F.E. and Stampfer, M.J. A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease. *Ann. Intern. Med*. 133: 933-941, 2000.
10. Grohe, C., Kahlert, S., Karas, R.H., Linz, W.K., Lobbert, K., Meyer, R. and Vetter, H. Modulation of hypertensive heart disease by estrogen. *Steroids* 61: 201-204, 1996.
11. Grohe, C., Kahlert, S., Lobbert, K., van Eickels, M., Neyses, L., Stimpel, M. and Vetter, H. Angiotensin converting enzyme inhibition modulates cardiac fibroblast growth. *J. Hyperten*. 16: 377-384, 1998.
12. Hirai, M., Komoriyama, K., Kumai, T., Masubuchi, Y. and Uematsu, A. Gonadectomy-induced reduction of blood pressure in adult spontaneously hypertensive rats. *Acta. Endocrinol. (Copenh)* 101: 154-160, 1982.
13. Iwase, M., Bishop, S.P., Uechi, M., Vatner, D.E., Shannon, R.P., Kudej, R.K., Wight, D.C., Wagner, T.E., Ishikawa, Y., Homcy, C.J. and Vatner, S.F. Adverse effects of chronic endogenous sympathetic drive induced by cardiac GS alpha overexpression. *Circ. Res*. 78: 517-524, 1996.
14. Kimmelstiel, C.D. and Konstam, M.A. Heart failure in women. *Cardiology* 86: 304-309,1995.
15. Li, H., Simon, H., Bocan, T.M. and Peterson, J.T. MMP/TIMP expression in spontaneously hypertensive heart failure rats: the effect of ACE- and MMP-inhibition. *Cardiovasc. Res.* 46: 298-306, 2000.
16. Li, H.Y., Bian, J.S., Kwan, Y.W. and Wong, T.M. Enhanced responses to 17β-estradiol in rat hearts treated with isoproterenol: Involvement of a cyclic AMP-dependent pathway. *J. Pharmacol. Exp. Ther*. 293: 592-598, 2000.
17. Ling, S., Dai, A., Williams, M.R., Myles, K., Dilley, R.J., Komesaroff, P.A. and Sudhir, K. Testosterone (T) enhances apoptosis-related damage in human vascular endothelial cells. *Endocrinology* 143: 1119-1125, 2002.
18. Marsh, J,D., Lehmann, M.H., Ritchie, R.H., Gwathmey, J.K., Green, G.E. and Schiebinger, R.J. Androgen receptors mediate hypertrophy in cardiac myocytes. *Circulation* 98: 256-261, 1998.
19. Nickenig, G., Baumer, A.T., Grohe, C., Kahlert, S., Strehlow, K., Rosenkranz, S., Stablein, A., Beckers, F., Smits, J.F., Daemen, M.J., Vetter, H. and Bohm, M. Estrogen modulates AT1 receptor gene expression *in vitro* and *in vivo*. *Circulation* 97: 2197-2201, 1998.
20. Ogita, H., Node, K., Liao, Y., Ishikura, F., Beppu, S., Asanuma, H., Sanada, S., Takashima, S., Minamino, T., Hori, M. and Kitakaze, M. Raloxifene prevents cardiac hypertrophy and dysfunction in pressure-overloaded mice. *Hypertension* 43: 237-242, 2004.
21. Shimamura, S., Endo, H., Kutsuna, H., Kobayashi, M., Hirao, H., Shimizu, M., Tanaka, R. and Yamane, Y. Effect of intermittent administration of sustained release isosorbide dinitrate (sr-ISDN) in rats with pressure-overload heart. *J. Vet. Med. Sci*. 68: 213-217, 2006.
22. Thawornkaiwong, A., Preawnim, S. and Wattanapermpool, J. Upregulation of $beta_1$-adrenergic receptors in ovariectomized rat hearts. *Life Sci*. 72: 1813-1824, 2003.
23. Thum, T. and Borlak, J. Testosterone, cytochrome P450, and cardiac hypertrophy. *FASEB J*. 16: 1537-1549, 2002.
24. van Eickels, M., Patten, R.D., Aronovitz, M.J., Alsheikh-Ali, A., Gostyla, K., Celestin, F., Grohe, C., Mendelsohn, M.E. and Karas, R.H. 17-beta-estradiol increases cardiac remodeling and mortality in mice with myocardial infarction. *J. Am. Coll. Cardiol.* 41: 2084-2092, 2003.
25. Vazquez, F., Rodriguez-Manzaneque, J.C., Lydon, J.P., Edwards, D.P., O'Malley, B.W. and Iruela-Arispe, M.L. Progesterone regulates proliferation of endothelial cells. *J. Biol. Chem*. 274: 2185-2192, 1999.
26. Vicencio, J.M., Ibarra, C., Estrada, M., Chiong, M., Soto, D., Parra, V., Diaz-Araya, G., Jaimovich, E., and Lavandero, S. Testosterone induces an intracellular calcium increase by a nongenomic mechanism in cultured rat cardiac myocytes. *Endocrinology* 147: 1386-1395, 2006.
27. Wagner, D., Metzger, R., Paul, M., Ludwig, G., Suzuki, F., Takahashi, S., Murakami, K. and Ganten, D. Androgen dependence and tissue specificity of renin messenger RNA expression in mice. *J. Hypertens.* 8: 45-52, 1990.
28. Xu, Y., Arenas, I.A., Armstrong, S.J. and Davidge, S.T. Estrogen modulation of left ventricular remodeling in the aged heart. *Cardiovasc. Res*. 57: 388-394, 2003.