Hormonal regulation of β -myosin heavy chain expression in the mouse left ventricle

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Abstract

We investigated the influence of sex hormones on the expression of α - and β -cardiac myosin heavy chain isoforms (α -MHC and β -MHC) in C57bl/6 mice of both sexes under physiological and pathological conditions. In the left ventricles (LVs) of fertile female mice, β -MHC expression was tenfold higher compared with the age-matched males, whereas no difference was found in α -MHC expression. These differences disappeared after ovariectomy or in immature mice. We also found a sex-related difference in expression of β -adrenoceptors (β 1-AR), as mRNA levels of this gene were 40% lower in fertile females compared with males of the same age but did not differ in prepubertal or ovariectomized animals. Interestingly, the deletion of both β 1- and β 2-ARs abolished sex difference of β -MHC expression, as mRNA levels in the LVs of knockout males were increased and reached values comparable to those of knockout females. Moreover, the β 1-AR antagonist metoprolol induced about a threefold increase in β -MHC expression in adult male mice. The capability of gender to regulate β -MHC expression was also evaluated in the presence of hemodynamic overload. Thoracic aortic coarctation (TAC) produced cardiac hypertrophy along with a 12-fold increase in β -MHC and a 50% decrease in β 1-AR expression in males but not in females, thus abolishing the gender difference observed in sham animals for such genes. By contrast, TAC did not change β 2-AR expression. In conclusion, our results show that the expression of β -MHC and β 1-AR in the LVs undergo gender-related and correlated changes under both physiological and pathological conditions and suggest a role of β 1-AR-mediated signaling.

Key Words

- ▶ gender
- ▶ gene expression
- ▶ β -myosin
- ▶ β adrenergic receptors
- ▶ cardiac hypertrophy

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Introduction

Pathological cardiac hypertrophy, which occurs in response to hemodynamic overload, myocardial damage, or defects in sarcomeric proteins, is associated with an enhanced risk of ventricular dysfunction and heart failure. Increased cardiomyocyte size accompanied with the reactivation of so-called fetal genes, such as atrial natriuretic peptide (ANP), skeletal α -actin, and β -myosin heavy chain (β -MHC), normally extinguished in adult

ventricular myocardium (Izumo *et al.* 1987, 1988), represents the major hallmarks of pathological cardiac hypertrophy. Gender has been shown to influence the quantitative and qualitative characteristics of pressure overload myocardial remodeling. Premenopausal hypertensive women have a lower prevalence of left ventricular hypertrophy (LVH) than their male counterparts (de Simone *et al.* 1995, Agabiti-Rosei & Muiesan 2002), with

left ventricular ejection fraction and wall thickness preserved (Cleland *et al.* 2003, Hogg *et al.* 2004, Regitz-Zagrosek *et al.* 2007). This improved ability of females to adapt to hemodynamic overload is also supported and confirmed by several animal studies (Pfeffer *et al.* 1982, Douglas *et al.* 1998, Weinberg *et al.* 1999, Skavdahl *et al.* 2005). Although gender influence on left ventricular remodeling has been studied extensively, very little is known about the role played by sex hormones on fetal gene expression. Furthermore, the few studies performed in human samples relate to elderly patients (Reiser *et al.* 2001, Villar *et al.* 2009). Currently, the reexpression of the fetal gene program is believed to be part of a complex process of adaptation and compensation, aimed to limit cardiac energy consumption and to support cardiac output under an increased hemodynamic load. Thus, a better understanding of molecular mechanisms regulating fetal gene expression could provide important clues for the development of new therapeutic strategies to prevent the transition from hypertrophy to heart failure. Recently, we observed that mRNA levels of β -MHC were significantly higher (≈ 10 -fold) in the hearts of female C57bl/6 mice than in those of age-matched male mice of the same strain (Patrizio *et al.* 2008). To date, however, the mechanism responsible for these differences remains to be determined. To this purpose, we evaluated the influence of ovarian hormones on α -MHC and β -MHC expression in the left ventricle (LV) from C57bl/6 mice of both sexes and different ages, under both physiological and pathological conditions, and from gonadectomized mice. Moreover, we investigated the role of β -adrenergic receptors (β -ARs), the activation of which negatively regulates fetal gene expression (Patrizio *et al.* 2007, 2008). We found that the LVs from fertile female mice show a greater expression of β -MHC when compared with age-matched male mice under physiological conditions, and that this difference depends on the presence of ovarian hormones and is related to a lower expression of the $\beta 1$ -AR. For the first time, we also show that gender affects cardiac β -MHC expression under an increased pressure load.

Materials and methods

Materials

Metoprolol tartrate salt was purchased from Sigma Chemical Co. Mouse monoclonal anti-MHC slow isoform (anti- β -MHC), mouse monoclonal anti-MHC fast isoform (anti- α -MHC), and mouse monoclonal anti-actin were obtained from Santa Cruz Biotechnology, Inc. Anti-mouse

IgG conjugated to HRP secondary antibodies and western blot-ECL detection system were purchased from Amersham International.

Animals

All animal care and experimental procedures were in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the local ethics committee of the Italian National Institute of Health. C57BL/6 mice of both the sexes, ovariectomized C57BL/6 females, and orchidectomized C57BL/6 males were obtained from Harlan (S. Pietro al Natisone, Italy). β -AR KO mice (Adrb1/Adrb2 null mice, D β KO) were from a mixed FVB/C57/129/DBA genetic background, which prevents, as described previously, the high prenatal mortality induced by the $\beta 1$ KO mutation on inbred strains (Rohrer *et al.* 1996). As the attempts to breed these mice on a congenic background have not been successful (Bernstein *et al.* 2005), according to previous studies in which D β KO mice were used (Bernstein *et al.* 2005, Ecker *et al.* 2006), we mated D β KO mice with C57BL/6 mice to produce F1 mice heterozygous for both knockout genes. Thereafter, F1 heterozygous mice were mated to obtain F2 WT and D β KO mice derived from the same mixed genetic background. Genotyping was performed on tail DNA using standard protocols.

Chronic administration of metoprolol

Metoprolol was administered in drinking water at the dose of about 100 mg/kg per day for 14 consecutive days. The dosage was chosen on the basis of literature data (Asai *et al.* 1999, Baumhäkel *et al.* 2008). In previous experiments, we found that cardiac functional changes in response to the infusion of isoprenaline, a β -AR agonist, at the dose of 30 ng/kg per min did not occur in metoprolol-treated mice (Musumeci *et al.* 2011). At the end of the protocol, the animals were killed, their hearts were quickly dissected, and LVs were weighed and were then frozen in liquid nitrogen and kept at -80°C .

Mouse model of LV pressure overload

Pressure overload on the LVs was induced by thoracic aortic coarctation (TAC) for 14 days, as reported previously (Rockman *et al.* 1991), with some modifications. Animals were anesthetized with isoflurane (1.5–2.0% in 100% of oxygen) and the degree of aortic stenosis was about 60%.

A control group of mice was subjected to a sham operation with an identical surgical procedure but the ligature was not tightened. To quantify the hemodynamic load imposed on the mouse LVs after aortic banding, LV systolic pressure was measured with a 1.4-Fr micromanometer-tipped catheter (Millar Instruments, mod. SPR 839, Houston, TX, USA) by direct catheterization of the LVs at the end of experiments. Data were analyzed with a software package for cardiovascular analysis (IOX 1.7; EMKA Technologies, Paris, France). In our experimental conditions, the survival rate was 100%, without operation-related complications.

Echocardiography

Two weeks after banding, echocardiographic examination was performed in mice intubated and anesthetized with isoflurane (1% in 100% of oxygen) and set in a supine position as described previously (Patrizio *et al.* 2007). Left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic diameter (LVEDD), and posterior wall end-diastolic thickness (PWT) were measured by an image analysis system (Metamorph; Universal Image Corporation, Downingtown, PA, USA). Percent endocardial fractional shortening (FS) was calculated as $(LVEDD - LVESD)/LVEDD \times 100$.

RNA isolation and quantification

Total RNA was extracted from both 12-week mouse LVs and neonatal mouse hearts using SV Total RNA Isolation System (Promega). cDNA out of total RNA was synthesized using the High-Capacity cDNA Archive kit (Applied Biosystems). RNA expression levels for α -MHC (*Myh6*), β -MHC (*Myh7*), β 1-AR (*Adrb1*), and β 2-AR (*Adrb2*) were quantified with Real-Time TaqMan RT-PCR using 7500 Real-Time PCR system (Applied Biosystems). TaqMan reactions were carried out in 96-well plates using cDNA, TaqMan universal PCR mastermix, preoptimized, and preformulated TaqMan gene expressions assays including specific primers and fluorescent probes for mouse, and water to a final volume of 50 μ l according to the manufacturer's instructions. The codes for each gene expressions assay were derived from the online Applied Biosystems catalogue for quantitative gene expression analysis. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. No reverse transcriptase and no template controls were used to monitor for any contaminating amplification. The Δ Ct was used for statistical analysis and $2^{-\Delta\Delta C_t}$ for data presentation (Livak & Schmittgen 2001).

Western blot analysis

Mouse LVs were lysed in Tris/HCl 50 mM supplemented with 1 mM EDTA, 1 mM EGTA, 0.5% Triton X-100, 0.1% SDS, 1 mM dithiothreitol, 0.2 mM phenylmethylsulphonyl fluoride, and the protease inhibitors leupeptin (10 μ g/ml) and aprotinin (30 μ g/ml). Insoluble material was removed by centrifugation at 1000 g at 4 °C for 10 min, and protein concentration was measured. For each condition, 25 μ g protein were separated by 7.5% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were then blocked with 5% nonfat milk and incubated with anti-MHC- α , anti-MHC- β (1:400 dilution), or with anti-actin (1:6000 dilution) for 1 h at 25 °C. After several washes, the membranes were incubated with secondary antibodies (anti-mouse IgG conjugated with HRP, 1:10 000) for 1 h at 25 °C and visualized by the Amersham ECL system. The optical density of the bands (arbitrary units, AU) was measured with a GS-700 Imaging Densitometer (Bio-Rad), was normalized with respect to actin, and referred to the corresponding control samples (taken as 1) run in the same gel.

Statistical analysis

Group means ($\pm$ S.E.M.) were calculated for all relevant variables. Statistical significance between the different experimental groups was performed by ANOVA followed by Bonferroni's *post hoc* test in all the experiments except the western blot analysis, in which the statistical significance was evaluated by a paired Student's *t*-test. Graphpad Prism software was used. A value of $P < 0.05$ was considered statistically significant.

Results

Fertile female mice express greater β -MHC levels compared with age-matched males

In the LVs of hearts from 12-week-old female mice, the levels of β -Mhc mRNA were significantly higher (about tenfold), compared with the age-matched male group, whereas no differences between the sexes were found in the expression of α -Mhc mRNA (Fig. 1A). By contrast, no significant changes in the expression of both α -Mhc and β -Mhc (Fig. 1A) have been detected in sexually immature animals (7 days old). The gender-based difference of β -MHC expression was confirmed by western blot analysis. Protein expression was found to be four times higher in fertile females compared with corresponding males,

Figure 1

(A) α -Mhc and β -Mhc mRNA expression in the (LVs total hearts) obtained from male (M) and female (F) mice 12-weeks-old 7 days old. Mean $\pm$ S.E.M. of eight and six animal samples are presented respectively. $*P < 0.05$ = significantly different from corresponding M at 12 weeks using ANOVA followed by Bonferroni's *post hoc* test. (B) Western blot analysis of β -MHC protein levels in the LVs obtained from male (M) and female (F) mice of 12 weeks old and 7 days old. Optical density of the bands was measured with a densitometer and referred to the corresponding males (taken as 1) run in the same gel. Upper: one representative out of three performed is shown. Lower: mean $\pm$ S.E.M. values of densitometric analysis of three distinct immunoblots. $*P < 0.05$ = significantly different from corresponding M at 12 weeks using a paired Student's *t*-test. NS, not significantly different using a paired Student's *t*-test.

whereas it did not differ in prepubertal mice (Fig. 1B), suggesting a possible involvement of sex hormones in the greater expression of β -MHC observed for adult females.

The gender-based difference of β -Mhc expression disappeared after ovariectomy

In order to test this hypothesis, we studied the expression of the two myosin isoforms in the LVs of hearts obtained from 12-week-old gonadectomized mice. As shown in

Fig. 2A, the mRNA expression of β -Mhc in orchidectomized males was the same as that of the control males. On the contrary, the expression of β -Mhc in ovariectomized females was greatly reduced compared with the control females and was even lower than that in the age-matched male group, suggesting a possible estrogen-mediated mechanism. Also in this case, the difference was specific for the β -isoform, as we did not observe significant changes in the expression of α -Mhc (Fig. 2A).

β 1-AR expression is lower in fertile female mice compared with age-matched males

We have previously shown both *in vivo* and *in vitro* that β -AR-mediated signaling can negatively regulate fetal gene expression induced by Gq activation (Patrizio *et al.* 2007, 2008). Therefore, to investigate the possible involvement of β -AR in the β -MHC sex-related differences, we measured β 1- and β 2-*Ar* expression levels under the different experimental conditions described earlier. In adult female mice, the expression of β -*Ar* mRNA was 40% lower compared with age-matched males, while in 7-day-old animals there was no significant difference of β -*Ar* expression (Fig. 2B) between the genders. Moreover, ovariectomized females expressed β 1-*Ar* levels similar to those of corresponding adult males (Fig. 2B). By contrast, the expression of β 2-*Ar* did not differ under any of the studied conditions (Fig. 2B), suggesting a specific involvement of the β 1-*Ar* subtype.

Genetic deletion of β -ARs abolished the sex difference in β -Mhc expression

To further investigate the role of β 1-*Ar* on β -Mhc expression, we used a C57BL/6-derived KO line of mice that carry the genetic deletion of both β 1- and β 2-*Ar* (D β KO). Interestingly, in D β KO mice, the RNA expression of β -Mhc in the female group differed only slightly (about 45% greater) from that measured in the WT group, but it was 16-fold greater in the male group (Fig. 3A), reaching values comparable to the levels of female expression. Western blot analysis confirmed that the differences between males and females in β -Mhc expression were abolished in the absence of β 1-*Ar*, as we did not observe significant changes in the α -MHC and β -MHC protein levels between D β KO mice of either gender (Fig. 3B).

The gender-based difference of both β -Mhc and β 1-*Ar* expression in TAC mice

In another set of experiments, we investigated the effect of hemodynamic overload on β -Mhc expression in the two

Figure 2

(A) *Mhc* isoforms mRNA expression in the left ventricles obtained both from ovariectomized females (Fovx, α -*Mhc* and β -*Mhc*) and orchidectomized males (Morx, β -*Mhc*) at 12 weeks of age. Mean $\pm$ S.E.M. of seven and five animal samples are presented respectively. * $P < 0.05$ = significantly different using ANOVA followed by Bonferroni's *post hoc* test. $^{\S}P < 0.05$ = significantly different from control male (M). (B) β 1- and β 2-*Ar* expression levels in males and females at 12 weeks and 7 days of age and in ovariectomized females. Mean $\pm$ S.E.M. of (n) animal samples are presented. * $P < 0.05$ = significantly different using ANOVA followed by Bonferroni's *post hoc* test. NS, not significantly different among the groups.

sexes. For this purpose, 12-week-old male and female mice were subjected to aortic banding (TAC) for 2 weeks. As shown in Fig. 4A, β -*Mhc* expression in the LVs of TAC females was similar to that expressed in sham females, whereas in males β -*Mhc* expression increased about 12-fold compared with their controls, reaching levels comparable to those of the female group. Moreover, TAC-treated males exhibited a 50% reduction of β 1-*Ar* expression levels, while in the female group the expression of this receptor was identical to that measured in the corresponding sham controls (Fig. 4B). On the contrary, the expression of β 2-*Ar* mRNA did not significantly differ between the male and female groups subjected to TAC (Fig. 4B).

TAC-induced LVH does not differ between female and male mice

It is also interesting to note that, despite the sharp gender-related difference of β -*Mhc* expression caused by aortic banding, the increase in the left ventricular mass assessed 14 days following TAC treatment did not differ between males and females (67 and 65% respectively), nor did we find any significant divergence in ventricular performance (assessed as endocardial FS) between the male and female groups (Table 1).

Metoprolol increases β -MHC expression in male mice

Finally, to verify whether there is a direct link between β 1-*Ar* signaling and the expression of β -*Mhc*, a selective β 1-*Ar* antagonist metoprolol was administered to adult mice, at a dose of 100 mg/kg per day for 14 consecutive days (Asai *et al.* 1999, Baumhäkel *et al.* 2008). As shown in Fig. 5, metoprolol increased β -*Mhc* expression by threefold in males, whereas it failed to affect β -*Mhc* mRNA levels in females.

Discussion

The main finding of this study is that the greater expression of β -*Mhc* in the LVs of fertile female mice, compared with age-matched males, must be attributed to the presence of ovarian hormones. Two independent lines of evidence support this conclusion. First, different from what is observed between female and male adult mice, in sexually immature mice (7 days old), β -*Mhc* levels are similar in both sexes; secondly, in young adult ovariectomized females, the β -*Mhc* expression decreases, reaching the levels of corresponding adult males. On the contrary, orchidectomy does not change the β -*Mhc* expression, excluding an involvement of male hormones in this gender difference.

The role played by sex hormones on fetal gene expression has been poorly investigated and the few studies performed in human samples relate to elderly patients (Reiser *et al.* 2001, Villar *et al.* 2009). In animals, it has been reported that estrogen administration in ovariectomized mice subjected to TAC limited the increase in the LV mass, ANP, and β -*Mhc* expression (Patten *et al.* 2008). We believe that this discrepancy with our results, as regards the β -*Mhc* expression, may be the result of experimental variations, such as differences in the experimental animal model, species, and gene analysis quantification. The functional significance of different

Figure 3

(A) α -Mhc and β -Mhc mRNA expression in the LVs obtained from C57BL/6 transgenic male and female mice deprived of both β 1- and β 2-Ars (DKO). Mean $\pm$ S.E.M. of six and eight animal samples are presented respectively. * P < 0.05 = significantly different using ANOVA followed by Bonferroni's *post hoc* test. (B) Western blot analysis of α -MHC and β -MHC in the LVs obtained from male and female DKO mice. Optical density of the bands was measured with a densitometer and referred to the corresponding males (taken as 1) run in the same gel. Upper: one representative out of three performed is shown. Lower: mean $\pm$ S.E.M. values of densitometric analysis of three distinct immunoblots. NS, not significantly different using a paired Student's *t*-test.

β -Mhc levels between fertile female and male mice remains to be elucidated, as well as the β -Mhc reexpression in pathological cardiac hypertrophy and other cardiac diseases. An advantageous adaptive response to pressure overload played by β -MHC reexpression was suggested some time ago (Izumo *et al.* 1988). β -Mhc is characterized by lower ATP activity and lower filament sliding velocity but can generate a cross-bridge force with a higher economy of energy consumption than α -Mhc (Krenz *et al.* 2003), thus preserving energy under hemodynamic stress conditions, such as hypertension or aortic stenosis (Lowes *et al.* 1997).

In agreement with an adaptive function of β -Mhc, it has recently been reported that, during pressure overload-induced cardiac hypertrophy, cardiomyocytes with only α -MHC develop hypertrophy after TAC, whereas the cells in which there is a new synthesis of β -MHC do not enlarge (López *et al.* 2011), suggesting that β -MHC expression may play an anti-hypertrophic role. Moreover, replacement of α -MHC with β -MHC was observed to reduce hypertrophy in a troponin T mutant mouse (Rice *et al.* 2010), and an increase in myocyte total myosin and myofibrils has been reported to improve heart function (Chang *et al.* 1997), whereas their loss caused a worse function in human cardiomyopathy (Zimmer *et al.* 1992).

On the other hand, a greater expression of β -MHC has been shown to depress cardiac contractility (Tardiff

Figure 4

(A) β -Mhc mRNA expression in the LVs obtained from male and female mice subjected to TAC for 14 days. Mean $\pm$ S.E.M. of six animal samples are presented. * P < 0.05 = significantly different using ANOVA followed by Bonferroni's *post hoc* test. NS, not significantly different. (B) β 1- and β 2-Ar RNA expression in TAC mice of both sexes. Mean $\pm$ S.E.M. of six animal samples are presented. * P < 0.05 = significantly different using ANOVA followed by Bonferroni's *post hoc* test. NS, not significantly different among the groups.

Table 1 Echocardiography measurements 14 days after surgery. The data represent the mean $\pm$ S.E.M. of results obtained from (n) animals of each condition

	C57 male Sham	C57 male TAC	C57 female Sham	C57 female TAC
BW (g)	28.0 $\pm$ 1.5 (5)	27.3 $\pm$ 0.3 (4)	22.1 $\pm$ 0.5 (6)	21.0 $\pm$ 0.5 (7)
LVW (mg)	92.0 $\pm$ 7.0 (5)	150.7 $\pm$ 0.5 (4)*	74.7 $\pm$ 1.8 (6)	117.3 $\pm$ 3.9 (7)*
VW/BW	3.3 $\pm$ 0.1 (5)	3.2 $\pm$ 0.2 (4)	3.2 $\pm$ 0.2 (6)	3.2 $\pm$ 0.2 (7)
LVEDD (mm)	3.2 $\pm$ 0.2 (5)	3.1 $\pm$ 0.2 (5)	3.9 $\pm$ 0.2 (6)	3.8 $\pm$ 0.3 (5)
LVESD (mm)	2.2 $\pm$ 0.2 (5)	2.1 $\pm$ 0.2 (5)	2.8 $\pm$ 0.2 (6)	2.6 $\pm$ 0.3 (5)
PWT	0.61 $\pm$ 0.07 (5)	0.91 $\pm$ 0.04 (5)*	0.68 $\pm$ 0.08 (6)	0.90 $\pm$ 0.1 (5)*
FS (%)	30.9 $\pm$ 3.8 (5)	32.4 $\pm$ 2.7 (5)	30.6 $\pm$ 2.2 (6)	31.3 $\pm$ 3.5 (5)

BW, body weight; LVW, left ventricular weight; PWT, posterior wall thickness; FS, endocardial fractional shortening. * $P < 0.05$ = significantly different from corresponding sham group using ANOVA followed by Bonferroni's *post hoc* test.

et al. 2000), resulting in a poor tolerance to mechanical or pharmacological cardiovascular stress (Krenz & Robbins 2004). In this regard, however, it has been suggested that caution must be used against genetic approaches that test the functional role of β -MHC by placing this isoform in most or all myocytes (López *et al.* 2011).

Our results also suggest that it is unlikely that β -Mhc expression may produce detrimental or maladaptive effects. In fact, the higher basal level of β -Mhc in females is not associated with hypertrophy or with reduced endocardial FS.

Even the data obtained by many interventions on adrenergic signaling are consistent with an adaptive function played by β -Mhc expression. The β -blocker propranolol was shown both to inhibit pressure overload-induced hypertrophy (Perlini *et al.* 2006, Patrizio *et al.* 2007) and to increase fetal gene expression (Patrizio *et al.* 2007, 2008), whereas a worse cardiac function was observed in an $\alpha 1$ -Ar KO model in which β -MHC and other fetal genes are not induced (O'Connell *et al.* 2006). Moreover, the $\alpha 1$ -Ar blockade was reported to cause survival benefits in the evolution of hypertensive heart disease (Perlini *et al.* 2006). Although these observations were obtained in a more advanced phase of hypertrophy than ours, these results may be understood, at least in part, as the capability of $\alpha 1$ -blockade to prevent the sympathetic overactivity-induced desensitization of $\alpha 1$ -Ar, known to selectively induce β -MHC expression.

Other important findings of this study strongly suggest the involvement of $\beta 1$ -Ar-mediated signaling in the regulation of β -Mhc by estrogens. We found that genetic deletion of both $\beta 1$ - and $\beta 2$ -Ar abolished the gender-related difference in β -Mhc gene expression and that fertile females have reduced cardiac mRNA levels of $\beta 1$ -Ar when compared with males, whereas the expression of $\beta 2$ -Ar was similar in both sexes. The inhibitory effects of

β -adrenergic signaling on β -Mhc expression in the hearts were previously reported. We showed that treatment with the β -blockers propranolol and metoprolol enhanced β -Mhc expression both in TAC-treated male mice and in rat-cultured cardiomyocytes stimulated with phenylephrine (Patrizio *et al.* 2008). In addition, here we found that metoprolol, a selective $\beta 1$ -AR antagonist, is able to induce a significant increase in β -MHC expression in males. On the other hand, in studies designed to evaluate the effects of β -blockade on the fetal gene profile in failing hearts, it was reported that β -blockers decrease β -Mhc expression in patients with heart diseases (Takeo *et al.* 2000, Lowes *et al.* 2002). This discrepancy was previously discussed (Patrizio *et al.* 2007). A possible explanation is

Figure 5

Effects of metoprolol (Meto) on β -Mhc mRNA expression in left ventricles obtained from male and female mice at 12 weeks of age. Mean $\pm$ S.E.M. of six animal samples are presented. * $P < 0.05$ = significantly different using ANOVA followed by Bonferroni's *post hoc* test. NS, not significantly different.

that our results, different from others, were obtained in physiological conditions and at a very early stage of the mouse model of TAC-induced cardiac hypertrophy, when cardiac function is still unchanged. The modulation of cardiac $\beta 1$ -Ar expression by ovarian hormones has already been reported in animal studies. Thawornkaiwong *et al.* (2003), Kam *et al.* (2004), and more recently Wu *et al.* (2008) have shown that ovariectomy upregulates $\beta 1$ -Ar gene expression and protein levels in the LVs of rats, an effect that was abolished by hormone replacement therapy (HRT). The mechanism by which estrogens reduce the synthesis of $\beta 1$ -AR has not been clarified to date. It has been suggested that sex hormones, through interaction with other proteins, affect the stability of $\beta 1$ -Ar mRNA in the heart (Kam *et al.* 2004). Further studies are needed to address this issue. In this respect, it is noteworthy that the anti-hypertrophic effect of estrogens has been shown to be linked to the inhibition of Ca^{2+} /calmodulin-dependent protein kinase II, known to be activated by $\beta 1$ -AR through a cAMP-independent signaling pathway (Ma *et al.* 2009) and then limiting the inhibitory action carried out by $\beta 1$ -Ar signaling on β -Mhc expression (Patrizio *et al.* 2008). The transition from compensated LVH to heart failure is characterized by a development of severe fibrosis, resulting in an increased deposition of collagen associated with a reduction of metalloprotease activity (Fielitz *et al.* 2004, Heymans *et al.* 2005, Tozzi *et al.* 2007).

It has been reported that estrogens attenuate the development of fibrosis and apoptosis induced in mice subjected to TAC (Fliegner *et al.* 2010). At present, the mechanism responsible for the anti-fibrotic effect of estrogens remains to be elucidated. Further research aimed at investigating whether β -MHC-positive cardiomyocytes, which in these experimental conditions were mainly observed in fibrosis areas (López *et al.* 2011), can play a role in counteracting extracellular matrix accumulation would be worthwhile exploring.

In conclusion, this study extends current knowledge regarding the regulation of β -MHC gene expression by demonstrating that gender and $\beta 1$ -AR-mediated signaling control cardiac β -MHC levels under physiological and pathological conditions. We suggest that the greater expression of β -MHC induced by ovarian hormones in the LV of fertile female mice could have a role in the anti-hypertrophic effect of estrogens in the lower rate of LVH observed in premenopausal hypertensive women (de Simone *et al.* 1995, Cleland *et al.* 2003, Hogg *et al.* 2004, Regitz-Zagrosek *et al.* 2007) and in the cardioprotective effect played by HRT in postmenopausal hypertensive patients. Moreover, in this perspective, the involvement of

$\beta 1$ -AR in the regulation of cardiac β -MHC by sex hormones would in part explain the ability of the antihypertensive therapy to potentiate the anti-hypertrophic effects of HRT (Modena *et al.* 1999, Agabiti-Rosei and Musiesan 2002).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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