Gender-specific patterns of left ventricular and myocyte remodeling following myocardial infarction in mice deficient in the angiotensin II type 1a receptor

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Submitted 28 May 2004; accepted in final form 4 March 2005

Bridgman, Paul, Mark A. Aronovitz, Rahul Kakkar, Michael I. Oliverio, Thomas M. Coffman, William M. Rand, Marvin A. Konstam, Michael E. Mendelsohn, and Richard D. Patten. Gender-specific patterns of left ventricular and myocyte remodeling following myocardial infarction in mice deficient in the angiotensin II type 1a receptor. Am J Physiol Heart Circ Physiol 289: H586–H592, 2005. First published March 11, 2005; doi:10.1152/ajpheart.00474.2004.—Left ventricular (LV) remodeling after myocardial infarction (MI) results from hypertrophy of myocytes and activation of fibroblasts induced, in part, by ligand stimulation of the ANG II type 1 receptor (AT₁R). The purpose of the present study was to explore the specific role for activation of the AT₁aR subtype in post-MI remodeling and whether gender differences exist in the patterns of remodeling in wild-type and AT₁aR knockout (KO) mice. AT₁aR-KO mice and wild-type littermates underwent coronary ligation to induce MI or sham procedures; echocardiography and hemodynamic evaluation were performed 6 wk later, and LV tissue was harvested for infarct size determination, morphometric measurements, and gene expression analysis. Survival and infarct size were similar among all male and female wild-type and AT₁aR-KO mice. Hemodynamic indexes were also similar except for lower systolic blood pressure in the AT₁aR-KO mice compared with wild-type mice. Male and female wild-type and male AT₁aR-KO mice developed similar increases in LV chamber size, LV mass corrected for body weight (LV/BW), and myocyte cross-sectional area (CSA). However, female AT₁aR-KO mice demonstrated no increase in LV/BW and myocyte CSA post-MI compared with shams. Both male and female wild-type mice demonstrated higher atrial natriuretic peptide (ANP) levels after MI, with female wild types being significantly greater than males. However, male and female AT₁aR-KO mice developed no increase in ANP gene expression with MI despite an increase in LV mass and myocyte size in males. These data support that gender-specific patterns of LV and myocyte hypertrophy exist after MI in mice with a disrupted AT₁aR gene, and suggest that myocyte hypertrophy post-MI in females relies, in part, on activation of the AT₁aR. Further work is necessary to explore the potential mechanisms underlying these gender-based differences.

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Echocardiography

Forty-one days after coronary ligation and 1 day before the terminal hemodynamic study, mice underwent transthoracic echocardiographic evaluation as previously described (24–26). Animals were anesthetized with a combination of ketamine and xylazine (10 mg/kg) administered by an intraperitoneal injection and placed on a warming pad to maintain body temperature between 36.5 and 37.5°C, and two-dimensional and M-mode recordings were obtained from the short-axis view at the midpapillary muscle level (24–26). All measurements were performed and analyzed by an individual blinded to the animal group.

Closed-Chest Hemodynamic Evaluation

Forty-two days after coronary ligation, hemodynamics were measured as described previously (25, 40). Mice were anesthetized with ketamine and xylazine, weighed, and placed supine on a warming pad to maintain adequate body temperature while under anesthesia. The right common carotid artery was cannulated with a 1.4-Fr micro-tip pressure transducer (Millar). After measurement of arterial and LV pressures, a 2-ng bolus of ANG II was administered, and LV pressure was recorded for an additional minute. The animal was euthanized via an injection of 0.6 meq KCl, arresting the heart in diastole.

Histological Analyses

Infarct size. Five-micrometer transverse sections of the LV were taken at 0.5-mm intervals from the apex to base (usually 4–7 slides/ventricle) using a standard microtome. All sections were stained with Sirius red. Histological images were captured using an Olympus BX40 microscope and a Hitachi VK-C370 digital video camera connected to a Power Macintosh 7100 AV computer (Apple). Infarct size was determined by measuring the infarcted portion of the LV epicardial and endocardial circumferences in all sections and expressing the infarct circumference as a percentage of total LV epi- and endocardial circumferences. (25, 26, 30)

Myocyte cross-sectional area. Cross-sectional areas (CSAs) of 100–125 cardiac myocytes were measured from hematoxylin and eosin-stained sections as previously described (40). Histological images were captured and analyzed by an individual blinded to gender and genotype. Myocytes cut in the cross-sectional plane at the level of the nucleus were traced, and the area was quantified using the image-analysis system described above.

Collagen content. From Sirius red-stained sections, 10 medium-power (×100) images of the noninfarct zone were digitally captured, and the collagen content was determined by the area occupied by red-stained collagen fibers and expressed as the percent area occupied by myocytes and collagen (25).

Semiquantitative RT-PCR

From each frozen LV segment obtained at the time of death, total cellular RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions and as previously described in detail (25). One microgram of RNA was treated with RNase-free DNase (RQ1, Promega) and reverse transcribed using Maloney murine leukemia virus reverse transcriptase (Invitrogen). For a comparative internal standard, primers for GAPDH were added to each PCR, and RT-PCR was performed to quantify the specific expression of atrial natriuretic peptide (ANP), collagen Type I, AT1αR, and AT1βR exactly as detailed previously (25). To assure that the number of cycles chosen for each PCR analysis was along the linear phase of the reaction, varying amounts of RNA (0.5–2.5 μg) were reverse transcribed and subjected to PCR. The resulting amount of product for all genes increased linearly with increasing amounts of input RNA (not shown). For a given gene, PCR experiments were performed two to three times for each sample, yielding highly reproducible results (correlation coefficients between experiments ≥ 0.9). All RT-PCR samples were run on 1–2% agarose gels stained with ethidium bromide and visualized under ultraviolet light. A digital image of the illuminated gel was obtained and the amount of a given PCR product was quantified by densitometric scanning using a commercially available system (Alpha Innotec). All products were expressed as a ratio to GAPDH.

Statistical Analysis

All data are presented as means ± SE unless otherwise indicated. Statistical analyses were performed using either Statview (version 4.5) or SPSS software. Survival data for the groups were analyzed by constructing cumulative hazard plots using the Kaplan-Meier estimate. Survival curves were compared using the log-rank test. Student’s two-tailed t-test was used to compare data from infarct versus sham animals within each gender and genotype. P < 0.05 was considered significant. Infarct sizes among genders and genotypes were compared using two-way ANOVA. Between-group differences for wild-type and transgenic mice and the effect of gender on specific parameters were analyzed using two-way ANOVA or three-way ANOVA as appropriate. For a given parameter, a P value of <0.05 was considered significant. Simple linear regression was used to correlate myocyte CSA with LV mass measurements.

RESULTS

Survival

Ninety mice underwent either left coronary artery ligation (n = 56) or sham procedure (n = 34). Among the wild-type mice, there were six acute deaths in the MI group and three acute deaths in the sham group. Four of the wild-type MI mice and one wild-type sham died during the 6-wk follow-up period. Among the AT1αR-KO mice, there were seven acute deaths in the MI group and one in the sham group. Two of the AT1αR-KO MI and one of the AT1αR-KO shams died during the 6-wk follow-up period. Of the survivors, six wild-type and three AT1αR-KO mice randomized to left coronary artery ligation displayed no histological evidence of MI and were excluded. Thus there were 12 shams and 12 MI mice available for final analysis within the wild-type group and 16 shams and 16 MIs available for final analysis within the AT1αR-KO group. There were no differences in survival between the wild-type and AT1αR-KO MI groups.

Infarct Size

Table 1 displays infarct size data for male and female wild-type and AT1αR-KO mice. Wild-type mice demonstrated an overall mean infarct size of 29.9 ± 3.1%, whereas AT1αR-KO mice had a mean infarct size of 31.7 ± 3.2% (P = not significant).

Hemodynamics

Table 1 displays the hemodynamic data. No differences in systolic blood pressure (SBP) were noted between the MI and sham groups. SBP in wild-type males was significantly higher.
groups with MI but was significant only in male and female AT1aR-KO mice exhibited significantly smaller LV vs. WT; ‡
mice (P/H110210.01 vs. shams). Similarly, male and female AT1aR-KO mice displayed increased LVEDD (both vs. shams), whereas female wild-type MI mice also developed increases in LVEDD (as compared with shams). However, female AT1aR-KO mice displayed no increase in LV/BW with MI. A significant interaction was present among procedure (sham or infarct), gender, and genotype (P < 0.05 by 3-way ANOVA), supporting that the influence of gender on MI-induced LV hypertrophy in the AT1aR-KO mice differed significantly from wild-type mice.

LV Mass, Myocyte CSA, and ANP Gene Expression

The data for LV/BW are shown in Fig. 1. With MI, male and female wild-type mice and male AT1aR-KO mice developed increased LV/BW compared with the respective sham groups (P < 0.05 for wild-type mice and P < 0.01 male AT1aR-KO mice), whereas the female AT1aR-KO mice did not develop an increase in LV/BW with MI. A significant interaction was present among procedure (sham or infarct), gender, and genotype (P < 0.05 by 3-way ANOVA), supporting that the influence of gender on MI-induced LV hypertrophy in the AT1aR-KO mice differed significantly from wild-type mice.

To determine whether the LV/BW measurements were reflective of differences in myocyte hypertrophy, myocyte CSA was quantified from sections of five hearts within each gender and genotype matched for infarct size and compared with the respective sham groups (Fig. 2, A and B). Myocyte CSA correlated with LV mass (r = 0.73, P < 0.01; Fig. 2C) and LV/BW (r = 0.69, P < 0.01). With MI, male and female wild-type mice and male AT1aR-KO mice developed significant increases in myocyte CSA compared with the respective sham groups (P < 0.01 vs. shams). However, female AT1aR-KO mice developed no increase in myocyte CSA after MI. As with LV/BW, there was a significant interaction between procedure, gender, and genotype (P < 0.05), supporting that the gender-specific effects on myocyte hypertrophy in the AT1aR-KO mice differed significantly from wild-type mice. Taken together, these data are consistent with significantly

Table 1. Infarct size and hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Male WT</th>
<th>Female WT</th>
<th>Male AT1aR-KO</th>
<th>Female AT1aR-KO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham (n = 6)</td>
<td>MI (n = 6)</td>
<td>Sham (n = 6)</td>
<td>MI (n = 6)</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(range)</td>
<td>23.5±4.1 (9–33)</td>
<td>35.9±3.1 (26–46)</td>
<td>34.0±0.7 (10–66)</td>
<td>30.0±2.3 (19–38)</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>349±18</td>
<td>354±21</td>
<td>300±36</td>
<td>334±11</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>147±15*</td>
<td>145±12*</td>
<td>119±14</td>
<td>117±10</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>8.0±1.1</td>
<td>14.3±2.2</td>
<td>6.9±1.1</td>
<td>9.5±1.2</td>
</tr>
<tr>
<td>Peaked +dP/dt</td>
<td>10,454±623</td>
<td>8,509±632</td>
<td>9,374±883</td>
<td>7,465±360</td>
</tr>
<tr>
<td>Rise in SBP with ANG II, mmHg</td>
<td>20.6±7.6</td>
<td>19.5±7.3</td>
<td>29.8±8.0</td>
<td>39.4±7.6</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of mice. HR, heart rate; SBP, systolic blood pressure; LVEDP, left ventricular (LV) end-diastolic pressure; dP/dt, change in pressure/change in time; WT, wild type; AT1aR-KO, ANG II type 1a receptor knockout group; MI, myocardial infarction. *P < 0.05 vs. female WT; †P < 0.01 vs. WT; ‡P < 0.05 vs. the respective sham group.

Table 2. LV chamber size and function

<table>
<thead>
<tr>
<th></th>
<th>Male WT</th>
<th>Female WT</th>
<th>Male AT1aR-KO</th>
<th>Female AT1aR-KO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham (n = 6)</td>
<td>MI (n = 6)</td>
<td>Sham (n = 6)</td>
<td>MI (n = 6)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>30.5±1.9</td>
<td>32.6±1.7</td>
<td>24.5±1.0</td>
<td>25.2±0.7</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>3.8±0.2</td>
<td>4.5±0.2*</td>
<td>3.7±0.1</td>
<td>4.4±0.1†</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>2.5±0.2</td>
<td>3.1±0.2*</td>
<td>2.4±0.1</td>
<td>3.1±0.1†</td>
</tr>
<tr>
<td>FS, %</td>
<td>36.1±2.3</td>
<td>30.4±2.1</td>
<td>35.5±2.5</td>
<td>29.7±1.1</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of mice. LVEDD, LV end-diastolic diameter; LVESD, LV end-systolic diameter; FS, fractional shortening. *P < 0.08 vs. shams; †P < 0.01 vs. shams; ‡P < 0.05 vs. shams; §P < 0.05 vs. male sham AT1aR-KO group. (P = not significant when LVEDD was indexed by body weight).
blunted LV and myocyte hypertrophy after MI in female AT1aR-KO mice.

We quantified the expression of the ANP gene, which increases in concert with cardiomyocyte hypertrophy. With MI, wild-type males and females developed a significant rise in ANP expression compared with shams (P < 0.02; Fig. 3). The overall higher level of ANP expression in wild-type females compared with males was statistically significant (P < 0.01 by two-way ANOVA). In contrast, within the AT1aR-KO groups, no increase in ANP gene expression was noted in male and females after MI. The blunted ANP response with MI in the AT1aR-KO group was statistically significant compared with wild-type mice (P < 0.02 by two-way ANOVA).

Collagen Type 1 Gene Expression and Collagen Content

We next analyzed the expression of collagen type 1. With MI, wild-type males and females demonstrated increased collagen type 1 expression compared with shams (P < 0.05, MI vs. shams; Fig. 4A). However, no increase in collagen type 1 gene expression was observed in either the male or female AT1aR-KO mice post-MI. The blunted collagen type 1 response with MI in the AT1aR-KO group was statistically significant compared with the wild-type group (P < 0.05 by two-way ANOVA). To explore whether lower collagen type 1 gene expression in the AT1aR-KO mice lead to diminished collagen in the noninfarct zone, we measured collagen content (percent area) from Sirius red-stained myocardial sections. Figure 4B demonstrates that MI led to a significant rise in noninfarct zone collagen content in male and female wild-type mice (P < 0.05 vs. shams for both male and female wild-type mice), but collagen content increased to a lesser extent in the male and female AT1aR-KO mice, which did not reach statistical significance. A strong trend (P = 0.08 by two-way ANOVA) for an
interaction between procedure and genotype supported that the blunted rise in collagen content with MI in the AT1aR-KO mice differed from wild-type mice.

**ANG II Receptor Gene Expression**

Expression of the AT1aR gene was present within the wild-type animals but absent within the AT1aR-KO group (Fig. 5). With MI, male wild-type mice developed a 2.5-fold increase in the expression of the AT1aR (0.53 ± .13 vs. 0.21 ± .01 arbitrary units, P < 0.05 vs. male shams; cf. Fig. 4). However, female wild-type mice displayed no increase in AT1aR gene expression with MI compared with shams (0.28 ± 0.03 vs. 0.26 ± 0.08 arbitrary units). The expression of the AT1bR was of much lower abundance within the myocardium but detectable in all wild-type and AT1aR-KO mice (not shown).

**DISCUSSION**

In the present study, male and female wild-type mice developed typical features of LV remodeling, including increased LV mass, chamber size, myocyte size, and ANP gene expression. The major novel finding of this study lies in the observed differences in LV and myocyte remodeling between male and female AT1aR-KO mice. Whereas male AT1aR-KO mice exhibited a similar degree of LV remodeling and myocyte hypertrophy as wild-type mice, female mice deficient in the AT1aR did not. There are numerous possible mechanisms to account for these findings. These data suggest that myocyte hypertrophy after myocardial injury in female mice is largely dependent on activation of the AT1aR. However, the lower systolic pressures observed in the female and male AT1aR-KO mice also raise the possibility that myocyte hypertrophy in females may rely more heavily on loading conditions, whereas males can develop LV and myocyte hypertrophy after MI independent of both AT1aR activation and loading conditions. This notion is consistent with previous findings in pressure overload models in which two groups (13, 14) demonstrated that AT1aR-KO mice develop LV hypertrophy after aortic...
banding similar to wild-type litters. In male C57BL/6J mice, we observed previously that administration of the AT1R antagonist losartan inhibited myocardial hypertrophy 6 wk post-MI (25). Taken together with the present study, these data support the possibility that AT1R activation may, in part, contribute to MI-induced LV and myocyte hypertrophy in male mice. Studies to explore this possibility further are currently underway.

Some of our findings contrast with those reported by Harada and colleagues, who investigated the effects of MI on LV remodeling in a different strain of AT1aR-KO mice (14, 36). In their study, which included only mice with large infarcts (>30%), AT1aR-KO mice demonstrated improved survival and significantly less LV dilatation 4 wk after MI. The smaller infarct sizes in our study may account for the lack of a survival advantage in our AT1aR-KO mice. Additionally, differences in the specific transgenic mouse strain and the shorter time in which remodeling was allowed to progress after MI in their study (4 vs. 6 wk in our study) may also have contributed to the disparate results observed here. In their study, Harada et al. did not provide details on the gender of mice included; thus a preponderance of females in the AT1aR-KO group may also have contributed to an overall reduction in post-MI remodeling.

The molecular responses to MI were different among the wild-type and AT1aR-KO mice. In line with findings of Harada et al. (14), we found that targeted deletion of the AT1aR receptor was associated with suppression of the MI-induced increase in collagen type I gene expression and collagen content within the noninfarct zone. Given that collagen type I production in the heart is specific to fibroblasts (8), these data support a role for activation of the AT1aR in fibroblast activation after MI. Wild-type males and females demonstrated the expected rise in ANP gene expression after MI. ANP gene expression was greater in both female sham and female-MI hearts compared with males, which is consistent with previous studies demonstrating an association between female gender and higher levels of ANP (1, 17, 21, 39). Although no increase in AT1aR gene expression was observed in the hearts of female wild-type mice, male wild-type mice developed an increase in AT1aR expression after MI. The regulation of AT1aR gene expression is complex and likely results from a combination of influences including myocardial stretch and autocrine and paracrine mechanisms. Our demonstration of a gender-specific effect on AT1aR and ANP gene expression in wild-type mice supports further that gender influences the molecular responses to myocardial injury.

An inhibitory effect on the development of LV and myocyte hypertrophy by female gender has been reported in numerous other transgenic mouse models (5, 9, 12, 19, 44). Taken together with the findings of this study, these data underscore the importance of stratifying data by gender to unveil possible gender-specific effects on the cardiac phenotype of interest within a given transgenic mouse strain.

In summary, these findings suggest that gender-specific patterns exist in the myocyte hypertrophic response to MI in mice with a disrupted AT1aR gene such that LV and myocyte hypertrophy in female AT1aR-KO mice was diminished. These data support the hypothesis that myocyte hypertrophy post-MI in females is largely dependent on activation of the AT1aR. Further studies are necessary to explore the potential mechanisms involved in these gender-based differences and whether sex hormones play a role in molecular responses to myocardial injury.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Richard Karas for helpful reviews of this manuscript.

The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official view of the National Institutes of Health or American Heart Association.

GRANTS

This study was supported by American Heart Association Grant 0256206T-RDP and National Heart, Lung, and Blood Institute Grant HL-03598. This study was also supported in part by a Merck Medical School grant.

REFERENCES


19. Kadokami T, MiMcTiernan CF, Kubota T, Frye CS, and Feldman AM. 


