17β-Estradiol Attenuates the Development of Pressure-Overload Hypertrophy

Martin van Eickels, MD; Christian Grohé, MD; Jack P.M. Cleutjens, PhD; Ben J. Janssen, PhD; Hein J.J. Wellens, MD; Pieter A. Doevendans, MD

Background—Cardiac hypertrophy is an independent risk factor for cardiovascular morbidity and mortality in men and in women. Epidemiological studies indicate that estrogen replacement therapy is cardioprotective; the mechanisms involved in this process, however, are poorly understood. We therefore studied the effect of 17β-estradiol (E₂) on the development of pressure-overload hypertrophy.

Methods and Results—Ovariectomized mice receiving E₂ or placebo underwent transverse aortic constriction (TAC) or sham operation. TAC led to a significant increase in ventricular mass compared with sham operation, whereas it had no effect on the degree of pressure overload, as determined by hemodynamic measurements. Furthermore, E₂ blocked the increased phosphorylation of p38-mitogen–activated protein kinase (MAPK) observed in the placebo-treated animals with TAC. No differences were observed in the phosphorylation of extracellular signal–regulated kinase (ERK) 1/2 and c-Jun N-terminal kinase (JNK) 1/2 between the groups. E₂ had no effect on the expression of angiotensin-converting enzyme (ACE) or the angiotensin II type 1 receptor. Ventricular atrial natriuretic peptide (ANP) expression was detected only in the animals with TAC. Compared with placebo, E₂ treatment led to an increased expression of ANP in animals with pressure overload.

Conclusions—Here, we show that E₂ attenuates the hypertrophic response to pressure overload in mice. This observation demonstrates that hormone replacement therapy with E₂ has direct effects on the heart and may be beneficial in the treatment of postmenopausal women to reduce cardiac hypertrophy. (Circulation. 2001;104:1419-1423.)

Key Words: hormones ■ hypertrophy ■ myocardium ■ sex

The increase of left ventricular mass represents the structural mechanism of adaptation of the heart in response to pressure overload. The resulting left ventricular hypertrophy is an important negative predictor of cardiac morbidity and mortality and displays significant sex-based differences. The role of estrogen in the development of cardiac hypertrophy, however, is poorly understood.

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We have shown previously that cardiac myocytes and cardiac fibroblasts contain both known estrogen receptor isoforms, called α and β. Via these receptors, estrogen can regulate the cardiac expression of endothelial and inducible NO synthase and connexin 43. Estrogen also modulates the activity of the mitogen-activated protein kinase (MAPK) pathways in cardiac myocytes. The MAPK signaling pathways consist of a sequence of successively acting kinases that ultimately result in the dual phosphorylation and activation of effector kinases such as p38-MAPKs, c-Jun N-terminal kinases (JNKs), and extracellular signal-regulated kinases (ERKs), which subsequently phosphorylate a large array of targets, leading to altered gene expression patterns. These signaling cascades play an important role in the initiation of cardiac hypertrophy and in the development of heart failure. Furthermore, we have shown previously that estrogen downregulates the activity of the renin-angiotensin system in the vasculature of normotensive and of hypertensive rats. The inhibition of the renin-angiotensin system has an antihypertrophic effect and is used widely in the therapy of patients with cardiac hypertrophy. In addition, it is known that estrogen can increase the expression of atrial natriuretic peptide (ANP). ANP has long been known to be a marker of cardiac hypertrophy. More recent studies, however, have suggested that ANP possesses antihypertrophic properties. Taken together, these data led us to the hypothesis that estrogen may modulate the development of cardiac hypertrophy. We therefore studied the effects of 17β-estradiol (E₂), the predominant estrogen in premenopausal women, on the development of cardiac hypertrophy.
development of pressure-overload hypertrophy and the activation of the pathways mentioned above.

**Methods**

**Animals**
Ten-week-old female C57BL/6 mice were housed under standard conditions. Animals were anesthetized with ketamine (100 mg/kg body weight [BW] IP) and xylazine (10 mg/kg BW IP) for ovarioctomy, pellet placement, and transverse aortic constriction (TAC). The study was approved by the animal ethics committee of the University of Maastricht.

**Estrogen Replacement**
One week after ovarioctomy, a 60-day-release pellet containing 0.18 mg E2 or placebo was implanted subcutaneously. E2 serum levels were measured with a radioimmunoassay (DPC Biermann) in a subset of animals. To measure the magnitude of proliferation of cardiac cells, a subgroup of animals with pressure overload received bromodeoxyuridine (BrdU) (2.5 mg per 21-day-release pellet) for the last 14 days of the study period. All pellets were purchased from Innovative Research of America.

**Surgical Procedures and Hemodynamics**
One week after the pharmacological intervention, TAC was performed, as described previously. Sham-operated animals underwent an identical operation without placement of the constricting suture. There was no difference in the mortality between the E2- and the placebo-treated animals.

Four and 8 weeks (n=7 and n=6 per group) after TAC or the sham procedure, animals were anesthetized with pentobarbital (100 mg/kg BW IP). Pressure-transducing catheters were introduced into the right and the left carotid arteries, and systolic pressure before and after the stenosis was measured as described previously.

**Tissue Preparation and Histology**
Hearts were arrested in diastole with CdCl2 (0.1 mol/L IV). For morphometric analysis, hearts were fixed in 10% formalin and embedded in paraffin as described previously. For protein extraction, hearts were excised, washed in ice-cold PBS, and frozen. All external fluid was completely removed before the organs were weighed. Transverse sections of the heart were stained with hematoxylin and eosin, picrosirius red, modified Azan, or anti-BrdU antibodies. The analysis of the collagen content was performed with a computerized morphometry system as described previously. Cross-sectional areas of 50 (sham) or 100 (TAC) myocytes in which the centrally located nucleus and a clear staining of the cell borders could be visualized were measured in the transversely cut papillary muscles of each animal.

<table>
<thead>
<tr>
<th>BW, g</th>
<th>4 Weeks</th>
<th>8 Weeks</th>
<th>4 Weeks</th>
<th>8 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>23.4±0.6</td>
<td>24.7±0.3</td>
<td>22.7±0.6</td>
<td>23.6±0.4</td>
</tr>
<tr>
<td>E2</td>
<td>23.1±0.5</td>
<td>23.5±0.5</td>
<td>23.7±0.9</td>
<td>22.5±0.7</td>
</tr>
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BW indicates body weight; VW, ventricular weight; TL, tibial length; Pp, prestenotic systolic pressure; and CSA, myocyte cross-sectional area. All values are mean±SEM. Four weeks, n=7; 8 weeks, n=6. *P<0.05 for TAC vs sham, †P<0.05 for E2 vs placebo.

**Immunoblot Analysis**
Total heart lysates (40 µg/lane) were analyzed by standard immunoblotting procedures as described previously. Equal loading was checked by stripping and reprobing the membrane with troponin C. The following primary antibodies were used: ACE (BMA Biomedicals AG); ANP (Phoenix Pharmaceuticals Inc); angiotensin type 1 receptor (AT1 R), ERK1/2, JNK, MKP1, p38-MAPK, phospho-JNK (Thr183/Tyr185), and troponin C (Santa Cruz Biotechnology Inc); and phospho-ERK1/2 (Thro202/Tyr204) and phospho-p-38 MAPK (Thr180/Tyr182) (New England Biolabs GmbH). Detection was performed with the enhanced chemiluminescence technique after incubation with a suitable secondary antibody coupled to horseradish peroxidase (ECL; Amersham Pharmacia Biotech). A computerized image acquisition system (Alpha Innotech Corp) was used for densitometric analysis.

**Statistical Analysis**
Data are shown as mean±SEM. Means were compared by ANOVA, followed by Bonferroni’s test for multiple comparisons. Differences were considered significant at P<0.05.

**Results**
Estrogen replacement led to a reconstitution of physiological estrogen levels (sham 121±20 pg/mL, TAC 119±17 pg/mL, n=5 per group). All measured E2 levels in animals receiving placebo were <5 pg/mL. Uterus weight was measured to demonstrate the effectiveness of ovarioectomy and E2 substitution in all animals (Table).

TAC led to a significant increase in ventricular mass 4 and 8 weeks after the intervention. The degree of ventricular hypertrophy was significantly lower in E2-treated than placebo-treated animals with pressure overload (Figure 1A). Compared with placebo, E2 treatment led to a significant reduction of the ventricular weight (VW), the VW/BW ratio, and the VW/tibial length ratio in animals with pressure overload (Figure 2 and Table). No differences were observed between the E2- and the placebo-treated animals that were sham-operated (Figure 2 and Table). The lung weight was increased in the animals with TAC; however, this increase reached statistical significance only in the placebo-treated animals (Table). There were no differences in BW between the groups (Table).

Because E2 can alter blood pressure, we tested whether the observed antihypertrophic effect of estrogen could be medi-
ated by a change in the degree of pressure overload. In our model of TAC, E2 had no influence on the degree of pressure overload as determined by the pressure gradient or the prestenotic pressure (Figure 2B).

Histological analysis revealed that pressure overload led to progressive cardiac fibrosis (Figure 1B). No differences in collagen content (E2 17.4±2.2% and placebo 14.1±1.7%) or proliferation of interstitial cells, measured by BrdU labeling (E2 1.57±0.37% and placebo 1.70±0.34%), however, were observed in the animals with ventricular hypertrophy. TAC led to a significant increase of myocyte size, as determined by myocyte cross-sectional area. This increase was significantly attenuated by treatment with E2 compared with placebo (Table).

To further elucidate the mechanism involved in the observed antihypertrophic effect of estrogen, we studied pathways involved in the development and progression of cardiac hypertrophy, which also have been shown to be regulated by E2. Immunoblot analysis revealed that E2 blocked the increased phosphorylation of p38-MAPK in ovariectomized animals with pressure-overload hypertrophy, whereas it exerted no effect in sham-operated animals (Figures 3A and 4A). No differences could be observed between the study groups with regard to the phosphorylation level of ERK1/2 and JNK (Figure 3A). In the sham-operated animals, the levels of AT1R expression were slightly lower in E2-treated animals; however, this difference did not reach statistical significance. In the hypertrophied hearts, no differences in the expression levels of AT1R or ACE were detected between the E2- and the placebo-treated animals (Figure 3B). Compared with placebo, E2 treatment led to an increase in ANP expression in the hypertrophied ventricles. No significant levels of ANP were detectable in the ventricles of sham-operated animals (Figures 3C and 4B).

**Discussion**

Here, we show for the first time that hormone replacement therapy with physiological doses of E2 can attenuate the development of pressure-overload hypertrophy. In contrast to a previous study in spontaneously hypertensive rats, the antihypertrophic effect observed in our study was not mediated by the blood pressure-lowering effect of E2.26 We did not observe E2-associated differences in cardiac fibrosis and cell proliferation in the heart. In fact, myocyte cross-sectional area was attenuated by estrogen treatment compared with placebo. Therefore, our study supports the hypothesis that E2 has direct effects on cardiac myocytes and the heart.

To further elucidate the mechanisms involved, we studied the activation of MAPK7–9,27 and the expression of ACE, AT1R,13,15 and ANP,14,17 all of which have been shown to
play important roles in the development and progression of cardiac hypertrophy and are estrogen responsive.

A previous study suggested that the activation of p38-MAPK is important for the maintenance of the hypertrophic response over a longer period of time.27 Therefore, the inhibition of the p38-MAPK phosphorylation by E2 treatment may represent one of the mechanisms by which E2 exerts its antihypertrophic effect in our model of pressure overload.

ERK1/2 and JNK were not activated in all the animals with pressure overload, possibly because these pathways may be more important in the induction of the hypertrophic response and have returned to baseline after 4 weeks.

Inhibition of the renin-angiotensin system plays an important role in the development of cardiac hypertrophy. On the basis of our previous findings that E2 reduces the expression of the AT1R, 11,12 we hypothesized that a similar mechanism could be involved in the observed antihypertrophic effect of E2. No such differences were observed, however, in the hypertrophied hearts.

Recent studies have identified the antihypertrophic properties of ANP.16,17 Furthermore, it has been shown previously that E2 increases the expression of ANP.14,28 In concordance with these results, E2 led to an increase in ANP expression in the ventricles of animals with pressure overload compared with placebo. These findings suggest that the antihypertrophic effect of E2 may be mediated by the increased expression of ANP.

The development of cardiac hypertrophy is a complex process involving signal integration of multiple pathways.7,29 Therefore, it may well be that the observed antihypertrophic effect of E2 is mediated by the modulation of not one but several of these pathways. To further elucidate the mechanisms involved in E2’s antihypertrophic effects, it will be necessary to identify the essential signaling molecules involved in the development of cardiac hypertrophy, their time course of activation, and the cross talk between them.

Overall, our results suggest that the lack of estrogen may be responsible for the increase in ventricular hypertrophy observed in postmenopausal women. Furthermore, these findings may constitute a novel approach in the treatment of postmenopausal women at risk of developing cardiac hypertrophy.

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References

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