Regulation of components of the brain and cardiac renin-angiotensin systems by 17β-estradiol after myocardial infarction in female rats

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Dean, Stephanie A., Junhui Tan, Roselyn White, Edward R. O’Brien, and Frans H. H. Leenen. Regulation of components of the brain and cardiac renin-angiotensin systems by 17β-estradiol after myocardial infarction in female rats. Am J Physiol Regul Integr Comp Physiol 291: R155–R162, 2006. First published February 2, 2006; doi:10.1152/ajpregu.00497.2005.—The present study tested the hypothesis that 17β-estradiol (E2) increases in angiotensin-converting enzyme (ACE) and ANG II type 1 receptor (AT1R) in the brain and heart after myocardial infarction (MI) and, thereby, inhibits development of left ventricular (LV) dysfunction after MI. Age-matched female Wistar rats were treated as follows: 1) no surgery (ovary intact), 2) ovariectomy + subcutaneous vehicle treatment (OVX + Veh), or 3) OVX + subcutaneous administration of a high dose of E2 (OVX + high-E2). After 2 wk, rats were randomly assigned to coronary artery ligation (MI) and sham operation groups and studied after 3 wk. E2 status did not affect LV function in sham rats. At 2–3 wk after MI, impairment of LV function was similar across MI groups, as measured by echocardiography and direct LV catheterization. LV ACE mRNA abundance and activity were increased severalfold in all MI groups compared with respective sham animals and to similar levels across MI groups. In most brain nuclei, ACE and AT1R densities increased after MI. Unexpectedly, compared with the respective sham groups the relative increase was clearest (20–40%) in OVX + high-E2 MI rats, somewhat less (10–15%) in OVX + Veh MI rats. However, because in the sham group brain ACE and AT1R densities increased in the OVX + Veh rats and decreased in the OVX + high-E2 rats compared with the ovary-intact rats, actual ACE and AT1R densities in most brain nuclei were modestly higher (<20%) in OVX + Veh MI rats than in the other two MI groups. Thus E2 does not inhibit upregulation of ACE in the LV after MI and amplifies the percent increases in ACE and AT1R densities in brain nuclei after MI, despite E2-induced downregulation in sham rats. Consistent with these minor variations in the tissue renin-angiotensin system, during the initial post-MI phase, E2 appears not to enhance or hinder the development of LV dysfunction.

ovariectomy; heart failure; estrogens

AFTER MYOCARDIAL INFARCTION (MI) in male rats, expression of brain and cardiac angiotensin-converting enzyme (ACE) and ANG II type-1 receptor (AT1R) is increased. In the heart, ACE mRNA and activity (8) and ACE and AT1R binding densities (24) increase severalfold in the noninfarcted and infarcted areas of the left ventricle (LV) after MI. In the brain, ACE and AT1R densities increase 10–20% after MI in several cardiovascular regulatory nuclei (24). Brain AT1R stimulation plays a critical role in determining sympathetic hyperactivity and progression to heart failure in male rats, inasmuch as chronic intracerebroventricular infusions of an AT1R antagonist normalize the increase in sympathetic tone and impairment of baroreflex function (30) and a significant portion of the LV remodeling and dysfunction after MI (17). Intracarotid injections of an ACE inhibitor or AT1R antagonist normalize the increased neuronal firing in the paraventricular nucleus of rats after MI (31). Transgenic rats with very low levels of angiotensinogen in the hypothalamus exhibit decreased sympathetic activation, reduced LV fibrosis, and less LV dysfunction after MI (28).

In female rats, 17β-estradiol (E2) plays a major role in the regulation of ACE and AT1R (for a review, see Ref 6). We recently showed that E2 deficiency by ovariectomy significantly increases ACE and AT1R densities in the heart by 50–100% and increases ACE and AT1R binding densities in several nuclei of the brain by ~20%. These increases are prevented by replacement of E2 at levels that normally occur during the estrous cycle (i.e., physiological replacement) and, in most cases, are reversed to decreases by supraphysiological E2 replacement (4).

Because of the upregulation of cardiac and brain ACE and AT1R by E2 deficiency and downregulation by high E2 in healthy female rats and the extensively demonstrated role of the brain and cardiac renin-angiotensin systems (RASs) in cardiac remodeling after MI in male rats, one may postulate that, in female rats, E2 status influences the extent of changes in brain and cardiac RASs and, thereby, the extent of cardiac remodeling and LV dysfunction after MI. The impact of estrogen status on changes in the circulatory and tissue RASs after MI has not been studied. The impact on cardiac remodeling and dysfunction has been examined in a few studies in mice and rats with conflicting results. Cavasin et al. (3) concluded that, in mice, estrogens prevent deterioration of cardiac function and remodeling after MI, whereas Van Eickels et al. (27) concluded that estrogen replacement increases ventricular remodeling and mortality in mice after MI, despite reducing infarct size and cardiomyocyte apoptosis. In ovariec-tomized (OVX) rats, Smith et al. (22) reported an increase in MI size with E2 replacement, whereas Nekooeian and Pang (18) reported that E2 replacement had no effect on MI size and caused minor changes in LV function (as measured under pentobarbital anesthesia).

The objectives of the present study were to determine 1) whether the overexpression of cardiac ACE and brain ACE and AT1R that occurs after MI is exacerbated in rats with E2 deficiency and attenuated in rats treated with high E2 and 2) whether such changes in the brain and cardiac RASs are...
associated with parallel changes in LV function in female rats during the early post-MI phase.

MATERIALS AND METHODS

General. Experiments were performed in two parts: protocol 1, in which OVX rats treated with vehicle or high-dose E2 were studied, and protocol 2, in which ovary-intact rats were studied. For protocol 1, 10- to 11-wk-old female Wistar rats were obtained from Charles River Breeding Laboratories (Montreal, QC, Canada). For protocol 2, 12- to 13-wk-old female Wistar rats were used to ensure that the rats in protocols 1 and 2 were age matched at the time of MI. Rats were maintained on a 12:12-h light-dark cycle and allowed free access to normal rat chow and water. All experimental procedures were carried out in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the University of Ottawa Animal Care Committee. All chemicals and reagents were purchased from Sigma (Oakville, ON, Canada) except where noted.

Ovariectomy and pellet implantation. Under isoflurane inhalation anesthesia, rats from protocol 1 underwent bilateral ovariectomy via a single dorsal incision (29) and were randomly assigned to immediately receive pellets containing vehicle or a high dose of E2 (15 mg, 250 µg/day, 60-day release; Innovative Research of America, Sarasota, FL). A comparable dose of E2 (5-mg pellets released over 21 days) resulted in plasma concentration of E2 of 894 ± 114 pg/ml (9), which is 5–10 times that during the estrous cycle. This dose of E2 given for 5 wk significantly reduces ACE activity and binding densities in the heart and in brain nuclei compared with sham-OVX or OVX rats (4). Pellets were implanted subcutaneously in the dorsal neck area. Ovary-intact rats were used in protocol 2.

 Coronary artery ligation. At 2 wk after ovariectomy and pellet implantation (protocol 1) or 3–4 days after entering the animal facility (protocol 2), rats were randomly assigned to sham or coronary artery ligation surgery, as described previously (19). Protocols 1 and 2, although performed separately, proceeded identically from this point. Under halothane inhalation anesthesia, the thorax was opened at the fourth and fifth intercostal spaces, and the left coronary artery was ligated 2–3 mm from its origin with a 6-0 silk suture attached to an atrumatic needle. Sham rats underwent a similar procedure without ligation. Buprenorphine (0.03 mg/ml, 0.1 ml/rat, twice daily for 3 days) was used for pain relief.

Echocardiography. A Philips Sonos 5500 echocardiography system with a 12-MHz transducer at a depth setting of 3–4 cm was used. At 2–2.5 wk after MI, under light isoflurane anesthesia, the rat was placed on its back and its chest was shaved. With the two-dimensional parasternal long-axis imaging plane as a guide, an LV M-mode tracing just below the tips of the mitral leaflets was obtained and recorded on videotape. Simultaneous M-mode recording of intraventricular septum (IVS) and posterior wall (PW) thickness and chamber size was performed for at least six cardiac cycles. LV internal dimensions in systole and diastole (LVdD and LVdD) were used to measure ejection fraction (EF) according to the following formula: $EF = \left[\frac{LVdD - LVdD}{LVdD}\right] \times 100\%$.

LV catheterization. At 3 wk after MI, under isoflurane anesthesia, a 2-F high-fidelity micromanometer catheter (model SPR-407, Millar Institute, Houston, TX) was threaded via the right carotid artery into the LV. Under minimal anesthesia, the waveforms of pressure changes were recorded for 1–2 min by a personal computer equipped with the data acquisition software AcqKnowledge (ACQ 3.2). LV peak systolic pressure (LVPSP), LV end-diastolic pressure (LVEDP), and positive and negative first derivatives of pressure (+dP/dt and −dP/dt) were calculated. After the recordings, this catheter was removed, and a polyethylene (PE)-50 catheter filled with heparinized saline was inserted.

Plasma and tissue collection. At 4–5 h after the measurement of LV function, blood was removed from conscious, unstressed rats via the arterial catheter for measurement of plasma renin activity (PRA) and plasma ANG I and ANG II concentrations, as previously described (15, 20). Rats were then euthanized by decapitation, and trunk blood was collected in chilled heparinized tubes for measurement of plasma ACE activity. Hearts were quickly removed and washed in ice-cold saline, and the atria and great vessels were removed. The right ventricle (RV) was dissected from the LV and weighed. After measurement of infarct size by planimetry (14), LVs from one group of MI rats were dissected into noninfarcted area, peri-infarct area, a ~3-mm-wide region in the border zone of the infarct scar, and infarct scar proper. These tissues and LVs from a group of sham rats were used to measure ACE mRNA abundance and ACE activity. The LVs from the remaining MI rats were dissected into noninfarcted area and infarcted area and, together with LVs from a second group of sham rats, were used to measure tissue ANG I and ANG II concentrations. All LV tissue pieces were weighed and frozen in liquid nitrogen. Tissues were stored at ~80°C.

Plasma and tissue ACE activity. ACE activity (as nanomoles His-Leu liberated per milliliter of plasma or per gram of total protein per minute of incubation at 37°C) was measured as recently described (4), with hippuryl-histidyl-leucine as the substrate. His-Leu liberation was also measured in duplicate sample tubes containing the ACE inhibitor captopril and subtracted from total activity to ensure specificity for ACE.

Real-time RT-PCR for ACE. LV tissues were ground in liquid nitrogen, and ~100 mg of tissue were used for total mRNA extraction using RNAwiz (Ambion). Up to 25 µg of nucleic acid were treated with DNase I to eliminate genomic DNA contamination (DNA-free; Ambion). One microgram of total RNA in RNAse-free water in a total volume of 28 µl was incubated with 15 U of avian myeloblastoma virus RT (Amersham Biosciences). The amounts of ACE and phosphoglycerate kinase-1 (PGK-1, as endogenous reference) were assayed using SYBR Green I (Roche Diagnostics, Baie D’Urfé, QC, Canada) and 2 µl of the RT product. The primers used in the reactions are as previously published (13). The reactions were carried out as follows: after 95°C for 10 min, 45 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 30 s and 80°C for 5 s (for ACE) and 40 cycles of 95°C for 0 s, 62°C for 37 s, and 83°C for 5 s (for PGK-1). These conditions yield single-product 934- and 898-bp target bands for ACE and PGK-1, respectively, on agarose gel electrophoresis. The target PCR product concentration of the samples was compared with the concentration of a standard curve run in the same assay. The standard curve was made from 0.1, 1, 10, and 100 pg of rat ACE cDNA plasmid for ACE and from 0.1, 1, 10, and 100 pg of rat PGK-1 cDNA plasmid for PGK-1. ACE mRNA abundance was expressed as the ratio of ACE to PGK-1 × 100.

PRA and plasma and LV ANG I and ANG II. PRA was determined by in vitro generation of ANG I followed by RIA as previously described (16). Plasma and LV ANG I and ANG II concentrations were measured by RIA after separation by HPLC, as previously described (20).

ACE and AT1R autoradiography. Binding densities for ACE and AT1R were determined using the ACE inhibitor derivative 125I-351ATI and 125I-ANG II, respectively, as recently described (4). Statistical analysis. Values are means ± SE. Comparisons were made using one- or two-way ANOVA followed by the Student-Newman-Keuls test where applicable. Mortality rates were analyzed using χ² test. All tests were performed using SigmaStat software (SPSS, Chicago, IL). The level of statistical significance was set at $P < 0.05$.

RESULTS

Mortality. Mortality was 0% in sham-operated groups (0 of 48 rats). All post-MI mortality occurred within 24 h of coronary artery ligation. Survival rates did not differ significantly between groups of MI rats: 50% (30 of 60), 60% (35 of 58),
and 43% (25 of 58) for ovary-intact, OVX + Veh, and OVX + high-E2 MI rats, respectively (P = 0.17).

Body weight. Body weights were related to E2 status. At the time of MI or sham surgery and at subsequent follow-up, body weight was increased in OVX + Veh rats and decreased in OVX + high-E2 rats compared with ovary-intact rats (Table 1). The increase in body weight after ovariectomy and the decrease with E2 treatment is well documented (10). MI did not affect body weight.

Uterus weight. In OVX + Veh rats, uterus wet weights were about one-third of those in ovary-intact rats (Table 1). In OVX + high-E2 rats, uterine weights were approximately three times those of ovary-intact rats. MI had no effect on uterus weight.

Infarct size. Infarct size was calculated first for all rats with small (<30%) and large (>30%) infarcts. For all infarcts combined, E2 status did not affect infarct size: 41 ± 2, 39 ± 2, and 45 ± 3% of LV for ovary-intact, OVX + Veh, and OVX + high-E2 rats, respectively. When rats with small infarcts were excluded, again E2 status had no effect: 42 ± 2, 42 ± 2, and 47 ± 3% of LV ovary-intact, OVX + Veh, and OVX + high-E2 rats, respectively. Rats with small infarcts were excluded from the main analysis. Three ovary-intact, eight OVX + Veh, and four OVX + high-E2 MI rats were excluded (P = 0.2, by χ2 test).

LV and RV weights. In sham and MI rats, LV wet weight was significantly lower in OVX + high-E2 rats than in the other two groups (Table 1). This trend was reversed when LV weights were indexed for body weight. Compared with the sham groups, MI groups showed modest increases in LV weight that were significant only in OVX + Veh rats. In the sham groups, RV wet weight was significantly lower in OVX + high-E2 than in ovary-intact and OVX + Veh rats, but this difference was not significant when RV weight was indexed for body weight. At 3 wk after MI, ovary-intact rats exhibited a significant increase in RV weight-to-body weight ratio, whereas increases were not significant in OVX + Veh and OVX + high-E2 rats.

LV function by echocardiography. E2 status had no significant effect on IVS and PW thicknesses, which were similar in sham and MI groups (Table 2). LV dimensions in diastole and systole were decreased in OVX + high-E2 sham rats, reflecting lower body weights in OVX + high-E2 than in OVX + Veh sham rats. LV dimensions increased significantly, particularly in systole, after MI in all three MI groups, and EF was decreased in all MI rats and to a similar extent across MI groups (Table 2).

LV function by Millar catheter. In sham rats, LVEDP, LVSP, +dP/dt, and −dP/dt were not affected by E2 status (Table 3). Heart rate tended (P = 0.07) to be lower in OVX + high-E2 rats than in the other groups. LVEDP and −dP/dt were similarly increased, and LVSP and +dP/dt were similarly decreased in all three MI groups. None of the parameters of LV function differed significantly across MI groups.

Circulatory RAS. Compared with ovary-intact sham rats, PRA, plasma ANG I, and plasma ANG II decreased in OVX + Veh rats and decreased markedly in OVX + high-E2 sham rats (Fig. 1). Only minor differences were noted in plasma ACE activity.

In ovary-intact and OVX + Veh MI rats, PRA, ANG I, ACE activity, and ANG II in plasma were unchanged compared with their respective sham groups. In contrast, in OVX + high-E2 MI rats, PRA was increased by 140%, ANG I concentration by 110%, and plasma ANG II concentration by 120% compared with OVX + high-E2 sham rats. Across the three MI groups, PRA and ANG I were lower in OVX + Veh than in ovary-intact and OVX + high-E2 rats. ANG II levels were similar across MI groups (Fig. 1).

Cardiac ACE. OVX + Veh sham rats exhibited a 100% increase in LV ACE mRNA and a 35% increase in LV ACE activity compared with ovary-intact and OVX + high-E2 sham rats (Fig. 2). In all MI groups, LV ACE mRNA abundance and

Table 1. Body, uterus, and heart weights of female rats 3 wk after MI

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>OVX + Veh</th>
<th>OVX + High E2</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>MI</td>
<td>Sham</td>
</tr>
<tr>
<td></td>
<td>(n = 16)</td>
<td>(n = 27)</td>
<td>(n = 16)</td>
</tr>
<tr>
<td>Body wt, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At OVX</td>
<td>237 ± 3</td>
<td>245 ± 3</td>
<td>239 ± 4</td>
</tr>
<tr>
<td>At MI</td>
<td>271 ± 4</td>
<td>267 ± 3</td>
<td>303 ± 5††</td>
</tr>
<tr>
<td>At echo</td>
<td>282 ± 6</td>
<td>285 ± 3</td>
<td>347 ± 6††</td>
</tr>
<tr>
<td>Final</td>
<td>294 ± 4</td>
<td>292 ± 2</td>
<td>359 ± 7††</td>
</tr>
<tr>
<td>Uterus wt, g</td>
<td>0.6 ± 0.03</td>
<td>0.7 ± 0.04</td>
<td>0.2 ± 0.02†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>OVX + Veh</th>
<th>OVX + High E2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>MI</td>
<td>Sham</td>
</tr>
<tr>
<td></td>
<td>(n = 6)</td>
<td>(n = 9)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>RV wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute, g</td>
<td>133 ± 3†</td>
<td>217 ± 26*</td>
<td>160 ± 8</td>
</tr>
<tr>
<td>Relative, mg/100 g body wt</td>
<td>46 ± 2</td>
<td>75 ± 9*</td>
<td>44 ± 2</td>
</tr>
<tr>
<td>LV wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute, g</td>
<td>628 ± 21</td>
<td>643 ± 31</td>
<td>702 ± 20</td>
</tr>
<tr>
<td>Relative, mg/100 g body wt</td>
<td>217 ± 5</td>
<td>220 ± 12</td>
<td>194 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats; OVX, ovariectomized; E2, 17β-estradiol; Veh, vehicle; MI, myocardial infarction; LV and RV, left and right ventricle; echo, echocardiography. *P < 0.05 vs. respective sham group. †P < 0.05 vs. respective intact group. ‡P < 0.05 vs. respective OVX + Veh group. Comparisons made by 2-way ANOVA.
ACE activity increased severalfold with increasing proximity to the infarct scar (Fig. 2). These increases were similar across all MI groups but tended to be the highest in the OVX + high-E2 MI rats.

Cardiac ANG peptides. LV ANG I and ANG II concentrations were similar in the three groups of sham rats (Fig. 3). ANG I and ANG II levels were not different in the remote LV of the MI groups compared with the respective sham groups. ANG I was increased by 100–200% in the combined peri-infarct-infarct area of each of the MI groups; the absolute levels of ANG I were not significantly different across MI groups. In ovary-intact MI rats, ANG II levels in the combined peri-infarct-infarct area were unchanged compared with the LVs of ovary-intact sham rats. In contrast, ANG II in the peri-infarct-infarct area was increased by 240% in OVX + Veh MI rats and by 140% in OVX + high-E2 MI rats compared with ANG II in the LV of the respective animals. The absolute ANG II levels in the peri-infarct-infarct area of OVX + Veh and OVX + high-E2 MI rats were similar but were increased compared with those of ovary-intact MI rats (Fig. 3).

Brain ACE. Compared with ovary-intact sham rats, OVX + Veh sham rats exhibited modest (<15%) increases in ACE densities in the subformical organ and median preoptic nucleus, and OVX + high-E2 rats exhibited significant decreases in ACE densities in all four brain nuclei studied (Table 4, Fig. 4).

Compared with their respective sham groups, OVX + high-E2 MI rats showed clear increases in ACE densities in all four brain nuclei, ovary-intact MI rats showed modest (<10%) increases in ACE densities in the paraventricular nucleus and median preoptic nucleus, and OVX + Veh MI rats showed only minor, nonsignificant increases. However, across the three MI groups, actual ACE densities in OVX + Veh MI rats were still ~10% higher than in ovary-intact MI rats and 10–15% higher than in OVX + high-E2 MI rats.

Brain AT1R. Compared with ovary-intact sham rats, OVX + Veh sham rats exhibited significant (<20%) increases in AT1R densities in all four brain nuclei and OVX + high-E2 sham rats exhibited significant decreases (Table 4, Fig. 4). Compared with their respective sham groups, OVX + high-E2 MI rats showed clear increases in AT1R densities in the organum vasculosum of the lamina terminalis, subformical organ, and paraventricular nucleus, whereas the ovary-intact and OVX + Veh MI rats showed modest (~10%) increases in some of the nuclei (Table 4). However, across the three MI groups, actual AT1R densities in all nuclei were still significantly higher (10–20%) in OVX + Veh rats than in ovary-intact and OVX + high-E2 rats.

**DISCUSSION**

The present study demonstrates that, in female OVX rats, E2 actually increases the activity of the circulatory RAS, amplifies increases in ACE and AT1R densities in brain nuclei, and does not inhibit activation of the cardiac RAS in MI vs. sham rats.

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**Table 2. Echocardiographic measurements in female rats 3 wk after MI**

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 13)</th>
<th>MI (n = 23)</th>
<th>Sham (n = 16)</th>
<th>MI (n = 21)</th>
<th>Sham (n = 12)</th>
<th>MI (n = 16)</th>
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<tbody>
<tr>
<td>Infarct size, % of LV</td>
<td></td>
<td>41 ± 2</td>
<td></td>
<td>43 ± 2</td>
<td></td>
<td>47 ± 3</td>
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<tr>
<td>IVSs, mm</td>
<td>2.2 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>3.5 ± 0.1</td>
<td>3.4 ± 0.1</td>
<td>3.3 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>PWd, mm</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>PWd,m, mm</td>
<td>3.3 ± 0.2</td>
<td>3.5 ± 0.1</td>
<td>3.1 ± 0.2</td>
<td>3.0 ± 0.1</td>
<td>3.1 ± 0.2</td>
<td>2.7 ± 0.1</td>
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<tr>
<td>LVIDd, mm</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Absolute, mm</td>
<td>5.5 ± 0.3</td>
<td>8.0 ± 0.3*</td>
<td>6.5 ± 0.2</td>
<td>7.7 ± 0.3*</td>
<td>5.4 ± 0.2†</td>
<td>7.4 ± 0.3*</td>
</tr>
<tr>
<td>Relative, mm/100 g body wt</td>
<td>1.9 ± 0.1</td>
<td>2.8 ± 0.1*§</td>
<td>1.9 ± 0.1</td>
<td>2.2 ± 0.1*§</td>
<td>2.5 ± 0.1‡</td>
<td>3.3 ± 0.2*</td>
</tr>
<tr>
<td>LVIDd, Absolute, mm</td>
<td>2.7 ± 0.3</td>
<td>5.4 ± 0.4*</td>
<td>3.0 ± 0.1</td>
<td>5.2 ± 0.5*</td>
<td>2.4 ± 0.3†</td>
<td>4.9 ± 0.4*</td>
</tr>
<tr>
<td>PWd,m, Relative, mm/100 g body wt</td>
<td>1.0 ± 0.1</td>
<td>1.9 ± 0.1*</td>
<td>0.9 ± 0.1</td>
<td>1.5 ± 0.1*§</td>
<td>1.1 ± 0.1</td>
<td>2.2 ± 0.2*</td>
</tr>
<tr>
<td>EF, %</td>
<td>88 ± 2</td>
<td>67 ± 4*</td>
<td>89 ± 1</td>
<td>67 ± 4*</td>
<td>90 ± 3</td>
<td>69 ± 5*</td>
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</table>

Values are means ± SE. Infarct size was measured from a subset of 1 of 23 intact rats; all others had “large” or “medium” infarcts by visual inspection. LV, left ventricle; IVS, interventricular septal thickness; PW, posterior wall thickness; LVID, LV internal dimension; EF, ejection fraction; s, systole; d, diastole. *P < 0.05 vs. respective sham group. †P < 0.05 vs. OVX + Veh sham group. ‡P < 0.05 vs. other sham groups. §P < 0.05 vs. other MI groups.

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**Table 3. LV function measured by Millar catheter in female rats 3 wk after MI**

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 8)</th>
<th>MI (n = 20)</th>
<th>Sham (n = 3)</th>
<th>MI (n = 7)</th>
<th>Sham (n = 3)</th>
<th>MI (n = 6)</th>
</tr>
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<tbody>
<tr>
<td>Infarct size, % of LV</td>
<td></td>
<td>41 ± 3</td>
<td></td>
<td>41 ± 3</td>
<td></td>
<td>41 ± 3</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>3 ± 2</td>
<td>16 ± 3*</td>
<td>5 ± 3</td>
<td>12 ± 2*</td>
<td>5 ± 1</td>
<td>13 ± 2*</td>
</tr>
<tr>
<td>LVSPSP, mmHg</td>
<td>129 ± 4</td>
<td>117 ± 3*</td>
<td>147 ± 12</td>
<td>132 ± 5</td>
<td>151 ± 7</td>
<td>130 ± 4*</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>8,284 ± 322</td>
<td>6,609 ± 234*</td>
<td>8,782 ± 531</td>
<td>7,309 ± 267*</td>
<td>8,311 ± 299</td>
<td>7,014 ± 213*</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>−7,663 ± 410</td>
<td>−5,683 ± 256*</td>
<td>−8,428 ± 664</td>
<td>−6,313 ± 324*</td>
<td>−8,057 ± 456</td>
<td>−5,661 ± 294*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>387 ± 10</td>
<td>374 ± 7</td>
<td>372 ± 18</td>
<td>348 ± 16</td>
<td>327 ± 23</td>
<td>317 ± 17</td>
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</table>

Values are means ± SE. Infarct size was measured from 13 of 20 intact rats; all others had “large” or “medium” infarcts by visual inspection. LVEDP, LV end-diastolic pressure; LVSPSP, LV peak systolic pressure; +dP/dt and −dP/dt, positive and negative first derivatives of pressure. *P < 0.05 vs. respective sham group (by 2-way ANOVA).
in contrast to the inhibition of the RAS by E2 in sham rats. As a result, post-MI levels of various components of the RAS showed only minor differences by E2 status. E2 status also did not affect the extent of LV dysfunction in the early post-MI phase.

Circulatory RAS. The changes in the circulatory RAS induced by ovariectomy and E2 in sham rats are consistent with those reported in previous studies of the effects of estrogen treatment on components of the RAS in OVX rats (1, 2, 21, 25). Together, these studies suggest that ovariectomy-induced E2 deficiency tends to decrease the activity of several components of the circulatory RAS and that high-E2 treatment decreases them further. E2 deficiency and E2 excess appear therefore to decrease plasma ANG I and ANG II levels, which may be a result of the opposing regulation of angiotensinogen (increased) vs. renin (decreased) by E2 (6).

**Fig. 1.** Components of the circulatory renin-angiotensin system (RAS) in female rats 3 wk after myocardial infarction (MI). Values are means ± SE; n = 5–6 in sham groups, n = 11–15 in ovary-intact MI group, n = 9–10 in ovary + vehicle (Veh) MI group, and n = 6–7 in ovariectomized (OVX) + high-17β-estradiol (E2) MI group for renin activity, ANG I, and ANG II and n = 9–24 for angiotensin-converting enzyme (ACE) activity. *P < 0.05 vs. respective sham group. †P < 0.05 vs. ovary-intact sham rats. ‡P < 0.05 vs. other MI groups. §P < 0.05 vs. sham OVX + Veh rats.

**Fig. 2.** ACE mRNA abundance and activity in left ventricle (LV) of female rats 3 wk after MI. rLV, remote LV; p-inf, peri-infarct area; inf, infarct area. Values are means ± SE of number of rats in parentheses. Lower number of rats in some groups is due to sample loss. *P < 0.05 vs. respective sham group. †P < 0.05 vs. respective ovary-intact group.
Our finding that components of the circulatory RAS in
erve-intact and OVX rats 3 wk after MI (5, 15, 23). In contrast, in OVX 
from those in the sham groups is similar to previous findings in 
high-E2 rats is due to sample loss. *P < 0.05 vs. respective sham group. †P < 
0.05 vs. respective other MI groups (2-way ANOVA).

Cardiac RAS. The three sham groups showed similar steady-
state concentrations of LV ANG I and ANG II, whereas LV 
ACE mRNA and activity were increased in OVX + Veh rats. The latter finding is consistent with previous studies (4, 7). In other studies (26, 32), an increase in LV ACE activity was also not 
associated with higher LV ANG II levels, and it appears that 
ACE is not necessarily rate limiting for the production of 
ANG II in the LV.

At 3 wk after MI, in all three groups of MI rats, LV ACE 
mRNA abundance and ACE activity were increased with 
proximity to the infarct scar to a maximum of five- to eightfold 
within the scar itself compared with the respective sham 
values. This upregulation of LV ACE after MI is consistent 
that reported previously (12, 24). Despite suppression of 
LV ACE mRNA and ACE activity in OVX + high-E2 vs. 
OVX + Veh sham rats (present study. Ref. 4), the increases in 
LV ACE mRNA and ACE activity after MI were not attenu-
ated in OVX + high-E2 rats and, if anything, were enhanced (Fig. 1). In the infarct-free LV 3 wk after MI, all three MI 
groups showed no significant changes in ANG I and ANG II 
concentrations, consistent with previous findings in male rats 
after MI. In contrast, in the infarct area, all three groups 
showed clear increases in ANG I levels, but ANG II levels 
were increased only in OVX + Veh and OVX + high-E2 rats, 
and not in ovary-intact rats. An explanation for these divergent 
changes and possible implications for scar formation is not 
readily apparent.

Brain RAS. In sham rats, the absence of E2 vs. supraphysi-
ological levels of E2 resulted in the expected (4, 11) up- 
and downregulation of ACE and AT1R densities in brain nuclei. 
Confirming our findings in male rats (24), at 3 wk after MI, 
ACE and AT1R densities were increased in several brain nuclei 
of female rats. The extent of these increases was opposite to the 
effect of E2 in sham rats: i.e., the largest increase occurred in 
the OVX + high-E2 group and the smallest in the OVX + Veh group. However, because of higher densities in the brain nuclei 
of OVX + Veh sham rats, densities were still 10–20% higher 
in OVX + Veh MI rats than in the other two MI groups. Other 
components of the brain RAS need to be measured, and 
functional studies are needed with, for example, central infu-
sions of an AT1R antagonist to assess whether E2 status influences the overall activity of the brain RAS and, thereby, sympathetic activity after MI. Such differences may be subtle, and more chronic follow-up may be required for these to impact on LV dysfunction.

RAS regulation and LV dysfunction after MI. At 3 wk after MI, infarct sizes, increases in LVEDP and LV dimensions, and decreases in LVPSP and EF were similar in all three E2-treated groups. LV dysfunction was therefore similar across MI groups. Changes in the circulating levels of E2 appear therefore not to affect infarct size or global LV dysfunction in female rats in the early post-MI phase. No previous study evaluated the impact of E2 status on LV function in the early post-MI phase. One study in rats examined the effects of E2 status on changes in in vivo LV function in the more chronic post-MI phase. Nekooiean and Pang (18) found that, at 7 wk after MI, infarct sizes were similar in OVX rats treated with E2 at physiological levels and in OVX rats treated with vehicle. The increase in LVEDP was 3 mmHg less after MI in OVX/E2 rats than in OVX/Veh rats, but LVPSP and +dP/dt were similarly decreased in both groups. Smith and colleagues (22) reported larger infarct sizes 10–11 wk after MI in OVX rats treated with regular E2 than in OVX rats treated with vehicle (42 vs. 27%), but only the latter showed a significant (2-fold) increase in LV cavity area. In vivo LV function was not measured, but MI and E2 status did not affect mean arterial pressure and LV function in vitro. These divergent findings are difficult to reconcile, and further studies on the possible impact of E2 status on cardiac remodeling and dysfunction during the more chronic post-MI phase are needed.

The absence of differences in the degree of LV dysfunction after MI in the present study may be consistent with the overall lack of effect of E2 status on the activity of the circulatory and cardiac RASs and minor effects on the brain RAS after MI. Although in sham rats E2 clearly downregulates all three RASs, after MI all three RASs are actually activated the most in rats on high E2, resulting in fairly similar RAS activity, irrespective of E2 status. Our study does not provide insights into how this relative activation of the circulatory, cardiac, and brain RAS occurs in OVX + high E2 vs. OVX + Veh rats after MI. This paradoxical response clearly requires further study.

Perspectives

Women exhibit lower circulating E2 levels after menopause or surgical ovariectomy, but no study has assessed whether components of the tissue RASs are regulated by E2 status in women, nor has the possible impact after MI been studied. This study demonstrates that in female rats the negative regulation of tissue ACE and AT1R in the heart and brain by E2 under normal physiological conditions does not lessen the activation of ACE and AT1R that occurs after MI and is not associated with attenuation of the development of LV dysfunction, at least in the early post-MI phase. This finding highlights the need to study regulation of local tissue RASs by E2 not only in otherwise healthy female subjects, but also in the presence of pathology, such as heart failure, hypertension, atherosclerosis, or diabetic nephropathy, where the activity of local RASs plays a role.

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