Endothelial Estrogen Receptor α Plays an Essential Role in the Coronary and Myocardial Protective Effects of Estradiol in Ischemia/Reperfusion

Julie Favre, Ji Gao, Jean-Paul Henry, Isabelle Remy-Jouet, Isabelle Fourquaux, Audrey Billon-Gales, Christian Thuilliez, Jean-François Arnal, Françoise Lenfant, Vincent Richard

Objective—To assess the coronary endothelial protective effects of 17β-estradiol (E2) and the role of estrogen receptor (ER) α in ischemia/reperfusion (I/R).

Methods and Results—E2 exerts protective effects in cardiac I/R. However, the implication in vivo of the endothelium and the cellular targets of the anti-ischemic effects of E2 are unknown. Mice were subjected to I/R (30 minutes of I and 1 hour of R) in vivo, after which acetylcholine-induced relaxation of isolated coronary segments was assessed ex vivo. I/R induced a coronary endothelial dysfunction in untreated ovariectomized mice that was prevented by long-term treatment with E2 in wild-type, but not in ERα−/−, mice. Chimeric mice inactivated for ERα in the hematopoietic compartment remained protected by E2. Further inactivation of endothelial ERα abolished the protective action of E2 on coronary endothelial function in Tie2-Cre(+) ERα0/0 mice. More importantly, E2 significantly limited infarct size in wild-type mice but not in mice deficient in endothelial ERα, even in the presence of hematopoietic ERα.

Conclusion—Endothelial ERα plays a crucial role in the E2-induced prevention of endothelial dysfunction after I/R. To our knowledge, we demonstrate for the first time, by using unique genetically modified mice, that targeting endothelial protection per se can confer cardiomyocyte protection in I/R. (Arterioscler Thromb Vasc Biol. 2010;30:2562-2567.)

Key Words: endothelium ▪ immune system ▪ ischemia ▪ estradiol ▪ estrogen receptor α

Estrogens favor several protective actions in cardiovascular diseases, including prevention of atheroma and the severity of its ischemic complications, in particular in brain and myocardial tissues. Cardiac ischemia/reperfusion (I/R) elicits injuries to the myocardium and the endothelium, leading to the development of an early coronary endothelial dysfunction. Considering the widely studied beneficial effects of 17β-estradiol (E2) on postischemic myocardial function,1–3 our first aim was to assess whether long-term in vivo E2 treatment exerts coronary endothelial protective effects during I/R in a previously developed murine model.4

Two nuclear receptors, estrogen receptor (ER) α and β, mediate the actions of E2; however, only ERα is necessary and sufficient to mediate most of the vasculoprotective effects of E2, such as the increase in basal NO production.5 Researchers have extensively documented the protective role of ERα in various situations, such as the acceleration of reendothelialization,6 atheroma,7,8 and the prevention of ischemia-induced skin necrosis.9 Thus, our second aim was to determine whether ERα mediates the coronary protective effect of E2. Despite the fact that ERβ took part in the E2 protection of ischemic myocardium,3,10 we chose to focus our study on ERα, considering its major role in endothelium.

Although the endothelium represents a major target for the cardiovascular protective effects of E2,11 ERs are also present in other cell types involved in response to I/R injury (eg, inflammatory cells, smooth muscle cells, and cardiomyocytes).12 Thus, the exact role of endothelial ERα in cardiac I/R is still unknown. Our third aim was to assess the cellular target of the E2-mediated coronary protection in I/R and especially the role of the endothelium. Finally, I/R-induced endothelial dysfunction and the corresponding altered NO production are known to favor neutrophil adhesion and platelet aggregation. The resulting vicious circle may play a central role in I/R-induced injury, by reinforcing endothelial dysfunction and aggravating myocardial injury, which leads to an increased infarct size.13 However, to the best of our knowledge, whether the targeting of endothelial protection does contribute to a modulation of myocardial injury has not been reported. Thus, our final aim was to determine whether selective abolition of E2-mediated endothelial effects influences E2-mediated myocyte protection (ie, infarct size).

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Methods

Experimental Animals

Female mice were ovariectomized (Ovx) or were left intact (UnOvx) at the age of 5 weeks to suppress endogenous production of estrogens. After at least 4 weeks of estrogen deprivation, Ovx mice either were or were not implanted with pellets releasing E2 (0.1 mg; 60-day release) 2 weeks before the induction of I/R. Thus, I/R was performed in mice at the age of 11 to 14 weeks.

Experiments were performed as follows: (1) in normal C57BL/6J mice (Janvier, Laval, France), (2) in mice with a global deficiency for the ERα (ERα knockout[E2−/−]), and (3) in mice with an ERα deficiency selectively targeted to the endothelium (together with hematopoietic cells) (Tie2Cre(+/−)ERαf/f). The latter mice were compared with their littermate wild-type (WT) controls (Tie2Cre(−/−)ERα) with a C57BL/6J background.

Murine Model of Coronary Endothelial Dysfunction After Cardiac I/R

The techniques for the induction of I/R, the evaluation of hemodynamic parameters, coronary endothelial function, infarct size, and myocardial production of reactive oxygen species (ROS) were previously described. Additional information is available in the supplemental data (available online at http://atvb.ahajournals.org).

Generation of Bone Marrow Chimeric Mice

To determine the cellular localization of ERα in E2-mediated endothelial protection, we developed bone marrow chimeric mice, as previously described. Briefly, 2 weeks after ovariectomy, WT mice were sublethally irradiated (9 Gy) and injected intravenously the next day with 107 bone marrow cells from the indicated donor mice (WT or ERα−/−). Six weeks after grafting, Ovx mice, either treated or not treated with E2, were subjected to I/R; and an evaluation of endothelial function was performed, as described in the supplemental data online. In agreement with previous data, we verified, in the present experiments, that bone marrow WT cells represented >90% of the hematopoietic population of chimeric mice 5 weeks after transplantation and that ERα mRNA abundance in the bone marrow of these mice, reconstituted with WT bone marrow, was not distinguishable from that of normal WT mice.

Transmission Electron Microscopy

Ultrastuctural changes induced by I/R were analyzed on isolated coronary arteries extracted from Ovx mice subjected to sham surgery or I/R, either treated or not treated with E2. Briefly, the heart was fixed in situ with left ventricle perfusion of formalin for 5 minutes. After heart excision, the left coronary artery was dissected out and fixed in 2% glutaraldehyde in 0.1 mol/L So¨rensen phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated in graded ethanol series, and embedded in resin (Epox 812–Araldite 502). Ultrathin sections were then cut (Reichert Ultracutmicro), mounted onto colloidin-coated copper grids, and poststained with uranyl acetate and lead citrate before being examined on an electron microscope.

Statistical Analysis

Data are expressed as mean±SEM. In vitro experiments, n represents the number of animals from which the arteries were taken. Relaxing responses were compared using 2-way ANOVA, followed by a Bonferroni posttest or a 2-way repeated-measures ANOVA. A 1-way ANOVA, followed by a Bonferroni posttest, was also performed. Differences were considered statistically significant at P<0.05.

Results

Uterine Weights and Hemodynamic Parameters

Uterine weights, used as bioassay for the action of estrogens, are shown in supplemental Table I. As expected, ovariectomy elicited uterine atrophy, whereas both endogenous and exogenous E2 induced a large increase in uterine weight.

![Figure 1. Rate pressure product (RPP) during I/R in UnOvx or Ovx female mice, either untreated or treated with E2 for 2 weeks. Data are given as mean±SEM. *P<0.05 for Ovx+E2 vs Ovx, and $P<0.05 for Ovx+E2 vs UnOvx.](http://atvb.ahajournals.org/)

Arterial blood pressure and heart rate were followed continuously during I/R. No significant differences in these hemodynamic parameters were observed between each group of mice before and during I/R. Figure 1 shows that heart rate (HR)–arterial blood pressure (MAP) product, an index of myocardial oxygen demand, was significantly elevated during the first minutes of ischemia in E2-treated Ovx mice compared with untreated Ovx mice and UnOvx.

Coronary Vascular Function

Vascular Diameters and Contractile Responses

Supplemental Table II shows no significant differences in either normalized coronary internal diameters or precontraction levels in response to serotonin (10−5 mol/L) in each group of mice.

Influence of Ovariectomy or E2 on Postischemic Coronary Endothelial Function

In sham-operated (control) female mice, the coronary relaxing responses to increasing concentrations of acetylcholine were not significantly affected by Ovx or Ovx+E2 compared with UnOvx (Figure 2A). In all 3 groups, these relaxing responses were abolished after in vitro NO synthase inhibition by N(G)-nitro-L-arginine.

Compared with control, I/R significantly altered relaxations to acetylcholine in Ovx mice (maximal relaxation for sham versus I/R, 75±4% versus 60±6%; P<0.05) (Figure 2, top). In contrast, this alteration was absent in UnOvx (sham versus I/R, 84±6% versus 75±6%) and was prevented by long-term E2 treatment in Ovx mice (sham versus I/R, 82±5% versus 84±5%). These coronary relaxations after I/R were abolished by in vitro incubation with N(G)-nitro-L-arginine, suggesting that E2 improves endothelial function by restoring NO production in I/R (Figure 2, middle).
The endothelium-independent relaxing responses to the NO donor sodium nitroprusside (Figure 2, bottom) did not differ between groups, showing that I/R or E2 treatment did not modify smooth muscle responsiveness to NO.

Ultrastructural Analysis

Compared with sham-operated coronary arteries, which displayed normal height and well-formed intercellular junctions (Figure 3A), I/R coronary arteries showed endothelial cells with electron-dense chromatin, nucleus condensation, and clear perinuclear cytoplasm (Figure 3B). Coronary endothelial desquamation (arrow), resulting in endothelium denudation (arrowhead) after I/R in Ovx mice, was also occasionally observed (Figure 3C). These I/R-induced ultrastructural alterations were absent in E2-treated mice (Figure 3D).

Implication of ERα in E2-Mediated Coronary Endothelial Function Protection

In contrast to WT mice (Figure 2), Figure 4A shows that long-term E2 treatment failed to improve relaxing responses to acetylcholine in coronary arteries isolated from ERα−/− mice subjected to I/R, demonstrating the key role of ERα in this protective action of E2.

We then sought to approach the cellular target(s) of E2. ERα is expressed in various cardiac cells and in circulating immune cells, which are known to contribute to I/R-induced coronary endothelial injury. Thus, we first evaluated the role of hematopoietic ERα by assessing the coronary protective action of E2 in chimeric mice, consisting in irradiated WT mice grafted with either WT or ERα−/− bone marrow (Figure 4B). E2 similarly improved coronary relaxation after I/R in mice deficient or not deficient in hematopoietic ERα. Therefore, ERα expression in hematopoietic cells and thereby in immune cells does not appear to be required for this protective action of E2.

We then sought to explore the role of endothelial ERα using Tie2Cre(+) ERα mice, which were previously demonstrated to be selectively deficient in both endothelial and hematopoietic ERα. Because hematopoietic ERα does not contribute to the E2 protective action, this mouse model appeared suitable to assess the specific role of endothelial ERα. The coronary endothelial protective effect of E2 in I/R was preserved in Tie2Cre(−) mice (WT controls) (Ovx versus Ovx+E2, 67±10% versus 88±6%; P<0.05) but was abolished in mice with endothelial ERα deficiency (ie, Tie2Cre[+] mice) (Ovx versus Ovx+E2, 66±9% versus 55±10%; P=0.44) (Figure 4C). As a result, a marked alteration in postischemic coronary relaxations was observed in E2-treated Tie2Cre(+) mice compared with their littermate controls (Tie2Cre[−]: 90±6% (P<0.05) versus Tie2Cre[+]; P<0.05). This demonstrates that endothelial ERα is necessary for the protective effects of E2.

Infarct Size

Figure 5 shows that ovariectomy was associated with a significant increase in infarct size after 1-hour reperfusion...
Cardiac Production of ROS

ROS were measured by electron paramagnetic resonance after 2-minute reperfusion in the previously ischemic myocardial tissue. Compared with untreated Ovx, E2 significantly decreased ROS generation (mM CM spin probe/mg protein: Ovx: 4.99±0.29 n=6; Ovx+E2 3.85±0.26 n=7; P<0.05).

Discussion

The protective action of estrogens against ischemia-induced damages was previously demonstrated in several animal models and included various tissues, such as brain, skin, and myocardial protection. Relaxing responses to acetylcholine in arteries isolated from mice subjected to I/R Ovx and treated (closed symbols) or not (open symbols) for a long time with E2. A, ERα-deficient mice (ERα−/−). B, Chimeric WT mice 6 weeks after irradiation and bone marrow grafting with WT (controls; top) or ERα−/− bone marrow (bottom). C, Tie2Cre(−) ERα−/− (controls; top) or Tie2Cre(+) ERα+/+ endothelial and hematopoietic deficient mice (bottom). Data are given as mean±SEM. *P<0.05 and **P<0.01 by repeated-measures ANOVA.

um,1,9,18 In particular, the protective action of E2 in cardiac I/R is largely demonstrated, but the cellular targets involved in this protection were still undefined. The present data demonstrated that the long-term activation of the endothelial ERα by E2 elicits both coronary endothelial and myocardial protective effects after cardiac I/R, and this does not require the concomitant activation of ERα expression in the hematopoietic compartment.

In the present study, I/R elicits endothelial structural injuries, including desquamation and necrosis, that are associated with altered coronary endothelial NO production in untreated Ovx mice. In contrast, after a 2-week treatment with E2, I/R was ineffective in affecting the endothelial layer, as underlined by preserved endothelial morphological features associated with maintained NO-mediated coronary endothelial relaxations. More important, this endothelial protective effect could also be detected with endogenous E2 because I/R did not induce any detectable endothelial dysfunction in intact female mice, in contrast to Ovx female mice, and to male mice.4 Thus, these effects represent another facet of the many endothelial protective aspects recently reviewed,11 including both endogenous and exogenous E2.

The protective effects of E2 may have resulted in part from an action on the immune system. Indeed, most of the previous studies19,20 reported a specific postischemic anti-inflammatory action on neutrophils after short-term E2 treatment. However, growing evidence suggests that the short-term effect of E2 could not be predictive of its long-term action. For instance, researchers21 showed that short-term in vitro treatment of macrophages by E2 led to an anti-inflammatory effect; however, in contrast, we observed an increased produc-
tiation of several proinflammatory cytokines by macrophages obtained from mice treated with E2 for a long time compared with those from Ovx mice. Altogether, these striking discrepancies illustrate the importance of the in vivo approach for understanding the pathophysiological effects of E2.

A prominent role of ERα was previously reported in several studies addressing estrogen-induced cardioprotection after I/R. In particular, ERα deficiency worsens global I/R-induced alteration in coronary flow and cardiac NO release in male mice; it also abolishes the endogenous cardiac protection displayed in female intact mice. We further demonstrate that global ERα deficiency in Ovx ERα−/− mice suppressed the coronary protective effect of E2 after I/R. Nevertheless, the E2-induced protection persisted despite the selective inactivation of ERα in hematopoietic cells because postischemic coronary relaxations of mice grafted with ERα−/− bone marrow remained protected by E2. However, because of the numerous and complex actions of estrogens on hematopoietic-derived cell populations, as immune cells, endothelial progenitor cells (EPCs), and platelets, we cannot exclude that various and potentially opposite actions of E2 are at work during the I/R injury and that these actions were not investigated in the present study; we suggest that the cellular target that accounts for the coronary protective action of E2 is not a radiosensitive bone marrow component.

The Tie2Cre mouse is known to be an effective approach for the extinction of genes in the endothelium. However, although commonly used, this approach has the limitation that it is also associated with gene extinction in hematopoietic cells; this is also observed when vascular endothelial (VE)-cadherin promoter is used to target the Cre recombinase to the endothelium. Although this problem is best addressed by performing experiments in chimeric mice in which bone marrow cells from Tie2Cre(−) ERα mice are transferred to Tie2Cre(+) ERα, as previously performed in other situations, we considered that these experiments were beyond the scope of the present study, especially because we demonstrated that hematopoietic ERα is not necessary for the protective action of E2. Based on this result, we can assume that the effects observed in Tie2Cre(+) ERα reflect inactivation of this gene selectively in the endothelium. Thus, the observation that the coronary protective effect of E2 is lost in Tie2Cre(+) ERα mice compared with their WT littermates (Tie2Cre[−]) demonstrates that endothelial ERα is required to prevent I/R-induced coronary endothelial dysfunction.

Altogether, the endothelium and, in particular, the endothelial ERα appear to be key cellular and molecular targets of the protective actions of E2 against I/R-induced coronary endothelial dysfunction. The last important question we raised in the present study concerned the potential role of endothelial ERα in the protective action of E2 on cardiomyocyte injury and especially infarct size. In agreement with a previous study, we observed that ovariectomy induced an increase in myocardial infarct size, which was completely reversed by long-term treatment with exogenous E2. More important, we found that the infarct size-limiting effect of E2 was abolished in Tie2Cre(+) mice in parallel to the loss of coronary endothelial protection. Furthermore, the fact that this equal loss of E2-mediated cardio-protection was also observed when ERα expression was restored in the hematopoietic lineage in irradiated Tie2Cre(+) mice strongly suggests that part of the cardiomyocyte protection depends on activation of endothelial ERα.

These data support a sequence of events in which activation of endothelial ERα by E2 triggers a protective action on coronary endothelial structure and function, which, in turn, limits infarct size. Such a cross-talk mechanism, in which endothelial integrity appears as an essential actor in cardiac protection against infarction, has already been strongly suggested in several studies, but the lack of a model that allows specifically targeting endothelial protection precluded a direct demonstration. Although we have not directly addressed this aspect (especially because of the numerous nonspecific effects that would be obtained in the present model by using NO synthase inhibitors in vivo), it is likely that this endothelium-mediated cardioprotection involves preserved NO production by E2. This protection may be due, in part, to the reduced cardiac oxidative stress, demonstrated by the decreased production of ROS observed during early reperfusion. Based on our knowledge of the signaling mechanisms of cardioprotection, NO may then signal in cardiomyocytes via protein kinases and may possibly secondarily protect mitochondria, resulting in decreased cardiomyocyte death. However, other indirect effects of endothelial protection may also occur (eg, reduced neutrophil-mediated cardiomyocyte injury).

The observation on the obligatory role of the endothelium for cardiomyocyte protection may appear contradictory to previous work showing a direct protective action of E2 on hypoxia/reoxygenation-mediated death of isolated cardiomyocytes. Several reasons could account for this apparent discrepancy. First, the in vitro data used large amounts of E2 administered immediately; in these pharmacological doses, it is possible that direct effects not observed in our study are elicited on cardiomyocytes. Second, the mechanisms of reperfusion injury to cardiomyocytes in vivo markedly differ from those involved in vitro because the immediate inflammatory response associated with severe oxidative stress appears to be operative in vivo but not in vitro; this phenomenon centrally involves the endothelium as both a target and a trigger of the inflammatory response. Third, another important aspect of reperfusion injury is the no-reflow phenomenon that may worsen I/R injury and that is likely to be reduced by E2 in our conditions (secondary to endothelial protection).

Although both ERα and ERβ have been noted to mediate cardiac protection after I/R, we did not explore the role of ERβ in the present study. Indeed, both ERα and ERβ agonists improve postischemic myocardial recovery also, a beneficial cardioprotective role of ERβ activation has been largely demonstrated in cardiac I/R. Furthermore, different studies showed that the cardiac endogenous protection encountered in isolated perfused hearts from female mice was abolished in either ERα or ERβ knockout mice. Thus, although ERα plays a prominent protective role in our experimental settings, and more generally mediates most of the endothelial effects of E2, we cannot exclude a potential role of ERβ in E2-mediated endothelial protection against I/R injury.

In conclusion, our study shows that, in a context of I/R, in vivo endothelial ERα activation plays a prominent protective role, not only by preventing endothelial dysfunction but also by limiting infarct size. Thus, endothelial ERα, but more
Coronary Protection by Estrogens After Reperfusion


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Supplemental Material

Methods:

Murine Cardiac Ischemia-Reperfusion:

Mice were anesthetized by intraperitoneal injection of a mixture of ketamine (150 mg/kg) and xylazine (6 mg/kg). Animals were ventilated with a rodent microventilator (Minivent 885, Hugo Sachs Electronics, March, Germany) and the body temperature maintained by use of a 37°C warm plate. Ischemia was achieved by ligating the left anterior descending artery proximal to its origin, using an 8-0 prolene suture which was tied off during 30 minutes and then removed to allow reperfusion for an hour. Successful ischemia was confirmed by visual observation of cyanosis, while successful reperfusion was confirmed by visualizing reactive hyperemia.

In vitro Vascular Studies

Coronary endothelial function was assessed on the basis of vascular studies performed previously \(^1, 2\). At the end of reperfusion, the heart was removed and immediately placed in cold, oxygenated Krebs buffer. A 1 mm long segment of the coronary artery distal to the site of occlusion was carefully dissected and mounted in a small vessel myograph for isometric tension recording (JP Trading Aarhus, Denmark). For this purpose, the artery was threaded onto two 25 µm tungsten wires. Normalization procedure was performed after an equilibration period, as previously described \(^3\). After a 30 minute equilibration period, the vessels were contracted with 10\(^{-5}\)M serotonin (which does not induce any endothelium-dependent relaxing responses in this model) before applying increasing concentrations of acetylcholine (10\(^{-9}\) to 3x10\(^{-5}\)mol/L). Arteries were then washed and incubated with the NO synthase inhibitor N\(^\text{G}\)-Nitro L-Arginine (LNNA 10\(^{-4}\)mol/L for 30 minutes) to assess the contribution of NO to the relaxing responses to acetylcholine. At the end of the experiment, concentration-response curves in response to a NO donor, sodium nitroprusside (SNP; 10\(^{-9}\) to 10\(^{-5}\) mol/L), were performed in order to assess the endothelium-independent relaxations.
Hemodynamic Parameters:

In some experiments, the left carotid artery was catheterized in order to continuously monitor arterial blood pressure through a Millar 1.2F pressure transducer. Heart rate, as well as systolic, diastolic and mean arterial pressures were recorded and used to calculate the rate pressure product (RPP; Mean Arterial Pressure X Heart Rate) which is indicative of myocardial oxygen consumption. These parameters were assessed 1 min before ischemia, 1, 5 and 30 minutes during ischemia and 5, 40 and 60 minutes during reperfusion.

Assessment of Infarct Size:

After the I/R period, the suture was tied again and the ischemic area was assessed after infusing 2% Evans blue in a retroperfused manner to delineate the area at risk (AAR). The left ventricle was then removed, gently frozen in cold isopentane and sliced into 1 mm cross sections. The slices were then incubated for 20 minutes with 1% triphenyltetrazolium chloride (TTC) solution (Sigma-Aldrich) at 37°C. The infarcted area was determined according to its pale, white coloration compared to viable (red) myocardium and evaluated, as well as the area at risk, using computerized planimetry.

Cardiac Production of Reactive Oxygen Species

Reactive oxygen species (ROS) production was evaluated by electron paramagnetic resonance (EPR) spectroscopy. Briefly, hearts were removed after 2 min reperfusion and quickly washed in a physiological solution. The centre of the previously ischemic area (left ventricular free wall) was separated and incubated at 37°C for 60 minutes in Krebs-HEPES buffer containing 5 mmol/L Diethyl dithiocarbamate, 25 mmol/L deferoxamine and the spin probe 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethyl pyrrolidine hydrochloride (CMH, 500 µmol/L, Noxygen, Elzach, Germany). Spectra of the oxidized product of CMH (CM°) were recorded from frozen samples with a Miniscopie MS-200 (Magnetech) with the following acquisition parameters: Bo-field 3350 G; microwave frequency 9.78 GHz; microwave power 1 mW; modulation amplitude 5 G; sweep time, 120 s. Spectra intensity was measured from the height of the central line and expressed in µmol/L CM° produced for 60 minutes, normalized per milligram of protein.
References:


**Supplemental Tables:**

**Table I:** Uterine weight (mg) measured in WT intact unovariectomized (UnOvx) and Ovx mice, either untreated or treated for two weeks by E2 (+E2). Data are mean ±SEM.

<table>
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<th></th>
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<th>Uterine weight (mg)</th>
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<td>UnOvx WT</td>
<td></td>
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<tr>
<td>Sham</td>
<td>10</td>
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<tr>
<td>I/R</td>
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<td>Ovx WT</td>
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<td>I/R</td>
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<tr>
<td>Ovx+E2 WT</td>
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<td></td>
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<tr>
<td>Sham</td>
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<td>119.3 ± 11.4</td>
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<tr>
<td>I/R</td>
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<td>113.9 ± 5.6</td>
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**Table II:** Normalized internal diameters (μm) and levels of precontraction to serotonin (mN/mm) obtained before the administration of acetylcholine. Data are mean ±SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Diameter (μm)</th>
<th>Contraction (mN/mm)</th>
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<tr>
<td><strong>UnOvx WT</strong></td>
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<tr>
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<tr>
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<tr>
<td>Sham</td>
<td>17</td>
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<tr>
<td>I/R</td>
<td>17</td>
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<td>1.71 ± 0.11</td>
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<tr>
<td><strong>Ovx+E2 WT</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>191 ± 4</td>
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<td>1.41 ± 0.19</td>
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