Distinct Roles of Estrogen Receptors α and β Mediating Acute Vasodilation of Epicardial Coronary Arteries

Tobias Traupe, Christoph D. Stettler, Huige Li, Elvira Haas, Indranil Bhattacharya, Roberta Minotti, Matthias Barton

Abstract—This study investigated the contribution of estrogen receptors (ERs) α and β for epicardial coronary artery function, vascular NO bioactivity, and superoxide (O$_2^-$) formation. Porcine coronary rings were suspended in organ chambers and precontracted with prostaglandin F$_{2alpha}$ to determine direct effects of the selective ER agonists 4,4′,4″-(4-propyl-[1H]pyrazole-1,3,5-triy)tris-phenol (PPT) or 2,3-bis-(4-hydroxyphenyl)-propionitrile (DPN) or the nonselective ER agonist 17β-estradiol. Indirect effects on contractility to U46619 and relaxation to bradykinin were assessed and effects on NO, nitrite, and O$_2^-$ formation were measured in cultured cells. Within 5 minutes, selective ERα activation by PPT, but not 17β-estradiol or the ERβ agonist DPN, caused rapid, NO-dependent, and endothelium-dependent relaxation (49±5%; P<0.001 versus ethanol). PPT also caused sustained endothelium- and NO-independent vasodilation similar to 17β-estradiol after 60 minutes (72±3%; P<0.001 versus ethanol). DPN induced endothelium-dependent NO-independent relaxation via endothelium-dependent hyperpolarization (40±4%; P<0.01 versus ethanol). 17β-Estradiol and PPT, but not DPN, attenuated the responses to U46619 and bradykinin. All of the ER agonists increased NO and nitrite formation in vascular endothelial but not smooth muscle cells and attenuated vascular smooth muscle cell O$_2^-$ formation (P<0.001). ERα activation had the most potent effects on both nitrite formation and inhibiting O$_2^-$ (P<0.05). These data demonstrate novel and differential mechanisms by which ERα and ERβ activation control coronary artery vasoreactivity in males and females and regulate vascular NO and O$_2^-$ formation. The findings indicate that coronary vascular effects of sex hormones differ with regard to affinity to ERα and ERβ, which will contribute to beneficial and adverse effects of hormone replacement therapy. (Hypertension. 2007;49:1364-1370.)

Key Words: atherosclerosis ■ endothelium ■ gender ■ hormone replacement therapy ■ nitric oxide ■ vascular smooth muscle

Vascular effects of estrogens (reviewed in References 1 and 2) can be divided in acute (nongenomic) and chronic (genomic) effects. 17β-Estradiol and selective estrogen receptor (ER) modulators directly induce relaxation in different vascular beds; however, whether vasodilatory effects occur dependent or independent of the endothelium remains controversial. Moreover, the natural estrogen 17β-estradiol may indirectly affect both vascular tone and vasoreactivity to different contractile or relaxant agonists, including U46619 or bradykinin. Endothelium-dependent effects of 17β-estradiol involve endothelial factors, including effects on reactive oxygen species, NO, and superoxide anion (O$_2^-$).

Two distinct subtypes of ERs have been cloned, ERα and ERβ. Both are located intracellularly and on the cell membrane and are present in vascular smooth muscle and endothelial cells. Nongenomic vascular effects of 17β-estradiol on endothelial cells are thought to be mediated by plasma membrane–bound and caveolar ERs, involving activation of protein kinases and endothelial NO synthase.

17β-Estradiol and other estrogenic compounds, including those found in conjugated equine estrogens used for hormone replacement therapy, bind to ERs. The contribution of ERα and ERβ to regulation of vascular tone in the coronary circulation is still obscure and only recently have suitable pharmacological tools become available, including 4,4′,4″-(4-propyl-[1H]pyrazole-1,3,5-triy)tris-phenol (PPT), a selective ERα receptor agonist, and 2,3-bis-(4-hydroxyphenyl)-propionitrile (DPN), a selective agonist for ERβ.

Both male gender and estrogen deficiency after menopause are independent cardiovascular risk factors (reviewed in References 1 and 2). The incidence of coronary artery disease is low in premenopausal women but increases after menopause and with aging, indicating protective effects of endogenous estrogens on the cardiovascular system. Importantly, the administration of exogenous nonhuman hormones, such as conjugated equine estrogens and methoxyprogesterone...
neacacetate in postmenopausal women, increases clinical complications, such as thrombosis in veins and coronary arteries. In humans, atherosclerosis typically develops in large conduit arteries, such as the epicardial coronaries. Porcine coronary arteries are widely used as a suitable experimental model of human coronary arteries because of the high anatomic and physiological similarities. Therefore, using a porcine coronary artery model of human coronary arteries because of the high anatomic and physiological similarities.22 Therefore, using a porcine coronary artery model of human coronary arteries because of the high anatomic and physiological similarities.22 Therefore, using a porcine coronary artery model of human coronary arteries because of the high anatomic and physiological similarities.22 Therefore, using a porcine coronary artery model of human coronary arteries because of the high anatomic and physiological similarities. Therefore, using a porcine coronary artery model of human coronary arteries because of the high anatomic and physiological similarities. Therefore, using a porcine coronary artery model of human coronary arteries because of the high anatomic and physiological similarities. Therefore, using a porcine coronary artery model of human coronary arteries because of the high anatomic and physiological similarities. Therefore, using a porcine coronary artery model of human coronary arteries because of the high anatomic and physiological similarities. Therefore, using a porcine coronary artery model of human coronary arteries because of the high anatomic and physiological similarities. Therefore, using a porcine coronary artery model of human coronary arteries because of the high anatomic and physiological similarities.

Methods

Tissue Preparations

Experiments were in accordance with the institutional guidelines and the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health. Porcine hearts of either sex were obtained from a local abattoir and immediately immersed in cold physiological Krebs–Ringer bicarbonate solution (in millimoles per liter): 118.6 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 25.1 NaHCO₃, 1.2 KH₂PO₄, 0.026 Na₂-EDTA, and 10.1 glucose. The left anterior descending coronary artery was dissected free from surrounding myocardium, cleaned of adherent fat and connective tissue, and cut into rings 4 to 5 mm in length. Six rings were prepared from each coronary artery. In a subset of rings, the endothelium was removed by gently rubbing the intimal surface with a wooden probe.

Vascular Function Experiments

Vascular rings were mounted onto stainless steel hooks and placed in organ chambers containing Krebs–Ringer solution (25 mL [pH 7.4], 37°C, 95% O₂, and 5% CO₂) as described. Rings were progressively stretched until optimal tension for generating force during isometric contraction was reached (4.0 g) and repeatedly exposed to KCl (60 mmol/L; iso-osmotically replaced) to determine a maximal contraction. Before the experiments, all rings were preincubated with the nonselective cyclooxygenase-inhibitor meclofenamate (1 μmol/L) for 30 minutes. A subset of rings was additionally incubated with N⁶-nitro-L-arginine methyl ester (L-NAME; 300 μmol/L) for 30 minutes to inhibit endothelial NO synthesis.

The integrity of vascular smooth muscle was determined by precontracting with acetylcholine (1 μmol/L), and the absence or presence of the endothelium was determined by the relaxant response to bradykinin (0.1 nmol/L to 1 μmol/L; data not shown); endothelium-independent relaxation was determined using sodium nitroprusside (300 μmol/L; data not shown). In rings precontracted with prostaglandin F₂α, direct vascular effects of 17β-estradiol, PPT, or DPN (10 μmol/L each) were recorded for 60 minutes. Ethanol at a final concentration of 0.2% (vol/vol) served as control. A subset of rings exposed to DPN was preincubated with inhibitors of endothelium-dependent hyperpolarization factors, charybdotoxin in combination with apamin (0.1 μmol/L each). After repeated washings, rings were incubated again with 17β-estradiol, PPT, or DPN (10 μmol/L) and precontracted with U46619 (1 nmol/L to 0.3 μmol/L). Endothelium-dependent relaxation was determined using bradykinin (0.01 nmol/L to 1 μmol/L), and endothelium-independent relaxation was determined using sodium nitroprusside.

Cell Culture Experiments

Human umbilical vein endothelial cells (HUVECs) were isolated by collagen digestion and cultured in endothelial cell growth medium (PromoCell). HUVEC-derived EA.hy 926 endothelial cells (a kind gift of Dr C J Edgell, University of North Carolina at Chapel Hill, Chapel Hill, NC) were grown as described.

Roles of Estrogen Receptors for Vasodilation

Human vascular smooth muscle cells (VSMC) were isolated using the explant technique and cultured as described. Cells of passages 3 to 6 were used for all of the experiments.

Determination of Endothelial NO Synthesis

To determine the effects of ER agonists, NO release by HUVECs was bioassayed using guanylyl cyclase–containing RFL-6 rat lung fibroblasts as reporter cells. HUVECs were treated with ER agonists (all 10 μmol/L) for 5 minutes. Thereafter, NO-containing conditioned media from the HUVECs were transferred to RFL-6 cells to stimulate guanylyl cyclase. The cGMP content of the RFL-6 samples was determined by radioimmunoassay.

In additional experiments, HUVECs, EA.hy 926 cells, or VSMCs were treated with ER agonists for 60 minutes, and nitrite accumulation in the cell culture supernatant was measured as an indicator of NO production by chemiluminescence using an NOA 280 nitric oxide analyzer (Sievers) or by the Griess reaction according to the instructions of the manufacturer (Caymann). Total protein content was determined (Bradford), and nitrite levels were normalized for protein.

The nitrite concentration in control cells was set 100%.

Vascular O₂⁻ Formation

Subconfluent VSMCs were starved for 24 hours and exposed to 17β-estradiol, PPT, DPN (all 10 μmol/L), or ethanol for 30 minutes. Three independent experiments were performed, with measurements being performed in triplicate. Cells were suspended in Krebs-HEPES buffer, and O₂⁻ generation was monitored using a chemiluminescence probe (L-012; 500 μmol/L, Wako Chemicals) in a luminometer (Lumat LB 9507) as described previously.

Drugs

Acetylcholine chloride, apamin, bradykinin, charybdotoxin, 17β-estradiol, L-NAME, prostaglandin F₂α, sodium nitroprusside dihydinate, and U46619 were from Sigma-Aldrich. DPN and PPT were from Tocris (Anawa). DPN, PPT, and 17β-estradiol were dissolved in 100% ethanol. All of the other substances were dissolved in water; stock solutions were diluted in Krebs solution to the required final concentration before use.

Calculations and Statistical Analysis

Data are expressed as mean±SEM, and n equals the number of animals. Contraction was expressed as the percentage of contraction to KCl 60 mmol/L, and relaxation was expressed as the percentage of precontraction. EC₅₀ values (as negative logarithm: pD₂), area under the curve, and maximal responses were calculated by nonlinear regression analysis.

One-way ANOVA, ANOVA for repeated measures (followed by Bonferroni–Dunn posthoc test), or unpaired Student’s t test was used when appropriate. A P<0.05 was considered significant.

Results

Selective ERα Activation Induces Rapid Endothelium- and NO-Dependent Relaxation

The selective ERα agonist PPT induced a rapid relaxation (49±5% within 5 minutes; P<0.001 versus ethanol [ETOHI]) that was not seen with either 17β-estradiol or the selective ERβ agonist DPN (Figure 1, original tracings, and Figure 2A). As shown in Figure 3A, the rapid response induced by PPT was abolished in rings without endothelium (5±1% versus 49±5%; P<0.001 versus PPT) or after pretreatment with the NO synthase inhibitor L-NAME (6±1% versus 49±5%; P<0.001 versus PPT). Data for males and females are presented separately in Table 1.
Sustained Relaxation to PPT and 17β-Estradiol Are Endothelium- and NO-Independent After 60 minutes of recording time, relaxation caused by PPT was increased further and became comparable to 17β-Estradiol (72±4%; P<0.001 versus ETOH), whereas DPN induced a smaller but significant response (40±4%; P=0.01 versus ETOH; Figure 1, original tracings and Figure 2B). Data for males and females are presented separately in Table 1. Denudation or inhibition of NO synthesis did not affect relaxation to PPT measured after 60 minutes (67±3% and 63±2%, respectively; P value not significant versus PPT). 17β-Estradiol induced a time-dependent relaxation (77±4% after 60 minutes; P<0.001 versus ETOH; Figure 1, original tracing and Figure 2B). Inhibition of NO synthesis with l-NAME did not affect this response (Figure 3C). Calculated values for area under the curve and maximal response are given in Table 2.

Sustained Relaxation Following ERβ Activation Involves Endothelium-Dependent Hyperpolarization The sustained response of DPN recorded after 60 minutes was unaffected by l-NAME (33±4% versus 40±4%; P value not significant; Figure 3B) and comparable to solvent control after removal of the endothelium (24±2% versus 40±4%; P=0.01 versus DPN alone). After pretreatment with l-NAME, charybdotoxin, and apamin, relaxation was completely inhibited (17±2% versus 33±3%; P=0.01 versus l-NAME alone). Calculated values for area under the curve and maximal response are given in Table 2.

Thromboxane-Mediated Contraction Is ERα-Sensitive Contractions to the thromboxane receptor agonist U46619 were attenuated in rings pretreated with either the unselective agonist 17β-estradiol or the ERα-selective agonist PPT (P<0.05 and P<0.001 versus ETOH; Figure 4A), whereas DPN had no effect. Maximal relaxations and sensitivity of endothelium-dependent relaxations to bradykinin were reduced after PPT and 17β-estradiol in rings precontracted with U46619 (P<0.05 versus ETOH; Figure 4B and Tables 3 and 4). Pretreatment with DPN had no effect.

Effects of ER Agonists on NO Synthesis in Human Vascular Cells Incubation with PPT, DPN, or 17β-estradiol (10 μmol/L each) increased NO production in HUVECs (cGMP generation in RFL-6 reporter cells) after 5 minutes of treatment (P<0.05 versus ETOH; Figure 5A). Nitrite concentration also significantly increased after treatment with PPT, DPN, or 17β-estradiol after 60 minutes (P<0.05 versus ETOH), the
strongest effect being observed after ERα activation with PPT (*P*<0.05 versus DPN and 17β-estradiol; Figure 5B). In HUVEC-derived EA.hy 926 hybridoma cells, nitrite synthesis after 60 minutes was increased only by 17β-estradiol (+147±20%; *P*<0.05 versus ETOH). In contrast, PPT, DPN, and 17β-estradiol did not induce nitrite formation in VSMCs (data not shown).

Inhibition of Smooth Muscle Cell O$_2^-$ Formation by ER Agonists

Treatment of VSMCs with 17β-estradiol or DPN reduced O$_2^-$ generation by −39±5% and −38±2%, respectively (*P*<0.001 versus ETOH). Interestingly, the ERα-selective agonist PPT had a much stronger inhibitory effect on O$_2^-$ generation than the other agonists (−62±0.5%; *P*<0.001 versus ETOH and *P*<0.01 versus DPN).

**Discussion**

This study demonstrates that, in epicardial porcine coronary arteries of males and females, selective activation of ERα by PPT involves a rapid, NO-dependent component, as well as sustained relaxant response, whereas selective ERβ activation causes sustained relaxation only. The sustained relaxing effect of combined ER activation by 17β-estradiol was similar to that of PPT; however, 17β-estradiol lacked rapid dilator effects. Rapid relaxation after ERα activation was NO-dependent, whereas sustained responses after activation of ERβ or both ERs were not. Activation of ERα, but not of ERβ, also reduced thromboxane A$_2$-receptor–mediated vasoconstriction. Finally, differential effects of selective ER agonists on endothelial NO bioactivity and on VSMC O$_2^-$ formation were observed.

This study is the first to demonstrate that, in epicardial coronary arteries, the dilator response after selective ERα activation involves 2 independent components. On the one hand, rapid, endothelium- and NO-dependent vasodilation occurs within the first minutes, which is followed by a sustained endothelium-independent dilator component reaching a maximum after ∼1 hour similar to that of the unselective ER agonist 17β-estradiol. Importantly, 17β-estradiol, which also activates ERβ, lacked the rapid NO-dependent dilator response, suggesting a possible inhibitory effect of ERβ on ERα-mediated NO-dependent activity. Endothelium- and NO-dependent relaxant effects have been demonstrated

**TABLE 1. Maximal Effects of Acute Relaxant Responses to ER Agonists in Porcine Coronary Arteries**

<table>
<thead>
<tr>
<th>Exposure Time</th>
<th>Agonist</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>ETOH</td>
<td>1±1</td>
<td>1±1</td>
</tr>
<tr>
<td></td>
<td>PPT</td>
<td>55±5*</td>
<td>43±8*</td>
</tr>
<tr>
<td></td>
<td>DPN</td>
<td>3±1†</td>
<td>7±2†</td>
</tr>
<tr>
<td></td>
<td>E$_2$</td>
<td>16±2†</td>
<td>20±5*</td>
</tr>
<tr>
<td>60 min</td>
<td>ETOH</td>
<td>27±4</td>
<td>21±3</td>
</tr>
<tr>
<td></td>
<td>PPT</td>
<td>73±5*</td>
<td>70±3*</td>
</tr>
<tr>
<td></td>
<td>DPN</td>
<td>36±3†</td>
<td>43±7†</td>
</tr>
<tr>
<td></td>
<td>E$_2$</td>
<td>76±6*</td>
<td>78±6*</td>
</tr>
</tbody>
</table>

Data are presented separately for arteries of male and female pigs. Data are mean±SEM, *n*=4–10. E$_2$ indicates 17β-estradiol.

*P*<0.05 vs ETOH; †*P*<0.05 vs PPT and E$_2$ (for each time point).

**TABLE 2. Acute Relaxant Effects of ER Agonists in Porcine Coronary Arteries**

<table>
<thead>
<tr>
<th>Agonist and Treatment</th>
<th>AUC</th>
<th>E$_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETOH</td>
<td>40±5</td>
<td>23±3</td>
</tr>
<tr>
<td>PPT</td>
<td>179±7*</td>
<td>66±2*</td>
</tr>
<tr>
<td>PPT+L-NAME</td>
<td>110±7*</td>
<td>61±2*</td>
</tr>
<tr>
<td>PPT denuded</td>
<td>115±6*†</td>
<td>65±3*</td>
</tr>
<tr>
<td>DPN</td>
<td>81±6*</td>
<td>38±2*</td>
</tr>
<tr>
<td>DPN+L-NAME</td>
<td>53±9</td>
<td>29±4</td>
</tr>
<tr>
<td>DPN denuded</td>
<td>52±3</td>
<td>24±2</td>
</tr>
<tr>
<td>DPN+L-NAME+CHAP</td>
<td>7±1†</td>
<td>14±2†</td>
</tr>
<tr>
<td>E$_2$</td>
<td>180±13*</td>
<td>74±4*</td>
</tr>
<tr>
<td>E$_2$+L-NAME</td>
<td>177±15*</td>
<td>73±4*</td>
</tr>
</tbody>
</table>

Area under the curve (AUC) and maximal responses (E$_{max}$) were calculated by nonlinear regression analysis. Data are mean±SEM, *n*=8 to 20.

*P*<0.05 vs ETOH; †*P*<0.05 vs agonist alone.
for both the ERα agonist PPT\textsuperscript{15,17,30} and combined ER agonist 17β-estradiol,\textsuperscript{31–35} yet none of these previous studies reported a rapid or NO-dependent dilator component to PPT, even at concentrations higher than those used in the present study.\textsuperscript{17,30} It has been shown previously that NO is released after short-term treatment with 17β-estradiol from HUVECs.\textsuperscript{12} Although there is evidence that NO release from endothelial cells is mediated by ER\textsubscript{α},\textsuperscript{36,37} more recent data suggest that both ER\textsubscript{α} and ER\textsubscript{β} can activate endothelial NO synthase and mitogen-activated protein kinases.\textsuperscript{38} In line with these observations, we show here that selective activation of either ER increases bioactive NO in HUVECs and also rapidly stimulates endothelial cell cGMP formation. It is noteworthy that again the strongest effect was seen after selective activation of ER\textsubscript{α}. Increased cGMP after 17β-estradiol was also detected in HUVEC-derived EA.hy 926 hybridoma cells,\textsuperscript{25} which, unlike in HUVECs,\textsuperscript{39} express only a truncated form of ER\textsubscript{α}.\textsuperscript{39} Thus, either selective or unselective activation of ERs appears to stimulate endothelial NO formation in most species. In the present study, the rapid NO-mediated dilator response in porcine coronary arteries was only seen after selective activation ER\textsubscript{α}, suggesting possible species differences in ER expression and/or function. Increased NO bioactivity may also be affected by antioxidant effects of estrogen agonists because of their phenolic structure.\textsuperscript{40} Our experiments using human VSMCs show that all of the ER agonists used inhibit vascular O\textsubscript{2}⁻ generation. Because the most potent effect was again seen with the ER\textsubscript{α} agonist PPT, it appears reasonable to speculate that scavenging of O\textsubscript{2}⁻ by PPT also indirectly contributes to the observed NO bioavailability and the rapid vasodilator component observed in the present study.

The maximum effect of the sustained vasodilator response to the ER\textsubscript{α} agonist PPT was equally potent but somewhat delayed compared with that induced by 17β-estradiol. In contrast, the maximum response to the ER\textsubscript{β} agonist DPN was less pronounced. This is in agreement with a study using aortic rings of female rats, in which the ER\textsubscript{α} agonist PPT acutely and concentration-dependently induced relaxations similar to those by 17β-estradiol,\textsuperscript{15} whereas the ER\textsubscript{β} agonist DPN had no effect.\textsuperscript{15} We found that the sustained dilator component to all 3 of the ER agonists was NO independent, which is in line with earlier studies using 17β-estradiol in coronary arteries of humans,\textsuperscript{41,42} dogs,\textsuperscript{43} or adult pigs of either sex.\textsuperscript{44,45} Therefore, the sustained dilator response must involve mechanisms distinct from NO, possibly inhibition of VSMC Ca\textsuperscript{2+} influx.\textsuperscript{3}

We surprisingly found that selective ER\textsubscript{β}-mediated epicardial coronary vasodilation was in part endothelium-dependent. Because combined cyclooxygenase and NO inhibition had no effect on the endothelium-dependent portion of the relaxant response to DPN, the results suggested a role for other vasodilator mechanisms, such as endothelium-mediated hyperpolarization. Indeed, experiments using inhibitors of endothelium-dependent hyperpolarization showed an attenuation of the relaxant responses after ER\textsubscript{β} activation, confirming this hypothesis. This mechanism may be particularly relevant to epicardial coronary arteries, which are known to produce high levels of endothelium-dependent hyperpolarizing factor,\textsuperscript{46} and to conditions when NO bioactivity is low, such as atherosclerosis or aging.

### TABLE 3. Acute Effects of ER Agonists on Contractions to U46619 in Porcine Coronary Arteries

<table>
<thead>
<tr>
<th>Vasconstrictor</th>
<th>Agonist</th>
<th>pD\textsubscript{2}</th>
<th>AUC</th>
<th>E\textsubscript{max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>U46619</td>
<td>ETOH</td>
<td>8.3±0.1*</td>
<td>52±4</td>
<td>67±4</td>
</tr>
<tr>
<td></td>
<td>PPT</td>
<td>8.2±0.0*</td>
<td>22±2†</td>
<td>31±2‡</td>
</tr>
<tr>
<td></td>
<td>DPN</td>
<td>8.2±0.0*</td>
<td>45±2</td>
<td>67±3</td>
</tr>
<tr>
<td></td>
<td>E\textsubscript{2}</td>
<td>7.9±0.0*</td>
<td>24±3‡</td>
<td>51±5</td>
</tr>
</tbody>
</table>

\textit{E}_\text{max} values (as negative logarithm: pD\textsubscript{2}), area under the curve (AUC), and maximal responses (E\textsubscript{max}) were calculated by nonlinear regression analysis. Data are mean±SEM, n=8 to 18.

*P<0.05 vs 17β-estradiol (E\textsubscript{2}); †P<0.05 vs ETOH and DPN.

### TABLE 4. Acute Effects of ER Agonists on Relaxant Responses to Bradykinin in Porcine Coronary Arteries

<table>
<thead>
<tr>
<th>Vasodilator</th>
<th>Agonist</th>
<th>pD\textsubscript{2}</th>
<th>AUC</th>
<th>E\textsubscript{max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin</td>
<td>ETOH</td>
<td>7.8±0.1*</td>
<td>280±11</td>
<td>98±0</td>
</tr>
<tr>
<td></td>
<td>PPT</td>
<td>7.3±0.1*</td>
<td>218±13</td>
<td>93±1†</td>
</tr>
<tr>
<td></td>
<td>DPN</td>
<td>7.4±0.0</td>
<td>237±4</td>
<td>96±0</td>
</tr>
<tr>
<td></td>
<td>E\textsubscript{2}</td>
<td>7.1±0.1†</td>
<td>198±9*†</td>
<td>94±1*</td>
</tr>
</tbody>
</table>

\textit{E}_\text{max} values (as negative logarithm: pD\textsubscript{2}), area under the curve (AUC), and maximal responses (E\textsubscript{max}) were calculated by nonlinear regression analysis. Data are mean±SEM, n=7 to 16. E\textsubscript{2} indicates 17β-estradiol.

*P<0.05 vs ETOH; †P<0.05 vs DPN.
We finally investigated whether ER agonists indirectly affect vasoreactivity to contracting and relaxing substances. The vasoconstrictor thromboxane $A_2$ is released in high concentrations from aggregating platelets at sites of coronary plaque rupture and is a key event in the pathogenesis of acute coronary syndromes. We found that contractions to the thromboxane $A_2$ receptor agonist U46619 were markedly attenuated after ER$\alpha$ activation only but unaffected by ER$\beta$ activation, indicating another indirect and NO-independent vasodilator function of ER$\alpha$. The effect of unselective ER activation in response to 17$\beta$-estradiol was less pronounced than that of selective ER activation and comparable to previous studies. This suggests that (1) activation of ER$\alpha$ alone is sufficient and (2) that ER$\beta$ possibly regulates vascular ER$\alpha$ function. Indeed, our results suggest that ER$\beta$ activation appears to attenuate the ER$\alpha$-mediated effects during combined ER activation with 17$\beta$-estradiol, which would be compatible with a functional cross-talk between both ERs. Whether selective ER agonists also affect function of vasoconstrictors such as endothelin-1 in epicardial coronary arteries remains to be determined in future studies.

**Perspectives**

The observed NO-dependent coronary dilator response and attenuation of $O_2^-$ anion formation after selective ER$\alpha$ activation may have therapeutic implications for human vascular disease, including acute coronary syndromes and restenosis, and possibly also for hormone therapy in postmenopausal women. Our results also show that the effects of ER activation were similar in coronary arteries from male and female pigs. Finally, because endogenous 17$\beta$-estradiol also contributes to vascular homeostasis in males, effects of endogenous estrogens on the epicardial coronary arterial circulation, a vascular bed that is highly susceptible to atherosclerosis in humans, may be possibly of similar relevance for vascular disease in men and women.

**Acknowledgments**

We thank Giochen Bearth and coworkers at Schlachthof Zürich for their help and Wilhelm Vetter for support.

**Sources of Funding**

This work was supported by the Swiss National Science Foundation (SCORE; 32.58421.99, 32.58426.99/1, and 3200-108258/1), the Hanne Liebermann Stiftung Zürich, and the University of Zürich.

**Figure 5.** Effects of ER agonists on cGMP formation and NO bioactivity in cultured human endothelial cells. Conditioned media from HUVECs exposed to ER agonists for 5 minutes increased cGMP formation in RFL-6 reporter cells (A). Each ER agonist increased HUVEC nitrite formation, the ER$\alpha$ agonist PPT having the most potent effect (B). Data are mean±SEM; n=3 independent experiments.

*P<0.05 vs ETOH; †P<0.05 vs DPN and 17$\beta$-estradiol ($E_2$).

**References**


Distinct Roles of Estrogen Receptors α and β Mediating Acute Vasodilation of Epicardial Coronary Arteries

Tobias Traupe, Christoph D. Stettler, Huige Li, Elvira Haas, Indranil Bhattacharya, Roberta Minotti and Matthias Barton

Hypertension. 2007;49:1364-1370; originally published online April 30, 2007; doi: 10.1161/HYPERTENSIONAHA.106.081554

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/49/6/1364

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/