Specific Contribution of Estrogen Receptors on Mitogen-Activated Protein Kinase Pathways and Vascular Cell Activation

Pedro Geraldes, Martin G. Sirois, Jean-François Tanguay

Abstract—Randomized clinical trials have not provided conclusive data that hormone replacement therapy confers cardioprotection against coronary artery disease in postmenopausal women. However, other studies have shown that estrogens can induce beneficial effects on the vasculature. Nevertheless, the specific contribution of estrogen receptors (ERs) α and β on vascular cells is not well characterized. Therefore, we used an antisense gene therapy approach to investigate the contribution of ERα and ERβ on p38 and p42/44 mitogen-activated protein kinase (MAPK) activation and on vascular cell responsiveness. Treatment of porcine smooth muscle cells (PSMCs) with platelet-derived growth factor-BB induced p38 and p42/44 MAPK activation and their migration and proliferation. These effects were prevented by pretreatment with 17β-estradiol (17βE). The inhibitory effects of 17βE on PSMCs were abrogated by the downregulation of ERβ protein expression with selective ERβ mRNA antisense oligomers, whereas the downregulation of ERα had no effect. However, treatment of porcine aortic endothelial cells with 17βE promoted p38 and p42/44 MAPK phosphorylation and their migration and proliferation. These effects were ERα dependent, as defined by antisense gene therapy. These results suggest that in PSMCs, 17βE reduces p42/44 and p38 MAPK activity through ERβ stimulation, whereas in contrast, in porcine aortic endothelial cells, 17βE induces p42/44 and p38 MAPK through ERα activation. 17βE may contribute to the vascular healing process and to the prevention of restenosis by improving reendothelialization through ERα activation and by decreasing smooth muscle cell migration and proliferation through ERβ stimulation. (Circ Res. 2003;93:399-405.)

Key Words: 17β-estradiol ■ estrogen receptors ■ vascular cells ■ mitogen-activated protein kinase

Cardiovascular diseases (CVDs) are the leading cause of mortality for postmenopausal women in industrialized countries, accounting for >30% of deaths.1 Epidemiological studies over the past years have suggested a protective effect of hormone replacement therapy (HRT).2 The beneficial effects of estrogens were initially attributed to a decreased level of LDL cholesterol and to an increased level of HDL cholesterol. However, the positive effects of estrogens on lipid profile account for only approximately one third of the observed reduction in the risk of mortality from CVD among HRT users.3 Other studies have demonstrated that estrogens have direct actions on the blood vessel wall.4 Physiological concentrations of estrogens can inhibit platelet and monocyte aggregations and stimulate NO production and reendothelialization.5 Despite the beneficial effects of estrogens, randomized double-blind studies have reported no overall benefit from HRT.6,7 A better understanding of the effects of estrogen on vascular cells might contribute to optimization of the vascular healing process.

Estrogen receptors (ERs) α and β are members of the steroid/thyroid hormone receptor superfamily of ligand-activated transcription factors.8 Estrogen receptors contain DNA and ligand binding domains, which are critically involved in regulating vascular structures and functions.9 Receptor-ligand interactions trigger a cascade of events, including dissociation from heat shock proteins, receptor dimerization, phosphorylation, and the association of the hormone-activated receptor with specific regulatory elements in target genes.9 ERα and ERβ are expressed in vascular endothelial cells (ECs) and smooth muscle cells (SMCs), and their activation may lead to distinct biological activities even though they share many functional characteristics.10 In a previous study, Pare et al11 showed in ERα and ERβ knockout mice that the protective effects of estrogens to vascular injury are ERα dependent. However, the exact contribution played by ERβ remains to be clarified. Previous experiments have shown that compared with wild-type mice, ERβ-deficient mice exhibit higher vasoconstriction and blood pressure.12 However, several limitations exist when using knockout animal preparations, whereas the disruption of a gene may influence the response of estrogens.
Recently, we reported that local delivery of 17β-estradiol (17βE) after coronary angioplasty in pigs promotes the vascular healing process by reducing neointimal formation and by improving the reendothelialization process and endothelial NO synthase (eNOS) expression. However, other specific effects of estrogens may induce nongenomic signaling pathways and may interact with intracellular second messenger pathways, such as mitogen-activated protein kinase (MAPK). Under in vitro conditions, we showed that 17βE prevents SMC proliferation and migration by inhibiting p42/44 and p38 MAPK activation, whereas it promotes these events in ECs. However, the specific contributions of ERα and ERβ on these events remain unknown. We used an antisense gene therapy approach to regulate the protein expression of ERα and ERβ and to better understand the specific contribution of each ER. In the present study, we report that 17βE promotes p42/44 and p38 MAPK phosphorylation through ERα stimulation on ECs, whereas on SMCs, the inhibitory effects of 17βE on p42/44 and p38 MAPK phosphorylation are mediated by ERβ activation.

Materials and Methods

Cell Culture

Porcine aortic ECs (PAECs) and porcine SMCs (PSMCs) were isolated from freshly harvested aortas, cultured, and characterized as described previously. PAECs and PSMCs were used between passages 3 and 8.

Antisense Oligonucleotide Gene Therapy

To distinguish the role played by ERα and ERβ in the migration and proliferation of PSMCs and PAECs as well as in the activation of p38 and p42/44 MAPKs, we treated the cells with antisense oligonucleotide sequences complementary to porcine ERα and ERβ mRNA (GenBank accession Nos. Z37167 and AF164957, respectively). A total of four different antisense oligodeoxyribonucleotide phosphorothioate sequences were used: two targeted porcine ERα mRNA sequences (antisense 1 [AS1-ERα], 5'-TCG GAC GGT TGT GAT CTG-3'; antisense 2 [AS2-ERα], 5'-GAC GCT TGT GGT TGT AGG-3') and two targeted porcine ERβ mRNA sequences (antisense 1. [AS1-ERβ], 5'-GTA GGA GAC AGG AGA GTT-3'; antisense 2 [AS2-ERβ], 5'-GCT AAA GGA GAG AGG TGT-3'). Two scrambled oligodeoxyribonucleotide phosphorothioate sequences (scrambled ERα [SCR-ERα], 5'-TGT ACG TCG GTG TGT CCG-3'; scrambled ERβ [SCR-ERβ], 5'-GAG TGG ACG TGA AGA AGT-3') were also used as negative controls. These sequences were selected because they had no more than three consecutive guanosines and had no capacity or minimal capacity to dimerize together and to form hairpins. All sequences were synthesized at the Armand Frappier Institute (Laval, Quebec, Canada). On synthesis, the oligonucleotides were dried, resuspended in sterile water, and quantified by spectrophotometry.

Western Blot Analyses of ERα and ERβ Expression and p42/44 and p38 MAPK Phosphorylation

The efficiency and specificity of our antisense oligomers to prevent the expression of targeted proteins were evaluated by Western blot analyses. Culture media of confluent PAECs and PSMCs (100-mm tissue culture plate) were removed, and the cells were rinsed with DMEM (Life Technologies Inc) and trypsinized (trypsin-EDTA, Life Technologies). The cells were resuspended in DMEM containing 5% FBS (Hyclone Laboratories) and antibiotics (penicillin and streptomycin, Sigma), and the cell count was obtained with a Coulter counter Z1 (Coulter Electronics). Cells were seeded at 1×10⁴ cells per 100-mm tissue culture plate (Becton-Dickinson) and stimulated for 24 hours in DMEM, 5% FBS, and antibiotics with or without antisense oligomers (10⁻⁷, 5×10⁻⁸, and 10⁻⁹ mol/L). Lipofectamine (5 μg/mL, Life Technology Inc) was used to improve the cellular uptake of antisense oligomers in PSMCs. Go synchronization was achieved by starving the cells for 48 hours in DMEM, 0.1% FBS, and antibiotics with or without antisense oligomers (10⁻⁷, 5×10⁻⁸, and 10⁻⁹ mol/L) daily, followed by starvation for 7 hours in DMEM, 0.1% FBS, and antibiotics with or without antisense oligomers (10⁻⁷, 5×10⁻⁸, and 10⁻⁹ mol/L) to induce an upregulation of ER expression. Culture medium was removed, and the cells were rinsed. PSMCs and PAECs were then stimulated with or without 17βE as previously described. Briefly, PSMCs were incubated on ice in DMEM with or without 17βE (10⁻⁶ mol/L) for 30 minutes and incubated at 37°C for 30 minutes. Cells were then rinsed, incubated in DMEM with platelet-derived growth factor (PDGF)-BB (10 ng/mL) for 30 minutes on ice, and incubated at 37°C for 5 or 30 minutes. PSMCs were then incubated on ice in DMEM with or without 17βE (10⁻⁶ mol/L) for 30 minutes and then incubated at 37°C for 5 or 30 minutes. Total proteins were isolated by the addition of 500 μL lysis buffer containing 10 μg/mL leupeptin (Sigma), 1 mmol/L phenylmethylsulfonyl fluoride (Sigma), 30 μg/mL aprotinin (Sigma), and 1 μg/mL NaN₃ (Sigma). Plates were incubated at 4°C for 30 minutes and scraped, and the protein concentration was determined with a protein assay kit (Bio-Rad). Proteins (100 μg) were separated by a 10% gradient SDS-PAGE (Protean II kit, Bio-Rad) and transblotted onto 0.45-μm polyvinylidene difluoride membranes (Millipore Corp). The membranes were blocked in 5% Blotto-TTBS (5% nonfat dry milk [Bio-Rad], 0.05% Tween 20, 0.15 mol/L NaCl, and 25 mmol/L Tris-HCl, pH 7.4) for 1 hour at room temperature with gentle agitation and incubated overnight in 0.5% Blotto-TTBS containing the desired antibody (rabbit polyclonal anti-human ERα or anti-human ERβ, 1:5 000 dilution, Santa Cruz Biotechnology, Santa Cruz, Calif, or rabbit polyclonal anti–phospho-p42/44 MAPK, 1:10 000 dilution, or anti–phospho-p38 MAPK, 1:5 000 dilution, New England Biolabs). Membranes were washed three times with TTBS and incubated with a horseradish peroxidase goat anti-rabbit IgG antibody (1:10 000 dilution, Santa Cruz Biotechnology) in 0.5% Blotto-TTBS for 30 minutes. Membranes were washed with TTBS, and horseradish peroxidase-bound to secondary antibody was revealed by chemiluminescence (Renaissance kit, NEN Life Science Products). K aleidoscope molecular weight and SDS-PAGE broad-range marker proteins (Bio-Rad) were used as standards. Membranes were stripped with Re-Blot Plus (Chemicon International Inc), and total p42/44 and p38 MAPK expression was performed using the desired antibody (rabbit polyclonal anti–p42/44 MAPK, 1:5 000 dilution, or anti–p38 MAPK, 1:5 000 dilution, New England Biolabs). Digital image densitometry (PDI Bioscience) was performed to determine the relative expression of ERα and ERβ proteins. Western blot analyses were performed in triplicate, and results of image densitometry are representative of these experiments.

Mitogenic Assay

Confluent PAECs and PSMCs were rinsed with DMEM and trypsORIZED. Cells were resuspended in 10 mL DMEM, 5% FBS, and antibiotics, and a cell count was obtained using a Coulter counter Z1. PAECs and PSMCs were initially seeded at 1×10⁴ cells per well in 24-well tissue culture plates, stimulated for 24 hours in DMEM, 5% FBS, and antibiotics with or without antisense oligomers (10⁻⁷, 5×10⁻⁸, and 10⁻⁹ mol/L), and starved for 48 hours in DMEM, 0.1% FBS, and antibiotics with or without antisense oligomers (10⁻⁷, 5×10⁻⁸, and 10⁻⁹ mol/L) for Go synchronization. The cells were then stimulated for 72 hours in DMEM, 1% FBS, and antibiotics with or without antisense oligomers (10⁻⁷, 5×10⁻⁸, and 10⁻⁹ mol/L) daily. After trypsORIZATION, cell number was determined using a Coulter counter Z1.
Chemotactic Assay
Cell migration was evaluated using a modified Boyden 48-well microchamber kit (Neuroprobe). Near-confluent PAECs and PSMCs were rinsed with DMEM and trypsinized. Cells were resuspended in DMEM, 5% FBS, and antibiotics, and a cell count was obtained. PAECs and PSMCs were seeded at 2.5x10³ cells per well in 6-well tissue culture plates, stimulated for 24 hours in DMEM, 5% FBS, and antibiotics with or without antisense oligomers (10⁻⁶ mol/L), and starved for 48 hours in DMEM, 0.1% FBS, and antibiotics with or without antisense oligomers (10⁻⁶ mol/L) daily and with or without 17βE (10⁻⁷ mol/L). Cells were harvested by trypsinization and resuspended in DMEM, 1% FBS, and antibiotics at a concentration of 2.5x10⁵ cells/mL. Fifty microliters of this cell suspension with or without antisense oligomers (10⁻⁶ mol/L) treated with or without 17βE (10⁻⁷ mol/L) was added to the higher chamber of the modified Boyden chamber apparatus, and the lower chamber was filled with DMEM, 1% FBS, and antibiotics plus the desired concentration of agonist, either 17βE (10⁻⁶ mol/L) or PDGF-BB. The two sections of the system were separated by a porous polycarbonate filter (5-µm pores size), pretreated with a gelatin solution (1.5 mg/mL), and assembled. Five hours after incubation at 37°C, the nonmigrated cells were scraped with a plastic policeman, and the migrated cells were stained using a Quick-Diff solution (Shandon Inc). The filter was then mounted on a glass slide, and migrated cells were counted using a microscope adapted to a video camera to obtain a computer-digitized image. Because of a slight variation of basal cell migration between experiments, data were reported as relative mean migrating cells compared with baseline.

Statistical Analysis
Data are mean±SEM. Statistical comparisons were determined by ANOVA, followed by an unpaired Student t test with the Bonferroni correction for multiple comparisons. A value of P<0.05 was considered to be significant.

Results
Modulation of ERα and ERβ Protein Expression by Antisense Oligonucleotide Gene Therapy
To evaluate the potency of antisense oligonucleotides to prevent the expression of targeted proteins, PSMCs and PAECs were treated either with antisense or scrambled oligomers (as described in Materials and Methods) after 72 hours of stimulation with 1% FBS, and the expression of each receptor was determined by Western blot analysis. In PSMCs, we observed a basal ERα protein expression, which was inhibited by a treatment with antisense oligomers (10⁻⁶ mol/L) targeting porcine ERα mRNA. The antisense oligomers AS1-ERα and AS2-ERα suppressed ERα protein expression by 88% and 89%, respectively, in PSMCs (Figure 1A). Similar treatment with antisense oligomers (AS1-ERβ and AS2-ERβ, 10⁻⁶ mol/L) targeted against ERβ mRNA also reduced the basal ERβ protein expression in PSMCs by 84% and 92%, respectively (Figure 1A). The same series of experiments was conducted in PAECs. The antisense oligomers AS1-ERα and AS2-ERα (10⁻⁶ mol/L) suppressed PAEC ERα protein expression by 94% and 95%, respectively (Figure 1C), and AS1-ERβ and AS2-ERβ (10⁻⁶ mol/L) downregulated ERβ protein expression by 90% and 97%, respectively (Figure 1C). Treatment with scrambled oligomers (SCR-ERα and SCR-ERβ, 10⁻⁶ mol/L) had no significant effect on basal ERα and ERβ protein expression (Figures 1A and 1C). We also performed a new set of experiments in which we showed that treatment with PDGF-BB (for 5 hours) did not alter the protein expression of ERα and ERβ (data not shown).

To ensure that the antisense oligomers designed to downregulate the expression of ERα would not affect ERβ expression and vice versa, we performed additional Western blot analyses to evaluate the specificity of our antisense oligomers. Treatment with antisense oligomers targeting ERα mRNA (10⁻⁶ mol/L) did not affect ERβ basal protein expression, whereas the antisense oligomers directed against ERβ mRNA (10⁻⁶ mol/L) did not alter the basal protein expression of ERα on PSMCs and PAECs (Figures 1B and 1D).

Contribution of ERα and ERβ on PSMC Proliferation
Because the expressions of ERα and ERβ were specifically blocked by antisense oligomers, we investigated the contribution of both receptors on PSMC proliferation. Stimulation of quiescent PSMCs with DMEM containing 1% FBS for 72 hours increased PSMC proliferation by 88%, from 5432±680 to 10216±546 cells per well (Figure 2). Treatment with 17βE (10⁻⁶ mol/L) prevented by 95% the PSMC proliferation mediated by 1% FBS. Treatment of PSMCs with AS1-ERβ and AS2-ERβ prevented the inhibitory effects of 17βE on PSMC proliferation (P<0.05), whereas the antisense oli-
Role of ERα and ERβ on p42/44 and p38 MAPK Phosphorylation in PSMCs

Because 17βE can influence p42/44 and p38 MAPK phosphorylation in PSMCs, we evaluated the specific contribution of ERα and ERβ in this regard. Treatment of PSMCs with PDGF-BB increased p42/44 (Figure 4A) and p38 (Figure 4B) MAPK phosphorylation, which was reversed by a 30-minute pretreatment with 17βE (10⁻⁸ mol/L). Treatment of PSMCs with antisense oligomers targeting ERα mRNA did not affect the inhibitory effect of 17βE at preventing the p42/44 and p38 MAPK phosphorylation induced by PDGF-BB. In contrast, a treatment with antisense oligomers directed against ERβ mRNA significantly blocked the effects of 17βE on p42/44 and p38 MAPK phosphorylation (P<0.05) (Figures 4A and 4B). In the same series of experiments, scrambled oligomers did not alter 17βE activity on these MAPKs (Figures 4A and 4B).

Contribution of ERα and ERβ on PAEC Proliferation

Stimulation of PAECs with DMEM containing 1% FBS increased their proliferation by 83%, from 7427±243 to 13566±1 931 cells per well within 3 days. Compared with treatment with 1% FBS, the addition of 17βE (10⁻⁸ mol/L) enhanced the proliferation of PAECs by 123% (Figure 5). To investigate the selective contribution of ERα and ERβ on the positive mitogenic effect of 17βE on endothelial cells, PAECs were treated with antisense oligomers targeting ERα or ERβ mRNA. AS1-ERα and AS2-ERβ significantly reduced the mitogenic effects of 17βE by 80% and 100%, respectively (P<0.05). Treatment with antisense oligomers directed against ERβ mRNA failed to alter the mitogenic activity of 17βE on PAECs. Again, PAEC proliferation induced by 17βE was not influenced by treatments with scrambled antisense oligomers (Figure 5).

Anticbachotactic Effects of 17βE on PAECs: Role of ERα and ERβ mRNA

Compared with treatment with 1% FBS, treatment of PAECs with 17βE (10⁻⁸ mol/L) for 5 hours promoted their migration by 363% (P<0.05) (Figure 6). Treatment with antisense oligomers (10⁻⁶ mol/L) directed against ERα mRNA prevented the chemotactic activity of 17βE (10⁻⁸ mol/L) on PAECs by...
75% and 76%, respectively (P<0.05) (Figure 6), whereas the inhibition of ER\(\beta\) protein expression did not prevent the 17\(\beta\)E activity on PAECs (Figure 6). Treatment with scrambled oligomers did not alter the chemotactic activity of 17\(\beta\)E (Figure 6).

**Role of ER\(\alpha\) and ER\(\beta\) on p42/44 and p38 MAPK Phosphorylation in PAECs**

We have previously demonstrated that 17\(\beta\)E induces a marked increase of p42/44 and p38 MAPK phosphorylation in PAECs. To determine the contribution of ER\(\alpha\) and ER\(\beta\) on these intracellular mechanisms, PAECs were treated with antisense oligomers targeting ER\(\alpha\) or ER\(\beta\) mRNA. PBS-treated PAECs showed a basal phosphorylation of p42/44 (Figure 7A) and p38 MAPK (Figure 7B). Stimulation with 17\(\beta\)E (10\(^{-8}\) mol/L) for 5 minutes increased p42/44 MAPK phosphorylation by 317%, and 30 minutes of stimulation with 17\(\beta\)E increased p38 MAPK phosphorylation by 254%. Treatment of PAECs with AS1-ER\(\alpha\) and AS2-ER\(\alpha\) prevented the p42/44 and p38 MAPK phosphorylation induced by 17\(\beta\)E (Figures 7A and 7B). In contrast, treatment with antisense oligomers targeting ER\(\beta\) mRNA did not significantly reduce the p42/44 and p38 MAPK phosphorylation mediated by 17\(\beta\)E. Treatment with scrambled oligomers did not influence 17\(\beta\)E activity on p42/44 and p38 MAPK phosphorylation (Figures 7A and 7B).

**Discussion**

Previous studies have demonstrated that the disruption of ER\(\alpha\) in mice reduces the cardioprotective effects of estrogens.
on restenosis. However, other investigators have indicated that ERβ, the major ER expressed within the vasculature, might contribute to the beneficial effects of estrogens. Previously, we demonstrated that local delivery of 17βE on a porcine coronary angioplasty reduces restenosis by improving the reendothelialization process, eNOS expression, and vascular healing. In addition, we showed under in vitro conditions that the beneficial effects of 17βE on restenosis may be explained by a reduction of PSMC p38 and p42/44 MAPK phosphorylation, migration, and proliferation combined with a positive effect of these mechanisms in PAECs. To the best of our knowledge, the specific contribution of each ER (ERα and ERβ) on MAPK phosphorylation and vascular cell migration and proliferation remained unknown.

In the present study, we demonstrated that these effects of 17βE on PAECs are mediated through ERα activation, whereas in PSMCs, 17βE activities are mediated through ERβ stimulation.

Regulation of ERα and ERβ Protein Expression by Antisense Gene Therapy

We used an antisense gene therapy approach to prevent selectively the protein expression of ERα or ERβ, which allowed us to evaluate separately the contribution of ERα and ERβ on intracellular pathways in native ECs and SMCs. Other investigators have used antisense gene therapy to decrease brain ERα. In their experiments, the intraventricular infusion of antisense decreased ER protein expression by 65% at 6 hours after infusion. In the present study, we observed that treatment of PSMCs or PAECs with selective antisense oligomers (10⁻⁶ mol/L) for 4 days decreased ERα and ERβ protein expression up to 97% (Figure 1). ERα and ERβ can form homodimers and heterodimers in living cells. By downregulating ERα or ERβ, we observed that 17βE can still induce selective effects on vascular cells, suggesting that heterodimerization is not necessarily required in the biological activities studied.

Biological Activities of 17βE Are Mediated Through ERβ in PSMCs

Usually activated by growth factors and cytokines, SMC proliferation and migration remain important targets for the prevention of in-stent restenosis. Many studies have indicated that estrogens prevent restenosis formation by inhibiting SMC proliferation and migration after balloon injury. We have previously demonstrated that local delivery of 17βE prevents restenosis on an angioplasty. In the present study, we observed that treatment with 17βE (10⁻⁶ mol/L) inhibits the PSMC migration and proliferation induced by PDGF-BB. In addition, the downregulation of ERβ protein expression reduced the inhibitory effects of 17βE on PSMC proliferation and migration. Our results support previous observations that gene knockout of ERβ leads to hyperproliferative disease. Recently, we have reported that treatment of PSMCs with 17βE reduces the p42/44 and p38 MAPK phosphorylation induced by PDGF-BB. To further evaluate the contribution of ERα and ERβ on PSMCs, we demonstrated that treatment with antisense oligomers targeting ERβ mRNA abrogates the inhibitory effects of 17βE on the p42/44 and p38 MAPK phosphorylation mediated by PDGF-BB. These results support previous observations that ERβ may be responsible for abnormal vascular contraction, ion channel dysfunction, and hypertension in mice deficient in ERβ. Lindner et al have also demonstrated that ERβ mRNA expression is induced after vascular injury, supporting a direct contribution of this receptor in the vascular effects of estrogen. In contrast to ERβ, the absence of ERα protein expression did not influence the inhibitory effects of 17βE on p42/44 and p38 MAPK phosphorylation in PSMCs.

ERα Activation by 17βE Induces MAPK Phosphorylation in PAECs

Various conditions, such as hypercholesterolemia, hypertension, inflammation, and estrogen deficiency, have been associated with endothelial dysfunction. Vessel wall impairment may contribute to the development of atherosclerosis and CVD. Several animal and in vitro studies have shown that estrogens improve endothelial function. We have demon-
strated that local delivery of 17βE improves vascular healing and reendothelialization by promoting EC proliferation and migration and eNOS expression. However, the respective contribution of ERα and ERβ to these effects of 17βE has not been specifically evaluated. In the present study, we showed that the beneficial effects of 17βE on PAEC migration and proliferation are mediated through ERα stimulation. Our results are in agreement with the study of Brouchet et al, who observed that ERα is required for estrogen-accelerated reendothelialization in an electric injury model. Estrogens can also interact with the MAPK pathway, and we have previously demonstrated that 17βE significantly induces p42/44 and p38 MAPK phosphorylation induced by 17βE. These results support previous work demonstrating a strong relationship between ERα activation by estrogens and MAPK activity in breast cancer cells. Furthermore, our results confirm that the principal actions of estrogen on ECs are not mediated through ERβ. Ilioukhan et al have postulated that estrogens upregulate ERα expression in ECs, supporting an important role for ERα in the biological effects of 17βE on the endothelium.

In conclusion, the properties of 17βE to promote p38 and p42/44 MAPK activation and the migration and proliferation of PAECs are directly mediated through ERα stimulation. In contrast, 17βE inhibits these same events in PSMCs, which are mediated through ERβ activation. Our results suggest that in different vascular cell types but on the same mechanisms, the effects of 17βE are not mediated through the same ER, which may explain the distinct biological activity of estrogens. The present study provides new insight into our understanding of the specific contribution of estrogens to the vascular healing process.

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