Estrogen Regulation of Endothelial and Smooth Muscle Cell Migration and Proliferation

Role of p38 and p42/44 Mitogen-Activated Protein Kinase

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Objective—Restenosis is a major limitation of percutaneous coronary intervention. Migration and proliferation of vascular cells remain a cornerstone in neointimal formation. The cardioprotection of estrogen is well recognized, but the intracellular mechanisms related to these beneficial effects are not completely understood.

Methods and Results—We investigated the effects of 17β-estradiol (17βE) on mitogen-activated protein kinase (MAPK) activity and the migration and proliferation of porcine aortic endothelial cells (PAECs) and porcine smooth muscle cells (PSMCs). Treatment with 17βE (10^{-8} mol/L) abrogated p38 and p42/44 MAPK phosphorylation mediated by platelet-derived growth factor-BB as well as the migration and proliferation of PSMCs. In contrast, treatment with 17βE (10^{-8} mol/L) induced the phosphorylation of p38 and p42/44 MAPK and the migration and proliferation of PAECs. Interestingly, the effects of 17βE on PSMCs and PAECs were reversed by selective estrogen receptor antagonists (tamoxifen, 4-OH-tamoxifen, and raloxifen). These results suggest that in PSMCs, 17βE inhibits chemotactic and mitogenic effects of platelet-derived growth factor-BB as well as p38 and p42/44 MAPK phosphorylation. In contrast, 17βE promotes in PAECs the phosphorylation of p42/44 and p38 MAPK as well as the migration and proliferation of these cells.

Conclusions—Treatment with 17βE has a dual beneficial effect: the improvement of vascular healing and the prevention of restenosis after angioplasty. **(*Arterioscler Thromb Vasc Biol*. 2002;22:1585-1590.)**

Key Words: 17β-estradiol ■ smooth muscle ■ endothelium ■ mitogen-activated protein kinase

Restenosis, occurring in 20% to 40% of patients, is currently the primary limitation of percutaneous transluminal coronary angioplasty.[1] Estrogens play an important role in bone maintenance, in the cardiovascular system, and in the growth, differentiation, and biological activity of various tissues.[2] Moreover, numerous in vivo studies in various animal models have demonstrated that the neointimal formation induced after balloon injury is increased in the absence of estrogen but is decreased in its presence.[3] The protective effects of 17β-estradiol (17βE) are related to favorable changes in the plasma lipid profile,[4] to the inhibition of vascular smooth muscle cell (VSMC) proliferation[5] and migration,[6] to the relaxation of coronary vessels through endothelial NO synthase (eNOS) activity,[7] and to the reduction of platelet and monocyte aggregation,[8] tumor necrosis factor-α release,[9] and extracellular matrix synthesis.[10] We have shown that local delivery of 17βE reduces the neointimal thickness produced by coronary balloon injury in a porcine model.[11]

Estrogen can bind 2 estrogen receptors (ERs), ERα and ERβ, which are expressed in all vascular cell types.[12] The classic genomic mechanism, or long-term effect of estrogen on vascular tissues, is dependent on a change in gene expression. Most recently, a second mechanism related to the direct effect of estrogen has been identified.[13] Several studies have demonstrated that 17βE can activate many intracellular signaling responses.[14] The mitogen-activated protein kinase (MAPK) cascade plays a central role in the cellular signal transduction pathway in response to vascular stimuli.[15] Well-characterized subfamilies of the MAPKs, extracellular signal–regulated kinase and p38 MAPK pathways, are involved in chemotactic and mitogenic activity in a variety of cell types.[16] These MAPKs are stimulated after arterial injury.[17] Therefore, we hypothesized that an acute administration of 17βE may influence these MAPK activities in vascular cells. Estrogens can modulate intracellular events through other ligand receptors[18] and reduce neointimal formation after injury in ER$\alpha\beta$ knockout mice.[19] These results suggest that estrogen may provide a protection against endothelial injury in the absence of ER$\alpha\beta$.

In the present study, we evaluated the activity of 17βE on endothelial and smooth muscle cell (SMC) proliferation and

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migration and p42/44 and p38 MAPK phosphorylation. Furthermore, we examined whether the actions of 17βE on p42/44 and p38 MAPK signal transduction pathways are ER dependent.

Methods

Cell Culture

Porcine aortic endothelial cells (PAECs) and porcine SMCs (PSMCs) expressing ERα and ERβ were isolated from freshly harvested aortas and cultured in DMEM (Life Technologies Inc) containing 5% FBS (Hyclone Laboratories) and antibiotics (penicillin and streptomycin, Sigma Chemical Co). PAECs were characterized by their cobblestone monolayer morphology. PSMCs were characterized by anti–smooth muscle α-actin monoclonal antibodies and by specific morphology for SMCs. PAECs and PSMCs between the third and eighth passages were used.

Mitogenic Assay

Confluent PAECs and PSMCs were rinsed with DMEM and trypsinized. Cells were resuspended in 10 mL DMEM, 5% FBS, and antibiotics, and a cell count was obtained with Coulter counter Z1 (Coulter Electronics). PAECs and PSMCs were initially seeded at 1.5×10^4 cells per well of 24-well tissue culture plates (Becton-Dickinson), stimulated for 24 hours in DMEM, 5% FBS, and antibiotics, and starved for 48 hours in DMEM, 0.1% FBS, and antibiotics. The initial cell number for growth was determined by using a Coulter counter. The cells were stimulated for 72 hours in DMEM, 1% or 5% FBS, and antibiotics with or without different concentrations of 17βE (Sigma), tamoxifen (Tam, Sigma), 4-OH-tamoxifen (4-OHT, Sigma), or raloxifen (Ral, Eli Lilly). After trypsinization, cell number was determined by using a Coulter counter.

Chemotactic Assay

Cell migration was evaluated by 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 2.5×10^5 cells per well of 6-well tissue culture plates, stimulated for 24 hours in DMEM, 5% FBS, and antibiotics, and starved for 48 hours in DMEM, 0.1% FBS, and antibiotics with or without 17βE (10^{-8} mol/L), Tam (10^{-7} mol/L), 4-OHT (10^{-7} mol/L), or Ral (10^{-7} mol/L). ER antagonists were added 5 minutes before 17βE. Cells were harvested by trypsinization and resuspended in DMEM, 1% FBS, and antibiotics at a concentration of 5×10^5 cells/mL. Fifty microliters of this cell suspension, which was treated with or without 17βE (10^{-8} mol/L), Tam (10^{-7} mol/L), 4-OHT (10^{-7} mol/L), or Ral (10^{-7}mol/L), was added in 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 basic fibroblast growth factor (bFGF) or platelet-derived growth factor (PDGF)-BB. The 2 sections of the system were separated by a porous polycarbonate filter (5-μm pore 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 by use of a Quick-Diff solution (Shandon Inc). The filter was then mounted on a glass slide, and migrated cells were counted by use of a microscope adapted to a video camera to obtain a computer-digitized image.

Western Blot Analysis of p38 and p42/44 MAPK Phosphorylation

Confluent PAECs and PSMCs were starved for 7 hours in DMEM and antibiotics. Culture medium was removed, and the cells were rinsed twice with ice-cold DMEM. PSMCs were incubated on ice in DMEM with or without 17βE (10^{-8} mol/L) for 30 minutes, incubated at 37°C for 5, 10, 15, and 30 minutes, and then brought back on ice. Cells were then rinsed with cold DMEM, incubated on ice in DMEM, BSA (1 mg/mL), and PDGF-BB (10 ng/mL) for 30 minutes, incubated at 37°C for 5 minutes, and then brought back on ice. PAECs were incubated on ice in DMEM with or without 17βE (10^{-8} mol/L) for 30 minutes, incubated at 37°C for 5, 10, 15, and 30 minutes, and then brought back on ice. For all the experiments on PSMCs and PAECs, Tam (10^{-7} mol/L), 4-OHT (10^{-7} mol/L), or Ral (10^{-7} mol/L) was added 5 minutes before 17βE treatment. Total proteins were prepared by the addition of 500 μL lysis buffer containing phenylmethylsulfonyl fluoride (1 mmol/L), leupeptin (10 μg/mL), aprotinin (30 μg/mL), and $NaVO_3$ (1 mmol/L, Sigma). Plates were incubated at 4°C for 30 minutes and scraped, and the protein concentration was determined with a protein kit (Bio-Rad). The same protein quantity for each cell type and condition was dissolved in Laemmli buffer, boiled for 5 minutes in reducing conditions, separated by 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 (containing 5% nonfat dry milk [Bio-Rad], 0.05% Tween 20, 0.15 mol/L NaCl, and 25 mmol/L Tris-HCl, pH 7.5) for 1 hour at room temperature with gentle agitation and incubated overnight at 4°C in 0.5% Blotto-TTBS with the addition of anti–phospho-p42/44 MAPK or anti–phospho-p38 MAPK rabbit polyclonal antiserums (α-pp42/44 [dilution 1:10 000] and α-pp38 [dilution 1:5000], respectively; New England BioLabs). Membranes were washed with TTBS and incubated at room temperature with an anti-rabbit IgG antibody coupled to horseradish peroxidase (dilution 1:10 000 to 1:20 000, 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). Kaleidoscope molecular weight and SDS-PAGE broad-range marker proteins (Bio-Rad) were used as standards for SDS-PAGE. Digital image densitometry (PDI Bioscience) was performed on x-ray film to determine the relative phosphorylation of p42/44 and p38 MAPK. All Western blot analysis was performed in triplicate, and the results of image densitometry are representative of these experiments.

Statistical Analysis

Data are mean±SEM. Statistical comparisons were performed by ANOVA, followed by an unpaired Student *t* test. A value of $P<0.05$ was considered significant.

Results

Effects of 17βE on PSMC Proliferation

First, we evaluated the effect of 17βE on PSMC proliferation. Stimulation of quiescent PSMCs with DMEM and 1% and 5% FBS significantly increased their proliferation from 9894±714 cells per well to 16 258±1441 and 42 500±2889 cells per well, respectively. Treatment with 17βE (10^{-8} mol/L) abrogated by 88% and 90% the PSMC proliferation mediated by 1% and 5% FBS, respectively (data not shown). Then, we investigated how different estrogen antagonists may interfere with 17βE activity. Treatment of quiescent PSMCs with DMEM and 1% FBS increased the PSMC cell count from 10 213±734 to 16 100±1 142 cells per well (Figure 1). Treatment with 17βE (10^{-8} mol/L) completely inhibited the 1% FBS mitogenic activity (Figure 1). The antimitogenic activity of 17βE on PSMCs was reversed by Tam (10^{-7} mol/L), 4-OHT (10^{-8} mol/L and 10^{-7} mol/L), and Ral (10^{-7} mol/L) by 75%, 81%, and 100%, respectively (Figure 1). In the absence of 17βE, treatment of PSMCs with these ER antagonists did not alter the mitogenic activity of 1% FBS (data not shown).

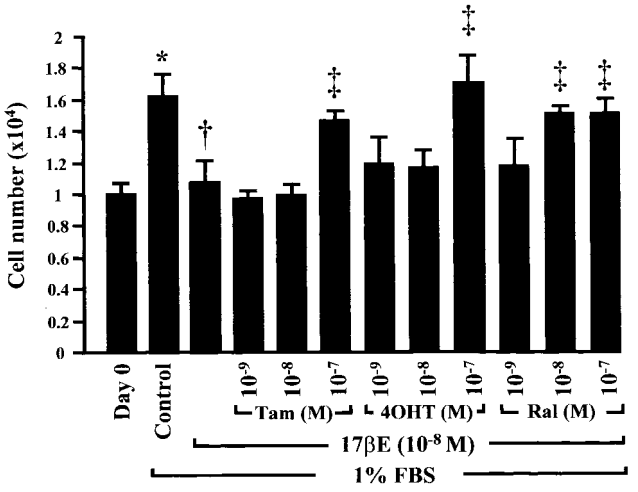


Figure 1. Effects of 17β-E and ER antagonists on PSMC proliferation. PSMCs were seeded at 1.5×10^4 cells per well and stimulated as described in Methods. The cells were then stimulated with 17β-E combined with Tam, 4-OHT, or Ral. Values are means of cell count obtained from 6 wells for each treatment. *$P<0.05$ compared with day 0; †$P<0.05$ compared with control; and ‡$P<0.05$ compared with 17β-E (10^{-8} mol/L).

Effects of 17β-E on PSMC Migration

By use of a modified Boyden chamber assay, PDGF-BB at 1, 5, and 10 ng/mL (compared with 1% DMEM) dose-dependently and significantly induced the migration of PSMCs by 96%, 137%, and 202%, respectively, 5 hours after treatment (data not shown). Treatment with 17β-E (10^{-8} mol/L) completely inhibited the chemotactic effect of 10 ng/mL PDGF-BB (Figure 2). To evaluate the interaction of ER antagonists with 17β-E on PSMC chemotactic activity, PSMCs were pretreated with Tam, 4-OHT, and Ral (10^{-9} to 10^{-7} mol/L) before adding 17β-E (10^{-8} mol/L). The anti-chemotactic effect of 17β-E was reversed completely by Tam, 4-OHT, and Ral at 10^{-7} mol/L (Figure 2). Treatment with these ER antagonists in the absence of 17β-E did not modify the effect of PDGF-BB on PSMC migration (data not shown).

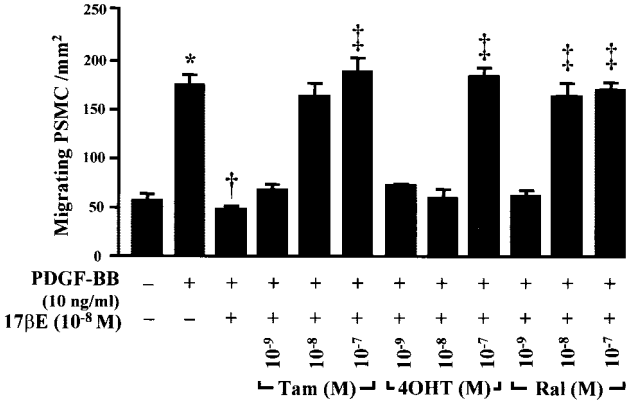


Figure 2. Effects of 17β-E and ER antagonists on PSMC migration. PSMCs were treated with Tam, 4-OHT, or Ral, and then 17β-E (10^{-8} mol/L) was added in the higher chamber of a modified Boyden chamber apparatus; the lower chamber was filled with DMEM and 1% FBS with PDGF-BB. Values are means of migrating cells per millimeter squared from 6 chambers for each treatment. *$P<0.05$ compared with day 0; †$P<0.05$ compared with control; and ‡$P<0.05$ compared with 17β-E (10^{-8} mol/L).

Effects of 17β-E on PSMC p42/44 and p38 MAPK Phosphorylation

Because PDGF-BB can induce SMC p42/44 and p38 MAPK phosphorylation, we investigated whether treatment with 17β-E might influence the phosphorylation of these MAPKs mediated by PDGF-BB. Treatment of PSMCs with PDGF-BB induced a rapid and transient phosphorylation of p42/44 MAPK within 5 minutes, which decreased below the basal level within 15 minutes (data not shown). Pretreatment with 17β-E (10^{-8} mol/L) inhibited time-dependently, with maximum inhibition at 30 minutes, the phosphorylation of p42/44 MAPK induced by 5-minute stimulation with PDGF-BB (Figure 3). Pretreatment with Tam, 4-OHT, or Ral (10^{-7} mol/L) 5 minutes before stimulation with 17β-E (30 minutes) reversed by 54%, 79% ($P<0.05$), and 100% ($P<0.05$), respectively, the inhibitory effect of 17β-E on PDGF-BB–mediated p42/44 MAPK phosphorylation (Figure 3).

The same series of experiments was performed on p38 MAPK phosphorylation induced by PDGF-BB. Stimulation of PSMCs with 10 ng/mL PDGF-BB (compared with PBS) induced the phosphorylation of p38 MAPK, which was maximal within 30 minutes as (data not shown). Treatment of PSMCs with 17β-E (10^{-8} mol/L) before stimulation with PDGF-BB (30 minutes) decreased the phosphorylation of p38 MAPK in a time-dependent manner and produced up to 85% inhibition of phosphorylation at 30 minutes after PDGF-BB treatment (Figure 3). Pretreatment with Tam, 4-OHT, and Ral (10^{-7} mol/L) reversed by 51%, 53%, and 32%, respectively, the effect of 17β-E (10^{-8} mol/L) on p38 MAPK induced by 30-minute stimulation with PDGF-BB (Figure 3). In the absence of PDGF-BB stimulation, treatment with 17β-E alone or with ER antagonists alone did not alter the basal phosphorylation of p42/44 and p38 MAPK on PSMCs (data not shown).

Effects of 17β-E on PAEC Proliferation

Quiescent PAECs were stimulated with DMEM and 1% FBS, which raised the basal cell count from $13\,328\pm560$ to $24\,244\pm843$ cells per well. The addition of 17β-E (10^{-10} to 10^{-7} mol/L) induced a dose-dependent proliferation of PAECs, with a maximum induction at 10^{-8} mol/L (data not shown). To investigate how ER antagonists may interfere with the positive effect of 17β-E on endothelial cell proliferation, quiescent PAECs were stimulated with DMEM and 1% FBS, which raised the cell count from $10\,512\pm832$ to $29\,138\pm870$ cells per well in 72 hours. Treatment of PAECs with 17β-E (10^{-8} mol/L) induced the proliferation of PAECs by 37% over 1% FBS treatment (Figure 4). Pretreatment with Tam, 4-OHT, and Ral (10^{-7} mol/L) completely inhibited the 17β-E mitogenic activity in PAECs (Figure 4). Treatment of these cells with ER antagonists in the absence of 17β-E did not affect the mitogenic effect of 1% FBS (data not shown).

Effects of 17β-E on PAEC Migration

Compared with treatment with 1% DMEM, treatment with bFGF at 1, 5, and 10 ng/mL dose-dependently induced the

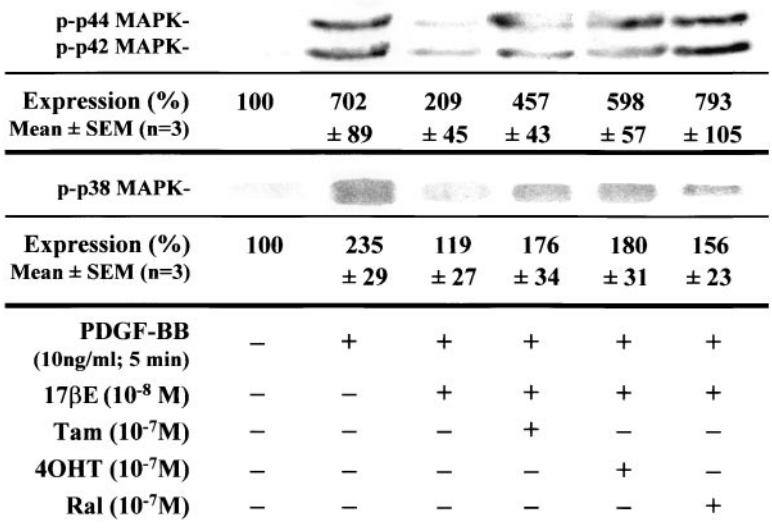


Figure 3. Effects of 17βE and ER antagonists on PSMC p42/44 and p38 MAPK activity. PSMCs were rinsed and pretreated for 5 minutes with Tam, 4-OHT, or Ral (10^{-7} mol/L) and then treated with 17βE (10^{-8} mol/L) for 30 minutes and stimulated with PDGF-BB for 5 minutes (for p42/44 MAPK) and 30 minutes (for p38 MAPK). Proteins were detected by Western blot analysis. Image densitometry results are given as relative expression (percentage) compared with PBS-treated cells.

migration of PAECs by 46%, 124%, and 114%, respectively, in 5 hours ($P<0.05$, data not shown). In another series of experiments, compared with bFGF (10 ng/mL) treatment alone, a combined treatment with 17βE (10^{-8} mol/L) significantly stimulated (by 121%) PAEC migration (Figure 5). Pretreatment of PAECs with ER antagonists (10^{-7} mol/L) 5 minutes before the addition of 17βE completely prevented the chemotactic activity of 17βE (10^{-8} mol/L) on PAECs (Figure 5). We also investigated whether the ER antagonists in the absence of 17βE had an effect on bFGF chemotactic activity. The ER antagonists did not alter the chemotactic activity of bFGF (10 ng/mL) on PAECs (data not shown).

Effects of 17βE on PAEC p42/44 and p38 MAPK Phosphorylation

Considering that 17βE can stimulate the proliferation and migration of PAECs, we evaluated the effect of 17βE on the phosphorylation of p42/44 and p38 MAPK in PAECs. Control (PBS-treated) PAECs showed a basal phosphorylation of p42/44 MAPK. Stimulation with 17βE (10^{-8} mol/L) increased time-dependently at 5, 10, 15, and 30 minutes the phosphorylation of p42/44 MAPK by 1122%, 1074%, 1420%, and 1835%, respectively (data not shown). Pretreatment with Tam, 4-OHT, or Ral (10^{-7} mol/L) 5 minutes before the addition of 17βE decreased by 36% ($P<0.05$), 44% ($P<0.05$), and 66% ($P<0.05$) the phosphorylation of p42/44 MAPK mediated by a 5-minute treatment with 10^{-8} mol/L 17βE (Figure 6). Similar to the results for p42/44 MAPK, treatment of PAECs with 17βE at 10^{-8} mol/L (compared with unstimulated [PBS-treated] PAECs) induced a time-dependent phosphorylation of p38 MAPK, with a maximum stimulation at 30 minutes (data not shown). Pretreatment with ER antagonists (10^{-7} mol/L) 5 minutes before the addition of 17βE inhibited by 84%, 81% ($P<0.05$), and 98% ($P<0.05$) the phosphorylation of p38 MAPK induced by a 30-minute treatment with 17βE at 10^{-8} mol/L (Figure 6). In the absence of 17βE stimulation, treatment with the ER antagonists did not alter the basal phosphorylation of PAEC p42/44 and p38 MAPK (data not shown).

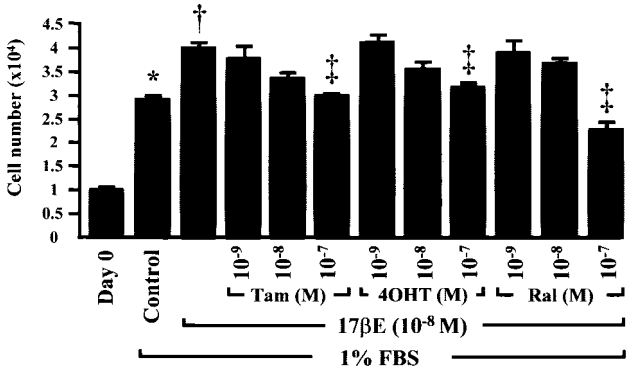


Figure 4. Effects of 17βE and ER antagonists on PAEC proliferation. PAECs were seeded at 1.5×10^4 cells per well and stimulated as described in Methods. The cells were treated with 17βE plus Tam, 4-OHT, or Ral in DMEM and 1% FBS. Values are means of cell count obtained from 6 wells for each treatment. Values are means of migrating cells per millimeter squared from 6 chambers for each treatment. *$P<0.05$ compared with PBS; †$P<0.05$ compared with bFGF (10 ng/mL); and ‡$P<0.05$ compared with 17βE (10^{-8} mol/L).

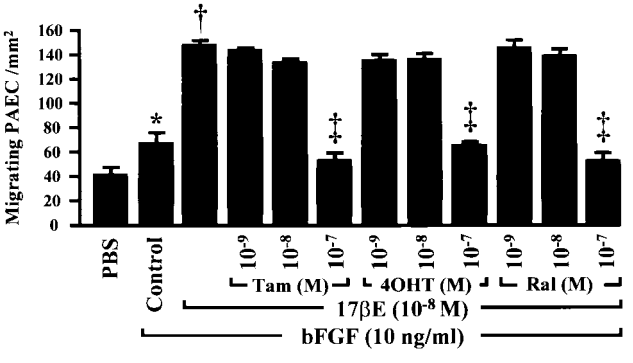
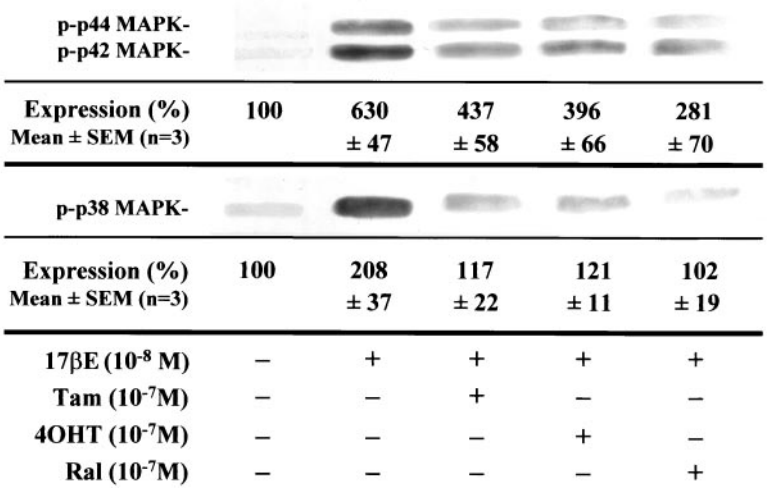


Figure 5. Effects of 17βE and ER antagonists on PAEC migration. PAECs were treated with 17βE, and Tam, 4-OHT, or Ral was added in the higher chamber of the modified Boyden chamber apparatus, and the lower chamber was filled with DMEM and 1% FBS with bFGF. Values are means of migrating cells per millimeter squared from 6 chambers for each treatment. *$P<0.05$ compared with PBS; †$P<0.05$ compared with bFGF (10 ng/mL); and ‡$P<0.05$ compared with 17βE (10^{-8} mol/L).



Expression (%) Mean ± SEM (n=3)	100	630 ± 47	437 ± 58	396 ± 66	281 ± 70
Expression (%) Mean ± SEM (n=3)	100	208 ± 37	117 ± 22	121 ± 11	102 ± 19
17βE (10^{-8} M)	−	+	+	+	+
Tam (10^{-7}M)	−	−	+	−	−
4OHT (10^{-7}M)	−	−	−	+	−
Ral (10^{-7}M)	−	−	−	−	+

Figure 6. Effects of 17βE and ER antagonists on PAEC p42/44 and p38 MAPK activity. PAECs were rinsed and pretreated for 5 minutes with Tam, 4-OHT, or Ral and then stimulated with 17βE for 5 minutes (for p42/44 MAPK) and for 30 minutes (for p38 MAPK). Proteins were detected by Western blot analysis. Image densitometry results are given as relative expression (percentage) compared with PBS-treated cells.

Discussion

Several studies have shown that estrogen treatment may have beneficial effects on the cardiovascular system by reducing postinjury neointimal formation in animal carotid arteries[20] and by improving some aspects of endothelial function in postmenopausal women.[21] We have previously demonstrated that a local delivery of 17βE on a porcine coronary angioplasty reduces the degree of restenosis by up to 50% and improves the reendothelialization, eNOS expression, and vascular healing.[11,22] In the present study, we observed that treatment with 17βE stimulates PAECs but reverses PSMC phosphorylation of p42/44 and p38 MAPK. Our results also suggest that these effects of 17βE are at least partially ER dependent.

Antimitogenic and Antichemotactic Effects of 17βE in PSMCs

VSMCs contribute to the pathological formation of restenosis by migrating from the media to the intima, proliferating, and depositing extracellular matrix proteins. PDGF, which is secreted from platelets and macrophages recruited at the early inflammatory lesion, has been described as playing an important role in restenosis. In animal experiments, PDGF-BB has been associated with SMC proliferation and migration.[1] In the present study, treatment with 17βE (10^{-8} mol/L) inhibited the proliferation and migration of PSMCs stimulated by PDGF-BB. Our results are consistent with those of other authors who were able to demonstrate that estrogen can inhibit VSMC proliferation[5] and migration[6] in in vitro and in vivo experiments. It is also well established that PDGF-BB activates ERKs, such as MAPKs.[16] We have demonstrated that PDGF-BB can induce the phosphorylation of p42/44 MAPK and p38 MAPK within 5 and 30 minutes, respectively, in PSMCs. To better understand the nongenomic effects of estrogen, we have shown that treatment of PSMCs with 17βE for a short period of time reduced the phosphorylation of p42/44 and p38 MAPK mediated by PDGF-BB by 100% and 85%, respectively. A previous study has also observed that estrogens mediate their inhibitory effects on SMCs by reducing p42/44 MAPK activity.[23] To determine whether the antimitogenic and antichemotactic effects of 17βE on PSMCs are ER dependent or independent, we pretreated these cells with ER antagonists before treatment with 17βE. We showed that pretreatment of PSMCs with ER antagonists reversed the effect of 17βE, ie, prevention of the phosphorylation of p42/44 and p38 MAPK induced by PDGF-BB. These ER antagonists are also called selective ER modulators and may have potential positive effects on cardiovascular diseases.[24] In contrast, we have not observed any beneficial effect of Tam, 4-OHT, or Ral alone in the prevention of PSMC proliferation and migration and MAPK activity mediated by PDGF-BB.

17βE Promotes Reendothelialization by Increasing the Proliferation and Migration of PAECs

Reendothelialization plays a critical role in restenosis. The improvement of endothelium regeneration will accelerate vascular healing after balloon injury and will reduce neointimal formation. A previous study has noted that the administration of estrogen in healthy young men is associated with enhanced arterial endothelial function.[25] We have shown that local delivery of 17βE improves reendothelialization and eNOS expression after angioplasty.[11] In the present study, we propose that 17βE increases reendothelialization by increasing the proliferation and migration of PAECs. Previous studies have demonstrated that estrogen can rapidly induce the activation of various pathways.[26] To examine the nongenomic effects of 17βE on PAEC proliferation and migration, we evaluated the MAPK activity of these cells after a brief administration of 17βE. Our results demonstrated that treatment of PAECs with 17βE increased p42/44 and p38 MAPK phosphorylation within 5 and 30 minutes of stimulation, respectively. These results support other studies that have observed that estrogen can preserve the actin cytoarchitecture during metabolic stress, induce the migration of endothelial cells by stimulation of the p38 MAPK pathway,[27] and stimulate p42/44 MAPK in human endothelial cells.[28] To determine whether these rapid activations of p42/44 and p38 MAPK by 17βE are ER dependent or independent, PAECs were pretreated with Tam, 4-OHT, or Ral. Our results suggest that pretreatment of PAECs with ER antagonists reverses the phosphorylation of p42/44 and p38 MAPK mediated by

17β-E. Interestingly, treatment of PAECs with ER antagonists alone did not affect the proliferation, migration, or MAPK activity of these cells.

In conclusion, an acute administration of 17β-E activates p42/44 and p38 MAPK, thus promoting the proliferation and migration of PAECs, and in contrast, it inhibits these events in PSMCs. Our results suggest that the beneficial effects of treatment with 17β-E on restenosis may be explained by a reduction of PSMC migration and proliferation combined with positive endothelial cell migrating and proliferating activity. These effects of 17β-E appear to be at least partially ER dependent.

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