

Estradiol Inhibits Smooth Muscle Cell Growth in Part by Activating the cAMP-Adenosine Pathway

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Abstract—Estradiol inhibits smooth muscle cell growth; however, the mechanisms involved remain unclear. Because estradiol stimulates cAMP synthesis and adenosine inhibits cell growth, we hypothesized that the conversion of cAMP to adenosine (ie, the cAMP-adenosine pathway) mediates in part the inhibitory effects of estradiol on vascular smooth muscle cell growth. To test this hypothesis, we examined the effects of estradiol (0.001 to 1 $\mu\text{mol/L}$) on serum-induced DNA, collagen, and total protein synthesis and cell number in the absence and presence of 1,3-dipropyl-8-*p*-sulfophenylxanthine (10 nmol/L; A_1/A_2 adenosine receptor antagonist), KF17837 (10 nmol/L; selective A_2 adenosine receptor antagonist), 8-cyclopentyl-1,3-dipropylxanthine (10 nmol/L; selective A_1 adenosine receptor antagonist), and 2',5'-dideoxyadenosine (10 $\mu\text{mol/L}$; adenylyl cyclase inhibitor). Estradiol inhibited all measures of cell growth, and the concentration-dependent inhibitory curves for estradiol were shifted to the right ($P < 0.05$) by 1,3-dipropyl-8-*p*-sulfophenylxanthine, KF17837, and 2',5'-dideoxyadenosine but not by 8-cyclopentyl-1,3-dipropylxanthine. Moreover, the inhibitory effects of estradiol were enhanced by stimulation of adenylyl cyclase with forskolin and by inhibition of adenosine metabolism with erythro-9-(2-hydroxy-3-nonyl)adenine plus iodotubericidin (adenosine deaminase and kinase inhibitors, respectively). Estradiol also increased levels of cAMP and adenosine, and these effects were blocked by 2',5'-dideoxyadenosine ($P < 0.05$). Our results support the hypothesis that estradiol stimulates cAMP synthesis and cAMP-derived adenosine regulates smooth muscle cell growth via A_2 adenosine receptors. Thus, the cAMP-adenosine pathway may contribute importantly to the antivasoocclusive effects of estradiol. (*Hypertension*. 2000;35[part 2]:262-266.)

Key Words: hormones ■ cyclic AMP ■ adenosine ■ muscle, smooth ■ receptors, adenosine ■ vascular remodeling

17 β -Estradiol is a potent inhibitor of vascular smooth muscle cell (SMC) growth¹⁻⁴; however, the mechanisms responsible for this inhibitory effect remain unclear. Recent studies from our group demonstrate that adenosine via the A_2 receptor is an effective inhibitor of vascular SMC growth⁵⁻⁷ and that in vascular SMCs, cAMP is an important determinant of adenosine production via a biochemical mechanism we refer to as the cAMP-adenosine pathway.⁶ This pathway involves the conversion of cAMP to AMP and hence to adenosine by the enzymes phosphodiesterase and 5'-nucleotidase, respectively. Moreover, our studies show that the inhibitory effects of exogenous cAMP on SMC growth are blocked by antagonism of A_2 receptors,⁶ thus implying that the cAMP-adenosine pathway regulates SMC growth. Since 17 β -estradiol increases cAMP levels in vascular SMCs,⁸ it is possible that the inhibitory effects of estradiol on SMC growth are mediated in part by the cAMP-adenosine pathway. This hypothesis was tested in the present study by determining whether estradiol increases cAMP and adenosine

levels in SMCs via a mechanism that is blocked by inhibition of adenylyl cyclase, by determining whether the inhibitory effects of estradiol on SMC growth are blocked by inhibition of adenylyl cyclase and antagonism of adenosine receptors, and by determining whether the effects of estradiol on SMC growth and adenosine levels are augmented by preventing the conversion of adenosine to inactive metabolites.

Methods

Aortic SMC Culture

Aortic SMCs were cultured as explants from the descending abdominal aortas obtained from anesthetized Sprague-Dawley male rats (Charles River, Wilmington, Mass) as described previously.⁷ SMC purity was characterized as described in detail previously.⁷ SMCs in primary cultures or first passage were used for all the experiments.

Treatments

DNA, collagen, total protein synthesis, and cell proliferation studies were performed under the following treatments: (1) 17 β -estradiol; (2) 17 β -estradiol plus 8-cyclopentyl-1,3-dipropylxanthine (DPCPX,

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a selective A₁ receptor antagonist); (3) 17 β -estradiol plus KF17837 (a selective A₂ receptor antagonist); (4) 17 β -estradiol plus 1,3-dipropyl-8-*p*-sulfophenylxanthine (DPSPX, a nonselective A₁/A₂ adenosine receptor antagonist); (5) 17 β -estradiol plus erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA, an inhibitor of adenosine deaminase) plus iodotubercidin (an adenosine kinase inhibitor); (6) 17 β -estradiol plus (R)-*p*-adenosine 3',5'-cyclic phosphorothioate (Rp-cAMP, cAMP-dependent protein kinase A inhibitor); (7) 17 β -estradiol plus 2',5'-dideoxyadenosine (DDA, adenylyl cyclase inhibitor), (8) EHNA plus iodotubercidin; (9) DPCPX; (10) DPSPX; (11) KF17837; (12) DDA; and (13) Rp-cAMP.

DNA, Collagen, and Total Protein Synthesis

[³H]Thymidine, [³H]proline, and [³H]leucine incorporation studies were performed as a measure of DNA, collagen, and total protein synthesis, respectively. SMCs were plated at a density of 2.5 × 10⁴ cells per well in 24-well tissue culture dishes and allowed to grow in DMEM/F12 containing 10% FCS under standard tissue culture conditions. For DNA synthesis and cell number studies SMCs were grown to subconfluence, whereas for protein and collagen synthesis studies SMCs were grown to confluence. The monolayers of SMCs were then growth arrested by feeding DMEM containing 0.4% bovine serum albumin for 48 hours. Growth was stimulated by treating growth-arrested SMCs with DMEM supplemented with 2.5% FCS and containing or lacking the various treatments as described above. For DNA and total protein synthesis after 20 hours of incubation, the cells were pulsed with [³H]thymidine (1 μ Ci/mL) or [³H]leucine (1 μ Ci/mL), respectively, for an additional 4 hours. For collagen synthesis, the cells were treated for 36 hours in the presence of [³H]L-proline (1 μ Ci/mL). The experiments were terminated by washing the cells twice with Dulbecco's PBS and twice with ice-cold trichloroacetic acid (10%). The precipitate was solubilized in 500 μ L of 0.3N NaOH and 0.1% SDS after incubation at 50°C for 2 hours. Aliquots from 4 wells for each treatment with 10 mL scintillation fluid were counted in a liquid scintillation counter, and each experiment was conducted with 4 separate cultures. [³H]Thymidine studies were conducted in subconfluent monolayers. However, to ensure that changes in collagen and protein synthesis were not due to a decrease in cell number, these experiments were conducted in confluent monolayers of cells in which changes in cell number were precluded. Cell counting was performed in cells treated in parallel to the cells used for the collagen and total protein synthesis, and the data were normalized to cell number.

Cell Proliferation

Cell counting was performed as a direct measure of cell proliferation. Trypsinized SMCs were suspended in DMEM/F12 containing 10% FCS and plated in a 24-well culture dish at a density of 1 × 10⁴ cells per well. After incubation for 18 hours, the cells were fed DMEM containing 0.25% FCS for 48 hours to growth arrest the cells. SMCs were then treated every 24 hours for 4 days with DMEM supplemented with 2.5% FCS and containing or lacking various treatments, as described above. The treatments were terminated on day 5, and cells were dislodged with trypsin-EDTA, diluted in Isoton-II, and counted with a Coulter counter. Aliquots from 3 wells were counted for each group with 4 separate cultures.

Adenosine and cAMP Measurements

Monolayers of SMCs grown to confluence in 12-well culture plates were washed twice with PBS and then incubated with buffered (HEPES, 25 mmol/L; NaHCO₃, 13 mmol/L) PBS containing or lacking the above treatments. After 10 minutes or 4 hours of incubation, the supernatants were collected, and the cells were treated with 1 mL of ice-cold propanediol and pooled together with the supernatants. Total adenosine and cAMP levels were then analyzed by high-performance liquid chromatography with a previously described method.⁹ In cells grown in parallel and treated similarly, the monolayers were inspected microscopically for intactness, the number of cells were counted, and the data were normalized to cell number.

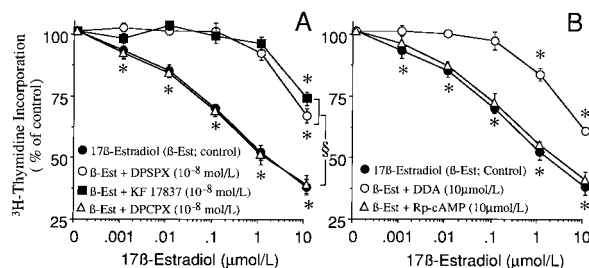


Figure 1. Inhibitory effects of 17 β -estradiol on 2.5% FCS-induced [³H]thymidine incorporation in the presence and absence of adenosine receptor antagonists (DPCPX, 10 nmol/L; DPSPX, 10 nmol/L; and KF17837, 10 nmol/L) (A), an adenylyl cyclase inhibitor (DDA, 10 μ mol/L) (B), and a cAMP-dependent protein kinase A inhibitor (Rp-cAMP, 10 μ mol/L) (B). Values are mean \pm SEM from 3 experiments conducted in quadruplicate. **P* < 0.05 vs control; §*P* < 0.05 vs estradiol.

Statistical Analysis

All experiments were performed in triplicate or quadruplicate with 4 separate cultures, and all data are presented as mean \pm SEM. Statistical analysis was performed with ANOVA, paired Student's *t* test, or Fisher's least significant difference test, as appropriate. A value of *P* < 0.05 was considered statistically significant.

Results

Treatment with FCS significantly (*P* < 0.05, paired Student's *t* test) stimulated [³H]thymidine, [³H]proline, and [³H]leucine incorporation and cell number by \approx 5- to 8-fold. Treatment with 17 β -estradiol significantly (*P* < 0.05, ANOVA) inhibited FCS-induced [³H]thymidine incorporation in a concentration-dependent manner (Figure 1). In this regard, significant inhibition occurred even with physiological concentrations of 17 β -estradiol (1 nmol/L), and a 50% decrease was observed at \approx 1 μ mol/L of 17 β -estradiol (Figure 1).

Similar to the effects on [³H]thymidine incorporation, 17 β -estradiol inhibited FCS-induced [³H]proline and [³H]leucine incorporation and cell number in a concentration-dependent fashion (Figures 2, 3, and 4, respectively; *P* < 0.05, ANOVA followed by Fisher's least significant test). The lowest concentration of 17 β -estradiol that significantly inhibited FCS-induced increases in cell number was 1 nmol/L, and this concentration inhibited cell number by 16 \pm 1.6%.

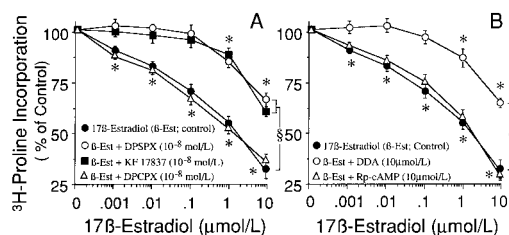


Figure 2. Inhibitory effects of 17 β -estradiol on 2.5% FCS-induced [³H]proline incorporation in the presence and absence of adenosine receptor antagonists (DPCPX, 10 nmol/L; DPSPX, 10 nmol/L; and KF17837, 10 nmol/L) (A), an adenylyl cyclase inhibitor (DDA, 10 μ mol/L) (B), and a cAMP-dependent protein kinase A inhibitor (Rp-cAMP, 10 μ mol/L) (B). Cell counts in SMCs treated with various combinations were not different from those in controls (<3% variation); cell counts in controls were 1.46 \pm 0.07 \times 10⁵ cells per well and in cells treated with various combinations ranged from 1.44 \times 10⁵ to 1.49 \times 10⁵ cells per well. Values are mean \pm SEM from 3 experiments conducted in quadruplicate. **P* < 0.05 vs control; §*P* < 0.05 vs estradiol.

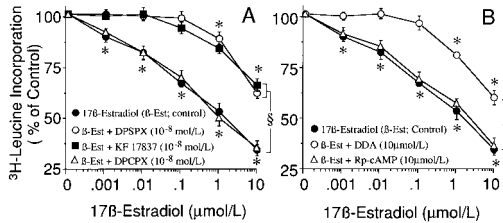


Figure 3. Inhibitory effects of 17β-estradiol on 2.5% FCS-induced [³H]leucine incorporation in the presence and absence of adenosine receptor antagonists (DPCPX, 10 nmol/L; DPSPX, 10 nmol/L; and KF 17837, 10 nmol/L) (A), an adenylyl cyclase inhibitor (DDA, 10 μmol/L) (B), and a cAMP-dependent protein kinase A inhibitor (Rp-cAMP, 10 μmol/L) (B). Cell counts in SMCs treated with various combinations were not different from those in controls (<3% variation); cell counts in controls were 1.43±0.04×10⁵ cells per well and in cells treated with various combinations ranged from 1.41×10⁵ to 1.46×10⁵ cells per well. Values are mean±SEM from 3 experiments conducted in quadruplicate. *P<0.05 vs control; §P<0.05 vs estradiol.

To investigate whether adenosine and its receptors participate in mediating the inhibitory effects of estradiol, the effects of 17β-estradiol (0.001 to 10 μmol/L) on SMC growth were examined in the presence and absence of DPCPX (10 nmol/L; a specific A₁ adenosine receptor antagonist), KF17837 (10 nmol/L; a specific A₂ adenosine receptor antagonist), and DPSPX (10 nmol/L; a nonselective A₁/A₂ adenosine receptor antagonist). The inhibitory effects of 17β-estradiol on FCS-induced [³H]thymidine, [³H]proline, and [³H]leucine incorporation and cell number were significantly reduced in SMCs pretreated with KF17837 and DPSPX but not DPCPX, and the inhibitory curves for 17β-estradiol were shifted to the right (Figures 1A through 4A; P<0.05, ANOVA followed by Fisher's least significant test).

To investigate whether cAMP and cAMP-dependent protein kinase A participate in mediating the inhibitory effects of estradiol, the effects of 17β-estradiol (0.001 to 10 μmol/L) on SMC growth were examined in the presence and absence of DDA (10 μmol/L; an adenylyl cyclase inhibitor) and Rp-

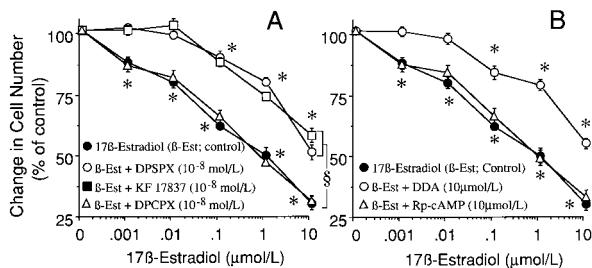


Figure 4. Inhibitory effects of 17β-estradiol on 2.5% FCS-induced cellular proliferation (cell number) in the presence and absence of adenosine receptor antagonists (DPCPX, 10 nmol/L; DPSPX, 10 nmol/L; and KF17837, 10 nmol/L) (A), an adenylyl cyclase inhibitor (DDA, 10 μmol/L) (B), and a cAMP-dependent protein kinase A inhibitor (Rp-cAMP, 10 μmol/L) (B). Cell counts in controls (SMCs treated with FCS) were 1.81±0.06×10⁴ cells per well on day 0 and 1.33×10⁵ cells per well on day 4. The cell counts in controls of various groups (ie, cells treated with FCS±DPCPX, DPSPX, KF17837, Rp-cAMP, or DDA) on days 0 and 4 were not different from those in controls (<4% variation). Values are mean±SEM from 3 experiments conducted in quadruplicate. *P<0.05 vs control; §P<0.05 vs estradiol.

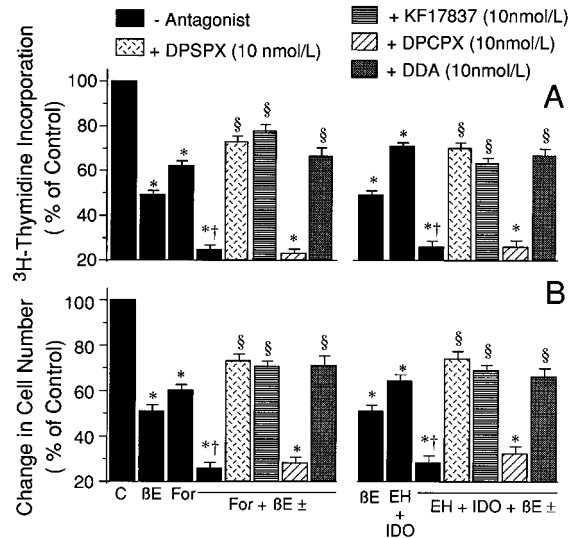


Figure 5. Modulatory effects of forskolin (For) (5 μmol/L) and EHNA (EH) (10 μmol/L) plus iodotubercidin (IDO) (0.1 μmol/L) on 17β-estradiol (βE) (1 μmol/L)-induced inhibition of 2.5% FCS-induced [³H]thymidine incorporation (A) and cell number (B) in the presence and absence of adenosine receptor antagonists (DPCPX, 10 nmol/L; DPSPX, 10 nmol/L; and KF17837, 10 nmol/L) and an adenylyl cyclase inhibitor (DDA, 10 μmol/L). Values are mean±SEM from 3 experiments conducted in quadruplicate. *P<0.05 vs control; §P<0.05 vs EHNA+iodotubercidin+17β-estradiol, forskolin+17β-estradiol; †P<0.05 vs 17β-estradiol, EHNA+iodotubercidin, or forskolin alone.

cAMP (10 μmol/L; a protein kinase A inhibitor). The inhibitory effects of 17β-estradiol on FCS-induced [³H]thymidine, [³H]proline, and [³H]leucine incorporation and cell number were significantly reduced in SMCs pretreated with DDA but not Rp-cAMP, and the inhibitory curves for 17β-estradiol were shifted to the right (Figures 1B through 4B; P<0.05, ANOVA followed by Fisher's least significant test).

To further investigate the role of cAMP and adenosine in mediating the inhibitory effects of estradiol on SMC growth, the effects of 17β-estradiol (1 μmol/L) on SMC growth were investigated in the presence and absence of forskolin (5 μmol/L; activates adenylyl cyclase and induces endogenous cAMP levels) and EHNA (10 μmol/L; prevents adenosine catabolism by inhibiting adenosine deaminase) plus iodotubercidin (0.1 μmol/L; elevates adenosine levels by inhibiting adenosine kinase activity). Analysis of the data by ANOVA followed by Fisher's least significant test revealed that the inhibitory effects of estradiol on [³H]thymidine incorporation and cell proliferation were significantly enhanced by either forskolin or EHNA plus iodotubercidin (Figure 5A and 5B). Moreover, the increase in the inhibitory effect of 17β-estradiol by forskolin or EHNA plus iodotubercidin was abolished in SMCs pretreated with DDA (10 μmol/L), DPSPX (10 nmol/L), or KF17837 (10 nmol/L) but not DPCPX (10 nmol/L) (Figure 5A and 5B).

To ascertain whether the inhibitory effects of 17β-estradiol were mediated by cAMP-derived adenosine, we assayed the levels of cAMP and adenosine in SMCs treated for 4 hours with various treatments (Figure 6). Compared with SMCs treated with PBS, both cAMP and adenosine levels increased

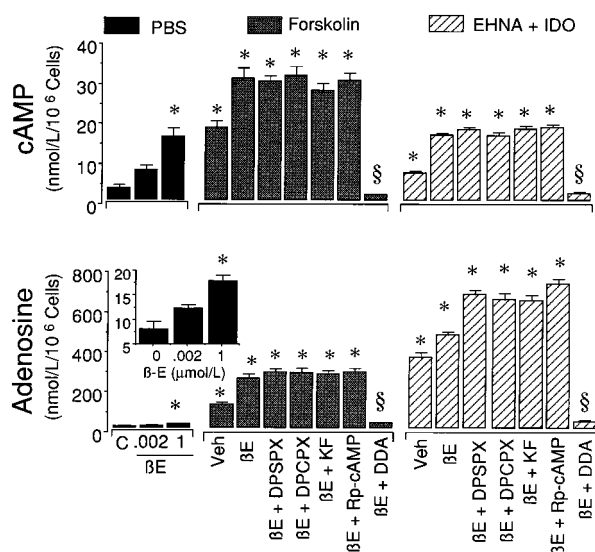


Figure 6. Modulatory effects of forskolin (5 $\mu\text{mol/L}$) and EHNA (10 $\mu\text{mol/L}$) plus iodotubercidin (0.1 $\mu\text{mol/L}$) on 17 β -estradiol (1 $\mu\text{mol/L}$ or as indicated in the figure)-induced adenosine and cAMP synthesis from SMCs treated for 4 hours in the presence and absence of adenosine receptor antagonists (DPCPX, 10 nmol/L; DPSPX, 10 nmol/L; and KF17837 [KF], 10 nmol/L), an adenylyl cyclase inhibitor (DDA, 10 $\mu\text{mol/L}$), and a cAMP-dependent protein kinase A inhibitor (Rp-cAMP, 10 $\mu\text{mol/L}$). Values are mean \pm SEM from 3 experiments conducted in quadruplicate. * $P < 0.05$ vs control; § $P < 0.05$ vs estradiol, forskolin, or EHNA plus IDO alone. Veh indicates vehicle; other abbreviations are as defined in Figure 5 legend.

significantly (paired Student's *t* test) in SMCs treated with 17 β -estradiol or forskolin. Moreover, the ability of 17 β -estradiol to increase adenosine levels was enhanced significantly ($P < 0.05$) by cotreatment with forskolin or EHNA plus iodotubercidin. The stimulatory effects of 17 β -estradiol with and without forskolin or EHNA plus iodotubercidin on cAMP and adenosine levels were inhibited by DDA but not by DPSPX, DPCPX, KF17837, or Rp-cAMP (ANOVA followed by Fisher's least significant test).

To determine whether the effects of 17 β -estradiol (1 $\mu\text{mol/L}$) on cAMP and adenosine levels were estrogen receptor mediated and nongenomic in nature, cAMP and adenosine levels were measured in SMCs treated for 10 minutes with 17 β -estradiol in the presence and absence of ICI 182780 (50 $\mu\text{mol/L}$; an estrogen receptor antagonist without partial agonist activity) and cycloheximide (10 $\mu\text{mol/L}$). Treatment of SMCs for 10 minutes with 17 β -estradiol increased cAMP as well as adenosine levels by almost 100%, and the stimulatory effects of 17 β -estradiol were blocked by ICI 182780 but not cycloheximide (data not shown).

Discussion

Our previous studies establish that SMCs synthesize adenosine and that exogenous as well as endogenous (SMC-derived) adenosine inhibits FCS-induced growth.⁵⁻⁷ Moreover, our prior studies demonstrate that SMCs express an active cAMP-adenosine pathway, ie, the conversion of cAMP to AMP and then to adenosine by phosphodiesterase and 5'-nucleotidase, respectively.⁶ Because 17 β -estradiol is known to increase cAMP levels in SMCs, we hypothesized

that the cAMP-adenosine pathway participates in mediating the well-known inhibitory effects of 17 β -estradiol on SMC growth.¹⁻⁴

The results of the present study indicate that adenosine importantly contributes to the inhibitory effects of 17 β -estradiol on SMC growth. Our findings that the inhibitory effects of 17 β -estradiol on SMC growth are significantly attenuated by KF17837, a specific A₂ adenosine receptor antagonist, and by DPSPX, a nonselective A₂ adenosine receptor antagonist, but not by DPCPX, a selective A₁ adenosine receptor antagonist, suggest that 17 β -estradiol inhibits SMC growth by a mechanism involving interactions of endogenous adenosine with A₂ adenosine receptors. Additional evidence for the role of adenosine in mediating the inhibitory effects of 17 β -estradiol are our observations that (1) treatment of SMCs with 17 β -estradiol increases adenosine levels; (2) the effects of 17 β -estradiol on SMC growth and adenosine levels are enhanced by EHNA and iodotubercidin, drugs that elevate endogenous adenosine levels by inhibiting adenosine deaminase and adenosine kinase activity, respectively; and (3) DPSPX and KF17837, but not DPCPX, block the inhibitory effects of 17 β -estradiol on SMC growth even in the presence of EHNA and iodotubercidin without influencing adenosine levels. Together, these findings provide evidence that 17 β -estradiol inhibits vascular SMC growth in part by increasing adenosine levels.

The results of the present study suggest that increased adenosine synthesis in response to 17 β -estradiol is derived from cAMP. This hypothesis is supported by our findings that 17 β -estradiol causes a concomitant increase in cAMP and adenosine levels and that inhibition of adenylyl cyclase attenuates 17 β -estradiol-induced increases in cAMP and adenosine levels. The observation that inhibition of adenylyl cyclase also attenuates the growth-inhibitory effects of 17 β -estradiol suggests that cAMP-derived adenosine participates in the mechanism by which 17 β -estradiol inhibits SMC growth.

The fact that inhibition of adenylyl cyclase prevents 17 β -estradiol-induced inhibition of vascular SMC growth is consistent with 2 hypotheses that are not mutually exclusive: (1) 17 β -estradiol increases cAMP levels, and the direct intracellular effects of cAMP via cAMP-dependent protein kinase mediates the growth-inhibitory actions of 17 β -estradiol; and (2) 17 β -estradiol increases cAMP levels, and cAMP is metabolized to adenosine, which mediates the growth-inhibitory actions of 17 β -estradiol. The present study supports the second, but not the first, mechanism since we observe that the growth-inhibitory effects of 17 β -estradiol are reduced by antagonism of adenosine receptors but are not reduced by blockade of cAMP-dependent protein kinase with Rp-cAMP. In this regard, our previous studies demonstrate that adenosine stimulates nitric oxide release from vascular SMCs via a pathway not involving adenylyl cyclase/protein kinase A.¹⁰ Since nitric oxide inhibits SMC proliferation,¹¹ this provides a protein kinase A-independent pathway via which 17 β -estradiol-induced adenosine could inhibit SMC growth.

Although the present studies focus on vascular SMCs, our previous studies demonstrate that not only vascular SMCs⁶

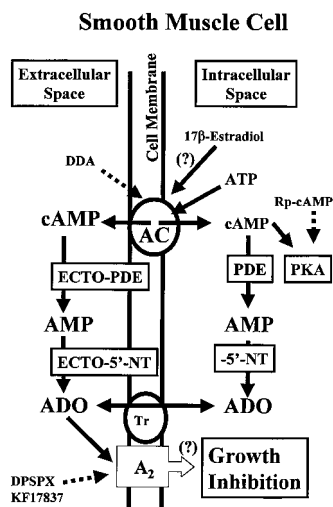


Figure 7. Hypothesis: Via activation of adenylyl cyclase, 17 β -estradiol stimulates cAMP synthesis and activates the cAMP-adenosine pathway (ie, the conversion of cAMP to AMP by phosphodiesterase [PDE] and hence to adenosine by 5'-nucleotidase [5'-NT]). This provides adenosine to A₂ receptors, which triggers the antimitogenic effects of 17 β -estradiol. "(?)" indicates mechanism is unknown; dashed lines, inhibition; AC, adenylyl cyclase; ADO, adenosine; and Tr, nucleoside transporter.

but also mesangial cells¹² and cardiac fibroblasts¹³ express the cAMP-adenosine pathway. Moreover, in all these cell types, cAMP-derived adenosine inhibits mitogen-induced cell growth.^{6,12,13} Therefore, it is possible that the mechanism revealed by the present study applies to a broader array of cardiovascular tissues/cell types.

The mechanism uncovered by the present study should be viewed in a wider context. Other mechanisms by which 17 β -estradiol induces its protective effects on the cardiovascular system include reducing apoptosis and inducing endothelial cell recovery and growth, improving endothelial-mediated degradation of LDL cholesterol, suppressing collagen and elastin synthesis, restoring endothelium-dependent vasodilator mechanisms after injury, reducing LDL levels, increasing HDL levels, preventing oxidation of LDL, releasing prostaglandins from vascular endothelial cells, reducing the adhesion of activated monocytes to the endothelium by inhibiting the expression of adhesion molecules, inhibiting cardiac fibroblast growth, and inhibiting MAP kinase activity.^{2,14} Our finding that 17 β -estradiol induces antiproliferative effects on SMCs via the cAMP-adenosine pathway provides evidence of yet another mechanism by which 17 β -estradiol induces cardioprotective effects.

The effects of 17 β -estradiol on cell function are thought to be mediated via estrogen receptor α and β and to involve genomic pathways. The finding that treatment of SMCs with 17 β -estradiol for 10 minutes increases cAMP and adenosine levels by \approx 100% and that these effects are blocked by the estrogen receptor antagonist ICI 182780, but not cycloheximide, suggests that stimulatory effects of 17 β -estradiol on cAMP/adenosine synthesis are mediated via a nongenomic pathway linked to estrogen receptor α or β .

What is the relevance of our in vitro finding that the cAMP-adenosine pathway participates in mediating the in-

hibitory effects of 17 β -estradiol on SMC growth? With the onset of menopause, circulating 17 β -estradiol levels decrease, and this would decrease adenosine levels in the vascular wall. Thus, the increased incidence of cardiovascular disease in postmenopausal women may be due in part to a reduction in the vascular levels of the cardioprotective autacoid adenosine.

In conclusion, we provide evidence that the cAMP-adenosine pathway, via activation of A₂ adenosine receptors, participates in mediating the inhibitory effects of 17 β -estradiol on vascular SMC growth (Figure 7). Thus, the cAMP-adenosine pathway may importantly contribute to the vasoprotective and cardioprotective effects of 17 β -estradiol.

Acknowledgments

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