Estradiol selectively stimulates endothelial prostacyclin production through estrogen receptor-α

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Abstract

Estradiol (E2) acts on the endothelium to promote vasodilatation through the release of several compounds, including prostanoids, which are products of arachidonic acid metabolism. Among these, prostacyclin (PGI2) and thromboxane A2 (TXA2) exert opposite effects on vascular tone. The role of different estrogen receptors (ERs) in the PGI2/TXA2 balance, however, has not been fully elucidated. Our study sought to uncover whether E2 enhances basal production of PGI2 or TXA2 in cultured human umbilical vein endothelial cells (HUVECs), to analyze the enzymatic mechanisms involved, and to evaluate the different roles of both types of ERs (ERα and ERβ). HUVECs were exposed to E2, selective ERα agonist (1,3,5-tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole, PPT) or ERβ (diarylpropionitrile, DPN) agonists and antagonists (unspecific: ICI 182 780; specific for ERα: methyl-piperidino-pyrazole, MPP). PGI2 and TXA2 production was measured by ELISA. Expression of phospholipases, cyclooxygenases (COX-1 and COX-2), PGI2 synthase (PGIS), and thromboxane synthase (TXAS) was analyzed by western blot and quantitative RT-PCR. E2 (1–100 nM) dose dependently increased PGI2 production (up to 50%), without affecting TXA2 production. COX-1 and PGIS protein and gene expressions were increased, whereas COX-2, phospholipases, and TXAS expression remained unaltered. All these effects were mediated through ERα, since they were produced not only in the presence of E2, but also in that of PPT, while they were abolished in the presence of MPP. In conclusion, E2, acting through ERα, up-regulates COX-1 and PGIS expression, thus directing prostanoid balance toward increased PGI2 production.

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Introduction

Epidemiological studies indicate that the incidence of cardiovascular disease is greater in men than in women throughout the world (Mendelsohn & Karas 2005). In addition, since these differences dissipate after menopause, estrogens have been considered as vasoprotective hormones. The protective effect detected in a considerable number of observational clinical studies (Barrett-Connor & Grady 1998) has not been confirmed by more recent randomized placebo-controlled trials designed to study the effects of hormonal therapy (Hulley et al. 1998, Grady et al. 2002). Nevertheless, a number of studies have demonstrated a favorable profile for estrogens in both experimental animal and in vitro models (Turgeon et al. 2006).

There is a body of evidence suggesting that estrogens exert cardiovascular effects via the promotion of endothelial vasodilator synthesis (Mendelsohn & Karas 2005). Recent studies, however, have described clinical complications, such as thrombosis in veins and coronary arteries, developed in postmenopausal women during the administration of exogenous hormones (Cano et al. 2007).

Estradiol (E2) is the most potent estrogen in humans, and exerts its actions mainly through binding and the activation of estrogen receptors (ERs). Two major subtypes of ERs (ERα and ERβ) have been identified, although the contribution of both receptors to the regulation of vascular effects is still obscure (Cano & Hermenegildo 2000). There is controversy over whether estrogenic effects are mediated through ERα, ERβ, or both. Recently, selective ERα agonist (1,3,5-tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole, PPT), selective ERβ agonist (diarylpropionitrile, DPN), and selective ERα antagonist (methyl-piperidino-pyrazole, MPP) have become available (Krom et al. 2007).

The prostanoids prostacyclin (PGI2) and thromboxane A2 (TXA2) play an essential role in the maintenance of vascular homeostasis. PGI2 is a vasodilator and an inhibitor of platelet aggregation; in contrast, TXA2 is a vasoconstrictor and a promoter of platelet aggregation. As a consequence of their opposing roles, an imbalance in PGI2 or TXA2 production has
been implicated in the pathophysiology of many thrombotic and cardiovascular disorders (Bunting et al. 1983). Both metabolites are products of arachidonic acid metabolism, preceded by phospholipases (cPLa2; PLA2G4A and PLA2G4B), and are metabolized by cyclooxygenases (COX-1 and COX-2). The COX product is metabolized by the terminal synthase enzymes, PGI2 synthase (PGIS) or thromboxane synthase (TXAS), to become PGI2 or TXA2 respectively (Hermenegildo et al. 2006). Because the balance between PGI2 and TXA2 production is central in the maintenance of vascular tone and platelet aggregation, it is important to determine the role of these enzymes in the regulation of prostanoid biosynthesis by the endothelium.

Although there is evidence that PGI2 release from endothelial cells is increased by E2 (Mikkola et al. 1995), less is known about the effect of E2 on the enzymes involved in the prostanoid pathway in cultured human umbilical vein endothelial cells (HUVECs), and the role of both ERs. Therefore, our aims were to study the synthesis of PGI2 and TXA2 metabolites when HUVECs are exposed to E2, and the enzymes involved in the production of both prostanoids. Moreover, we sought to uncover the role of ERα and ERβ in the observed effects.

Materials and methods

Cell culture and experimental design

Primary HUVECs were isolated by collagenase treatment of human umbilical veins from 12 healthy donors as described previously (Monsalve et al. 2007). Briefly, HUVECs were grown in 75-cm² flasks (Orange Scientific, Waterloo, Belgium) in human endothelial cell-specific medium EBM-2 (Lonza, Verviers, Belgium) supplemented with EGM-2 (Lonza) in an incubator at 37°C with 5% CO₂.

Cells were identified as endothelial by their characteristic cobblestone morphology and by the presence of von Willebrand factor by immunocytochemistry using a specific antibody (F-3520; Sigma). At the second passage, 100% of the cells in the cultures were positive for von Willebrand factor. Cells from passages 4 to 6 were seeded into 6-well plates with fibronectin-treated coverslides for immunocytochemistry, into 24-well plates for PGI2 and TXA2 measurements, into 96-well plates for the measurement of cell viability, and into 25-cm² flasks for western blot and mRNA isolation. When cells reached 75% of confluence, culture medium was exchanged for a phenol red-free Medium 199 (Gibco, Invitrogen) supplemented with steroid-deprived (by charcoal/dextran treatment) 20% fetal bovine serum (Gibco), and maintained for 24 h. Then, the culture medium was eliminated and immediately replaced with phenol red-free Medium 199.

The desired concentrations of E₂ (Sigma) were obtained by successive dilutions of a stock solution with ethanol. The desired concentrations of the selective ERα agonist (PPT), the selective ERβ agonist (DPN), the selective ERα antagonist (MPP), or the ER antagonist (ICI 182 780), all of them from Tocris Bioscience (Ellisville, MI, USA), were obtained by successive dilutions of a stock solution with DMSO. Control cells were exposed to the same vehicles (<0.1% ethanol or 0.1% DMSO respectively). Each experiment was performed in a different cell culture.

This investigation conforms to the principles outlined in the Declaration of Helsinki, and was approved by the institutional review board at our center, and written informed consent was obtained from all donors.

Assay of PGI2 and TXA2

After treatment with the desired products, the medium was collected and stored at −20°C until TXA2 or PGI2 was measured. Culture wells were then washed with PBS, and adherent cells were collected in 0.5 M NaOH for protein determination by the modified Lowry’s method using BSA as a standard (Lowry et al. 1951). The amount of PGI2 produced, calculated as the concentration of stable hydrolysis product, 6-keto-prostaglandin-F1α, was assessed in duplicate using a commercial EIA kit (Cayman Chemical, Ann Arbor, MI, USA). The production of PGI2 was expressed as ng PGI2/mg protein.

TXA2 is rapidly hydrolyzed non-enzymatically to form the stable metabolite thromboxane B2, which was assessed in duplicate using a commercial EIA kit (Cayman Chemical), and the results were expressed as pg thromboxane/mg protein.

Immunoblotting

HUVECs were treated in 25-cm² flasks for 24 h with the desired products. A volume of 150 μl of lysis buffer (0.1% triton X-100, 0.5% sodium deoxycholate acid, 0.1% SDS, 0.1% phenylmethanesulfonylfluoride, or phenylmethylsulfonyl fluoride in 100 ml of PBS containing protease inhibitors: 1 μg/ml leupeptin, 0.5 μg/ml pepstatin, and 1 μg/ml bestatin) was added and maintained at 4°C for 30 min. Then, the cells were collected using a cell scraper, boiled for 5 min, and sonicated for 10 s. Protein content was measured (Lowry et al. 1951), and the samples were frozen at −20°C until assay.

Equal amounts of protein (60–150 μg) were then separated by 10% of SDS-PAGE, and the protein was transferred to PVDF sheets (Bio-Rad). Immunostaining was achieved using specific antibodies anti-ERα (sc-8002; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-ERβ (sc-8974; Santa Cruz
Biotechnology), anti-COX-1 (cat 236003; Calbiochem, Darmstadt, Germany), anti-COX-2 (cat 160107; Cayman Chemical), and anti-prostaglandin I synthase (cat 100023; Cayman Chemical). Development was performed with alkaline-phosphatase-linked appropriate secondary antibodies (from Sigma), followed with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate, p-toluidine salt color development reaction. Blots were digitalized using a Gelprinter PLUS (TDI, Madrid, Spain), and the densities of spots were analyzed with the program Image Gauge 4.0 (Science Lab. 2007, Fuji Photo Film, Tokyo, Japan). Equivalent protein loading and transfer efficiency were verified by staining for β-actin (Sigma).

**RNA isolation and quantitative real-time PCR (qRT-PCR) assay**

Total RNA was extracted by using TRizol reagent (Invitrogen) following the manufacturer’s instructions. Reverse transcription (RT) was carried out using SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen) by using a personal Mastercycler Eppendorf Thermocycler (Eppendorf, Hamburg, Germany). One microgram of total RNA was reverse transcribed to cDNA following the manufacturer’s instructions. For each RT, a blank was prepared using all the reagents except the RNA sample (for which an equivalent volume of diethyl pyrocarbonate (DEPC)-treated water was substituted), and was used as a non-template control in qRT-PCR experiments.

The qRT-PCR data for PGIS, TXAS, PLA2G4A, PLA2G4B and GAPDH (endogenous control) were obtained with TaqMan assays (Hs00168766_m1, Hs00233423_m, Hs00233352_m1, Hs00192661_m1, and 4326317E respectively) performed with TaqMan Universal Mastermix (Applied Biosystems, Foster City, CA, USA). Reaction mix was prepared in RNase-free tubes of 0.2 ml by adding a volume of TaqMan Universal PCR Master Mix and TaqMan Gene Expression Assay. The sample of cDNA obtained from the RT was incorporated with the necessary quantity of DEPC-treated water to get a final concentration of 40 ng approximately (range: 10–100 ng). The appropriate volume of each reaction mixture was transferred for each RT, a blank was prepared using all the reagents except the RNA sample (for which an equivalent volume of diethyl pyrocarbonate (DEPC)-treated water was substituted), and was used as a non-template control in qRT-PCR experiments.

In the case of COX-1 and COX-2, qRT-PCR assays were performed using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems) with a heated lid (105°C), an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. To amplify cDNA, the RT samples were diluted 1/10. In each reaction, a total of 1 μl from each RT tube was mixed with 12.5 μl of SYBR Green PCR master mix (Applied Biosystems) containing nucleotides, Taq DNA polymerase, MgCl2, and reaction buffer with SYBR green; 1.5 μl of 5 μM adequate primers and DEPC-treated water were added to a final volume of 25 μl. In parallel, fivefold serial dilutions of well-known DNA concentrations were run as calibration curves. After the amplification process was ended, the melting curves program was used to assure that all the amplicons were obtained at the same temperature, and to assure there was no amplification of other products. Primers were designed using the Primers Express Software (Applied Biosystems), and were synthesized by Custom Primers (Life Technologies). The sequence of the GAPDH sense primer was 5’-CTGCTCCTCCTGTTCAGCAGT-3’ and that of the antisense primer was 5’-CCGTTGACCTGACCTTCAC-3’ (NCBI#: NM_002046), giving rise to an expected PCR product of 100 bp. The COX-1 primers, 5’-CTCTACAGTGCGCTCACAAC-3’ for the sense primer and 5’-GCAACTGCTTCTTCTTTTG-3’ for the antisense (NCBI#: AF440204), were designed to amplify a 168 bp PCR product. For COX-2, the primers used were 5’-ATCATAAGCGAGGCCAGCT-3’ for the sense primer and 5’-AAGGGCGAGTTACGCTGTC-3’ for the antisense one, and a 101 bp product was expected (NCBI#: D28235). Data were analyzed with the ABI PRISM Sequence Detection v. 1.7 analysis software (Perkin Elmer, Nieuwerkerk, The Netherlands). Duplicates showing more than a 5% variation were discarded. To validate a qRT-PCR, standard curves with r > 0.95 and slope values between −3:1 and −3:4 were required.

The amounts of COX-1 and COX-2 were relatively quantified based on the work of Pfaffl MW (Pfaffl et al. 2002). In some samples, PCR bands were purified using a MiniElute PCR Purification Kit (Qiagen), and were then sequenced to prove that the amplified products corresponded to previously published COX-1, COX-2, and GAPDH sequences. Agarose gel electrophoresis were also performed to demonstrate that qRT-PCR yielded a unique band.

**Cell viability measurement**

The possible toxic effect of the compounds that were used on HUVECs was discarded by the measurement of cell respiration, an indicator of cell viability, which was assessed by mitochondria-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan (Oviedo et al. 2004).

Cell viability after all treatments (E2, ICI 182 780, PPT, DPN, MPP, and their combinations) was the same as that of the control cells that were maintained without treatments (data not shown).
Statistical analysis

Values shown in the text and figures are mean ± S.E.M. ANOVA was applied for comparisons of means, and then Bonferroni's test was performed. *P* values <0.05 were considered significant. The statistical analysis was carried out using the Prism 4 software (GraphPad Software Inc., San Diego, CA, USA).

Results

HUVECs express both subtypes of ERs

The first step of the present work was to ensure the presence of ERs on HUVECs. Western blot analysis confirmed the presence of ERα and ERβ (Fig. 1). Moreover, not only the full-length 66 kDa ERα but also the short 46 kDa ERα is expressed in cultured endothelial cells, although the 66 kDa ERα is the main form. There were no differences in ER protein expression between different cell cultures. In addition, no changes in protein expression of both types of ERs were observed after exposure to E2, ICI 182 780, or E2 plus ICI 182 780 (Fig. 1).

Selective ERα activation increased PGI2 production

To investigate the effects of E2 on PGI2 and TXA2 production, HUVECs were first exposed to 1 and 10 nM of E2 during different incubation times up to 48 h (Fig. 2). Exposure to 1 nM E2 increased PGI2 production, compared with the control values, after 24 h (*P*<0.05), and the production remained augmented up to 48 h, whereas 10 nM E2 increased PGI2 production after 8 h (*P*<0.05 versus control). Differences between 1 and 10 nM E2 effects were only significant at 24 h (*P*<0.05). TXA2 remained unchanged after incubation with either 1 or 10 nM E2 at all the tested incubation times (data not shown). Therefore, the remaining experiments were performed at 24 h of incubation.

Exposure of endothelial cells to three different, physiological and near-physiological concentrations of E2 (1–100 nM) resulted in a dose-dependent increase of PGI2 production (*P*<0.001 E2 10 or 100 nM versus control, *P*<0.05 E2 1 nM versus control), increasing up to 50% when cells were exposed to 100 nM (Fig. 3). Moreover, the effect of 100 nM was significantly higher (*P*<0.05) than that of 1 nM. These effects were mediated through ER activation, since the treatment of cells with the unspecific E2 receptor antagonist ICI 182 780 (1 μM) completely reverted the effect of E2 at different concentrations (Fig. 3). To test whether the increased PGI2 production induced by E2 was mediated through ERα or ERβ activation, HUVECs were exposed to the selective ER agonists, PPT and DPN (Fig. 4). Treatment of cells with the ERα agonist PPT (1–100 nM) dose dependently increased PGI2 production. The effect afforded by 1 nM PPT was...
Figure 3 Dose-dependent increase of PGII production by endothelial cells after exposure to estradiol through ER activation. Steroid-deprived HUVEC were exposed to different concentrations (1–100 nM) of E2 with or without ICI 182 780 (ER-unspecific antagonist) for 24 h. Culture medium was then collected, and PGII concentration was measured as described in Materials and methods. Data are expressed as the percentage of the control values, and are mean ± S.E.M. of 10–17 duplicated determinations corresponding to four different experiments performed in cells from different cultures. Average control values (1–100 nM) did not modify PGII production.

Therefore, it seems that E2 increased PGII acting through ERα. To ensure the role of ERα, cells were exposed to E2, PPT, and DPN, with or without the selective ERα antagonist MPP (Fig. 5). Treatment of cells with the ERα agonist PPT (10 nM) increased PGII production to the same levels as those for E2 alone. Moreover, the ERα antagonist MPP (1 μM) completely abolished both the E2 and the PPT effects (P<0.05 versus same treatment without MMP). Therefore, E2 effects on PGII production are mediated through ERα activation. This effect was selective for PGII production, since E2 or different ER agonists do not modify production of the related vasoconstrictor compound TXA2 (Fig. 6).

**E2 did not modify either PLA2G4A or PLA2G4B mRNA expression**

To examine the role of the first enzymes involved in prostanoid production, the gene expressions for both PLA2G4A and PLA2G4B enzymes were measured. Treatment of cells with E2 did not change their expressions (data not shown).

**Selective ERα activation increased COX-1 expression and production**

Experiments were performed in order to study the role of both enzymes COX-1 and COX-2. COX-1 mRNA expression was significantly increased by 15–20% in cells exposed to E2 or PPT respectively (P<0.001 E2 versus control, P<0.05 PPT versus control; Fig. 7A), without changes in the presence of the ERβ agonist DPN, suggesting that E2 increases COX-1 expression through ERα activation. Moreover, to confirm previous results on gene expression, the analysis of the protein content revealed that the COX-1 protein was significantly increased in cells exposed to either E2 or PPT (P<0.05 versus control; Fig. 7B and C) without changes in the presence of DPN. Exposure of endothelial cells to E2 did not modify either COX-2 expression or production (data not shown).

**E2 increases PGIS but not TXAS expression**

To complete the analysis of the E2 effects on the enzymes implicated in prostanoid production, the last enzymes responsible for PGII and TXA2 prostanoid production were evaluated. PGIS mRNA levels increased by 170% (Fig. 8A), and protein PGIS production increased by 50% in cells treated with E2 or PPT (Fig. 8B and C). Moreover, in both cases, the effect was abolished in the presence of ER antagonist ICI 182 780 (1 μM) or selective ERα antagonist MPP (1 μM). These results demonstrate an E2-increased expression and protein content of the PGIS enzyme through ERα. In contrast, the treatment of cells with E2 did not modify TXAS mRNA expression (data not shown).

**Discussion**

In the present study, HUVECs were used to determine the role of E2 in the regulation of prostanoid synthesis. The molecular mechanisms of E2 action in prostanoid synthesis pathway were determined by measuring
the gene expression of PLAG4A2, PLAG4B2, COX-1, COX-2, PGIS, and TXAS; the protein production of COX-1, COX-2, and PGIS; and the production of PGI2 and TXA2.

Some authors have found that HUVECs do not express ERα (Toth et al. 2008), although other investigators have demonstrated the presence of both ERα and ERβ mRNAs in HUVECs (Wagner et al. 2001). Data presented in Fig. 1 demonstrate the expression of both ERα and ERβ proteins in HUVECs, thus confirming previous reports (Harris 2007, Oviedo et al. 2007). The full-length 66 kDa ERα is the main form that is expressed in HUVECs, but the short 46 kDa ERα is also present, as it has been previously described (Haynes et al. 2003). The 46 kDa ERα is sufficient to mediate several vascular effects of E₂ in vivo, including both nuclear actions to increase NO production and reendothelialization process (Billon-Galés et al. 2009) and rapid, membrane-initiated effects such as the activation of endothelial nitric oxide synthase (Kim & Bender 2009).

Time-course analysis, in which E₂ effects on PGI2 are evident only after 8 or 24 h (10 and 1 nM E₂ respectively), suggests an ER-mediated genomic effect. Our study demonstrates that in HUVECs, activation of ERα by E₂ or PPT induces a dose-dependent up-regulation of PGI2 through increased COX-1 and PGIS gene and protein expression (Fig. 9). The vasoconstrictor metabolite TXA2, however, remained unaltered in the presence of E₂. The observed effects, significant at 1 and 10 nM E₂, are within physiological levels, with the same being achieved in plasma from women of fertile age (Hermenegildo et al. 2002). Consequently, the 10 nM E₂ dose was chosen to carry out the experiments.

Our results are in agreement with several papers describing a relationship between estrogens and elevated PGI2. Thus, PGI2 appears to be involved in the atheroprotective effect of E₂ (Arnal et al. 2004). Furthermore, PGI2 levels are elevated during

Figure 5 Estradiol-increased PGI2 production through ERα activation. Steroid-deprived HUVECs were exposed to 10 nM of E₂, PPT, or DPN, with or without 1 μM MPP for 24 h. Then, the culture medium was collected, and PGI2 concentration was measured as described in Materials and methods. Data are expressed as the percentage of the control values, and are mean ± S.E.M. of 11–19 duplicated determinations corresponding to four different experiments performed in cells from different cultures. Average control values for all experiments were 19 ± 0.16 ng/mg protein (range: 0.31–1.97 ng/mg protein).

*P<0.05 versus control, †P<0.05 vs 10 nM E₂, and ‡P<0.05 vs 10 nM PPT.

Figure 6 Estradiol did not modify TXA2 production. Steroid-deprived HUVECs were exposed to 10 nM of E₂, PPT, or DPN, with or without 1 μM MPP for 24 h. Then, the culture medium was collected, and TXA2 concentration was measured as described in Materials and methods. Data are expressed as the percentage of the control values, and are mean ± S.E.M. of 11–19 duplicated determinations corresponding to four different experiments performed in cells from different cultures. Average control values for all experiments were 992 ± 100 pg/mg protein (range: 397–1617 pg/mg protein).

*P<0.05 versus control, †P<0.05 vs 10 nM E₂, and ‡P<0.05 vs 10 nM PPT.

Figure 7 Estradiol-increased COX-1 mRNA and protein expression through ERα activation. Steroid-deprived HUVECs were exposed to 10 nM of E₂, PPT, or DPN for 24 h. For mRNA expression (A), total RNA was extracted, and the relative expression of COX-1 was quantified by qRT-PCR as described in Materials and methods. For immunoblotting assay (B and C), cells were collected in a lysis buffer, and immunoblotted with specific anti-COX-1 antibody as described in Materials and methods. A typical immunoblotting image (C) and relative levels assessed by densitometry of bands (B) are presented. Data are expressed as the percentage of the control values, and are mean ± S.E.M. of five to ten values corresponding to four experiments performed in cells from different cultures. *P<0.05 versus control and **P<0.001 versus control.
pregnancy (Rupnow et al. 2002). In contrast, the decreased urine PGI2 metabolite observed after menopause is reversed in women receiving estrogen replacement therapy (Mueck et al. 2001).

Endothelium is the primary source of PGI2 production, and estrogens have been demonstrated to increase PGI2 levels. For example, E2 increases the release of PGI2 in a variety of artery preparations, including ovine uterine arteries, mesenteric arteries from ovariectomized rats, rat cerebral blood vessels, and aorta from ovariectomized monkeys (Hermenegildo et al. 2006). Regarding cultured endothelial cells, some discrepancies exist. E2 has been reported to increase PGI2 in HUVECs (Mikkola et al. 1995), and to show a neutral or even a decreasing effect in bovine coronary endothelial cells (Stewart et al. 1999).

TXA2 is produced primarily by platelets, but there is evidence of the synthesis of the vasoconstrictor metabolite by the endothelium (Sellers & Stallone 2008). E2 did not change TXA2 production in our experiments. The literature indirectly suggests that estrogen would have a beneficial effect by decreasing the production of thrombogenic compounds. For instance, there is a decrease in the formation of TXA2 in men after the use of high-dosage i.m. estrogen therapy (Henriksson et al. 1996). Moreover, the ratio of in vivo PGI2 to TXA2 formation increased twofold during estrogen replacement therapy (Mueck et al. 2001). Nevertheless, it has also been documented that estrogen increases platelet activation with the liberation of TXA2 in women treated with hormone replacement therapy (Oliveira et al. 2005), and that estrogen enhances the constrictor prostanoid function in female rat aorta (Li et al. 2008).

The relative role of both subtypes of ERs in prostanoid production has been scarcely documented, with only the involvement of ERβ in the E2-increased production of PGI2 in fetal ovine pulmonary artery endothelium being described so far (Sherman et al. 2002).

Despite cPLA2’s important role as the hormone-sensitive substance in PGI2 production in response to various agonists such as angiotensin II or bradykinin, and its high expression levels in HUVECs and umbilical smooth muscle cells (Ost et al. 1998), it does not appear to be regulated by E2. Our results are in agreement with previous studies showing no effect in either E2-treated cerebral arteries (Ospina et al. 2002) or in human myometrial cells prepared from second trimester pregnant women after stimulation with E2 (Korita et al. 2004).

The present study found increased levels of COX-1 mRNA and protein expression in E2-treated HUVECs, with a concomitant elevation of PGI2 synthesis, through ERα activation. That notwithstanding, COX-2 mRNA expression did not change in response to E2 treatment, in contrast to the reported increase in COX-2 expression in other cell types (Li et al. 2008). This discrepancy may be due to the different cell types used or the different methods of estrogen delivery. In summary, our results suggest that estrogen has a beneficial effect on prostanoid production in HUVECs, with a preferential increase in PGI2 synthesis through ERα activation.
and protein expression did not appear to be modified in HUVECs treated with E2. Subsequently, controversy exists over the effects of E2 on COX expression. Even though E2 has been reported to up-regulate COX-1 expression in ovine fetal pulmonary artery endothelial cells (Jun et al. 1998), it has also been reported to selectively increase COX-2 expression in HUVECs (Akarasereenont et al. 2000), and not to modify COX-1 or COX-2 expression in bovine coronary endothelial cells (Stewart et al. 1999). Moreover, E2 has been reported to increase COX-1 expression through ERα in ovine endothelial cells transfected with the human COX-1 promoter (Gibson et al. 2005). Our data describe for the first time an ERα-dependent increase of COX-1 expression in human cells, similar to that reported for other vascular enzymes responsible for vasodilator production, such as endothelial nitric oxide synthase (Traupe et al. 2007).

To complete the prostanoid pathway, PGIS mRNA levels and protein content were increased in HUVECs exposed to E2 through ERα, whereas TXAS mRNA expression remained unmodified. An E2-increased PGIS and COX-1 protein expression has already been described in rat cerebral artery (Ospina et al. 2002), and E2-up-regulated COX-2 and TXAS expression has been described in rat aorta (Li et al. 2008). Although it has been proposed that PGIS preferentially couples with COX-2 for the production of PGI2 (Ueno et al. 2001) in cultured cells, our data have indicated that PGIS would be up-regulated mainly with COX-1 in HUVECs exposed to E2 in accordance with a published outcome for sheep (Rupnow et al. 2002). TXAS, in turn, can be up-regulated along with COX-2 (Doroudi et al. 2000), and COX-2 can be up-regulated by TXA2 (Caughey et al. 2001), suggesting a closer relationship between COX-2 and TXAS. In our results, both COX-2 and TXAS remained unaltered after exposure to E2.

Molecular, cellular, and animal studies have convincingly demonstrated that E2 has favorable effects on vascular cells, with many of them being achieved through ER-dependent signaling responses. Both ERα and ERβ may have distinct roles in the vascular wall (Meyer & Barton 2009). Most of the beneficial vasculo-protective actions of E2 are mediated by ERα. Our results add new information that supports a role for ERα in E2-mediated vasodilatory effects. Therefore, E2 stimulates not only nitric oxide production but also PGI2 production through ERα, via both genomic and non-genomic pathways (Chen et al. 1999, Traupe et al. 2007). In addition, ERα agonists have been shown to improve endothelial dysfunction in rat blood vessels (Widder et al. 2003). Moreover, ERα mediates atheroprotective effects (Hodgkin et al. 2001). Finally, impaired vascular function and premature coronary artery disease were noted in a man with a disruptive mutation in the ERα gene (Sudhir et al. 1997).

In conclusion, the present study demonstrates that E2 increases vasodilatory response mediated by ERα in HUVECs, since E2 selectively up-regulates the protein expression of COX-1 and PGIS enzymes, which would turn the prostanoïd balance toward PGI2 production, without affecting TXA2 expression or TXA2 production (Fig. 9).

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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