Progestogens stimulate prostacyclin production by human endothelial cells

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BACKGROUND: The effects of progestogens on endothelial physiology are poorly studied. Prostacyclin is a potent vasodilator synthesized by two isoforms of cyclooxygenase (COX) in endothelium. We examined the effects of two clinically used progestogens, progesterone and medroxyprogesterone acetate (MPA), on prostacyclin production by cultured human umbilical vein endothelial cells (HUVEC) and the possible role of progesterone receptors and both COX enzymes. METHODS: Cells were exposed to 1–100 nmol/l of either progesterone or MPA and prostacyclin production was measured in culture medium. RESULTS: Both progestogens significantly increased prostacyclin release in a time- and dose-dependent manner, being higher than control after 24 h. Progesterone and MPA, both at 10 nmol/l, increased mRNA expression and protein content of both COX. All these effects were mediated through progesterone receptor activation, since they were abolished by treatment of cells with the progesterone receptor antagonist RU-486. Selective inhibitors of COX-1 and -2 (SC-560 and NS-398 respectively) reduced basal prostacyclin release, and eliminated increased production in response to progestogens. In combination with estradiol, progestogens had an additive effect without eliminating estradiol-induced prostacyclin production. CONCLUSIONS: Our results support the hypothesis that progesterone and MPA increased HUVEC prostacyclin production in a progesterone receptor-dependent manner, by enhancing COX-1 and COX-2 expression and activities.

Key words: cyclooxygenase/endothelial function/hormones/prostaglandins/vasoactive agents

Introduction

Vascular endothelium plays a leading role in vascular physiology. The endothelium is crucial to the modulation of vessel tone and to the control of platelet adhesion and aggregation, two key factors in the initiation and development of atherosclerosis (Ross, 1999). These actions are mainly mediated through the release of such vasorelaxing factors as prostacyclin and nitric oxide, or of vasoconstrictors, such as endothelin-1, angiotensin II or thromboxane A₂.

Prostacyclin is a potent endogenous anticoagulant for platelets and a strong vasodilator. Prostacyclin is a prostaglandin produced from free arachidonic acid through the catalytic activity of two different cyclooxygenases (COX), termed COX-1 and COX-2. COX represent the main control mechanism for prostacyclin production. COX-1 is considered to be expressed in a constitutive manner, whereas COX-2 is inducible by mitogens, cytokine growth factors and endotoxins and is over-expressed in inflammatory processes (Smith et al., 2000; Parente and Perretti, 2003).

Clinical and experimental data support the consideration of endothelium as a target for sexual hormones (Mendelsohn and Karas, 1999). Estrogen receptors have been found in endothelium and estradiol actions have been exhaustively studied (Mendelsohn and Karas, 1999; White, 2002). For instance, estradiol enhances endothelial prostacyclin production through both COX-1 (Jun et al., 1998) and COX-2 (Akarasereenont et al., 2000) and reduces production of vasoconstrictors, reducing blood pressure (Dubey et al., 2002).

Much less is known, however, regarding progestogen actions on the vascular wall. Several types of endothelial cells express progesterone receptors (PR) (Vazquez et al., 1999). Progestogens have been shown to regulate many physiological processes that impact on the atherosclerotic progression. For instance, progestogens can inhibit vasorelaxation (White et al., 1995) and decrease endothelial cell proliferation (Vazquez et al., 1999). Also, PR can regulate the vascular injury response (Karas et al., 2001).

Progestogens are a second component of hormone substitution in post-menopausal women, and are recommended in women with uterus to reduce the risk of endometrial cancer. The effects of exogenous administration of progestogens in combination with estradiol on endothelial function are also...
unclear, given that in some studies, progestogens counteract estradiol’s beneficial vascular effects, while in other trials progestogens do not (see reviews Cano, 1999; Dubey et al., 2002; Ganz, 2002).

Cardiovascular effects of hormone therapy are presently under discussion (Herrington and Klein, 2003; Kuller, 2003). A better knowledge of the endothelial effects of two clinically used progestogens, progesterone (the natural progestogen) and medroxyprogesterone acetate (MPA), could contribute to clarify the physiological and clinical effects. Moreover, all progestogens do not have similar effects on vascular tone (Sarrel, 1999).

The objectives of the work were the following: (i) to study the effects of two progestogens, progesterone and MPA, on prostacyclin production by endothelial cells; (ii) to uncover whether these actions are mediated by PR; (iii) to illuminate whether the observed effects are due to regulation of one or both COX isozymes; and (iv) to test whether progestogens interact with estradiol effects.

Materials and methods

Cell culture and experimental design

Primary HUVEC were isolated by collagenase treatment of human umbilical veins as described in Jaffe et al. (1973). briefly, HUVEC were grown in 25 cm² flasks (Orange Scientific, Belgium) in human endothelial cell-specific medium EBM-2 (Clonetics, BioWhittaker, USA), supplemented with EGM-2 (Clonetics), in an incubator at 37°C with 5% CO₂.

Cells were identified as endothelial by their characteristic cobblestone morphology and the presence of von Willebrand factor by immunocytochemistry using a specific antibody (F-3520; Sigma, Spain).

Cells from passages 4–6 were seeded onto 6-well plates with fibronectin-treated coverslips for immunocytochemistry, onto 24-well plates for prostacyclin measurements, onto 96-well plates for measurement of cell viability, and onto 25 cm² flasks for western blot and mRNA isolation. When cells were at 75% of confluence, culture medium was exchanged for a phenol red-free medium 199 (Gibco-BRL, Life Technologies, UK) supplemented with 20% charcoal/dextran-treated fetal bovine serum (Gibco-BRL) and maintained for 24 h. The culture medium was then eliminated and immediately replaced with phenol red-free medium 199. The desired concentrations of progesterone, MPA and estradiol were obtained by successive dilutions of a stock solution (1 mmol/l in ethanol) with phenol red-free medium. The desired concentrations of RU-486, SC-560 and NS-398 were obtained by successive dilutions of a stock solution (1 mmol/l in dimethylsulphoxide (DMSO)) with phenol red-free medium. Therefore, cells were exposed to <0.1% ethanol and/or <0.1% DMSO. Control cells were exposed to the same concentrations of ethanol and/or DMSO as treated cells.

Immunoblotting

HUVEC were treated in 25 cm² flasks for 24 h with the desired products. Flasks were then washed twice with pre-warmed medium 199. A volume of 150 µl of lysis buffer (0.1% Triton X-100, 0.5% sodium deoxycholate acid, 0.1% sodium dodecyl sulphate (SDS), in 100 µl of phosphate saline buffer containing protease inhibitors: 1 µg/ml leupeptin, 0.5 µg/ml pepstatin and 1 µg/ml bestatin) was added and incubation was maintained at 4°C for 30 min. Cells were then collected using a cell scraper, boiled for 5 min and sonicated for 10 s. Protein content was measured (Lowry et al., 1951) and samples were frozen at −20°C until assay.

Equal amounts of protein (ranging 40–125 µg) were then separated by 10% SDS–polyacrylamide gel electrophoresis, and the protein was transferred to PVDF sheets (PVDF Transfer Membrane Westman, Schleicher & Schuell, Germany). Immunostaining was achieved using specific antibodies anti-PR (sc-538; Santa Cruz Biotechnology, USA), anti-COX-1 (sc-1752; Santa Cruz Biotechnology) or anti-COX-2 (cat no. 160107; Cayman Chemical, USA). Development was performed with alkaline phosphatase-linked anti-goat antibody (for COX-1) or anti-rabbit antibody (for PR and COX-2) (both from Sigma), followed with nitroblue tetrazolium (NBT)/5-bromo-4-chloro-3-indolyl phosphate, p-toluidine salt (BCIP) colour development reaction. Blots were digitized using a Gelprinter Plus (TDI, Spain), and the densities of spots were analysed with the program 1-D Manager. Equivalent protein loading and transfer efficiency were verified by staining for β-actin (from Sigma).

Assay of prostacyclin

After incubation with the desired products, medium was collected and stored at −20°C until prostacyclin assay. Culture wells were then washed with PBS and adherent cells were collected in 0.5 mol/l NaOH solution for protein determination by the modified Lowry method using bovine serum albumin as standard (Lowry et al., 1951).

The amount of prostacyclin produced, calculated as the concentration of stable hydrolysis product, 6-keto-prostaglandin F₁, was assessed in duplicate by a commercial enzyme immunoassay kit (Cayman Chemical). The production of prostacyclin was expressed as ng prostacyclin/mg protein.

With the aim of discerning which of two COX isoenzymes was implicated in prostacyclin production, selective COX-1 (0.1 mol/l SC-560; Cayman Chemical) or COX-2 (1 µmol/l NS-398; Cayman Chemical) inhibitors were added to some wells.

RNA isolation and real-time RT–PCR assay

Total cellular RNA was extracted by using the TRIzol® reagent (Invitrogen, USA) following the manufacturer’s instructions. RT was carried out using SuperScript™ First-Strand Synthesis System for RT–PCR (Invitrogen) by using a personal Mastercycler Eppendorf Thermocycler (Eppendorf, 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 diethylpyrocarbonate-treated water was substituted) and was used as non-template control in real-time PCR experiments.

Primers for quantitative RT–PCR were designed using the Primer Express Software (Applied Biosystems, USA) and synthesized by Custom Primers (Life Technologies, Spain). The sequence of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sense primer was 5'-CTGCCCTCTCTGTGCGACAGT-3' and that of the antisense primer was 5’-CCGTTGACTCCGACCTTC-3’ (NCBI: NM_002046) giving rise to an expected PCR product of 100 bp. The COX-1 primers were designed to amplify a 168 bp PCR product and these were the following: 5'-TACTCAGTGGGCTCAAGC-3' for the sense primer and 5'-GCAACTCTTCTCTTTGTTTG-3' for the antisense primer (NCBI: AF440204). Related to COX-2, primers used were the following: 5’-ATCATAAGGGGAAAAAGCT-3’ for the sense primer and 5’-AAGGGCAAGTTACGCTGC-3’ for the antisense primer, and a 101 bp product was expected (NCBI: D28235).
RT–PCR was 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 1/100 and 1/200. 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 each 2.5 μmol/l specific primers and double-distilled water were added to a final volume of 25 μl. Each sample was amplified in duplicate for COX-1, COX-2 and GAPDH. In parallel, 5-fold serial dilutions of well-known cDNA concentrations were run as calibration curves. Data were analysed with the ABI Prism 1.7 analysis software (Applied Biosystems). Duplicates showing >5% variation were discarded. To validate a RT–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 normalized to the corresponding values of the housekeeping gene GAPDH to estimate and compare the relative COX-1 and COX-2 expression among samples. Experiments were performed four times.

In some samples, PCR products were purified by using MiniElute PCR Purification Kit (Qiagen, USA) and were sequenced to prove that the amplified bands corresponded to previously published sequences. Agarose gel electrophoreses were also performed to demonstrate that RT–PCR yielded a unique band.

**Cell viability measurement**

Cell respiration, an indicator of cell viability, was assessed by mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. Experiments were performed in parallel and with the same protocol described for prostacyclin production, but were performed in 96-well plates. At the end of each experiment, medium was discarded and cells were incubated with 0.1 mg/ml MTT dissolved in phenol red-free medium 199 for 3 h. Medium was then removed by aspiration and formazan contained in cells was solubilized with 100 μl DMSO. The extent of reduction of MTT to formazan was quantified through the measurement of optical density at 540 nm by using a microplate reader (Bio-Rad, USA). Results were expressed as relative percentage of formazan produced by cells maintained in phenol red-free medium 199 without treatments.

**Figure 1.** Progesterone receptor expression in cultured endothelial cells. Human umbilical vein endothelial cells were exposed to different combinations of progesterone (10 nmol/l) or medroxyprogesterone acetate (10 nmol/l) with 10 μmol/l RU-486 (mifepristone, a progesterone receptor antagonist) for 24 h. Equal amounts of protein (range: 80–125 μg) were subjected to 10% acrylamide gels electrophoresis and immunoblotted with a specific antibody anti-PR, as described in Materials and methods. A typical immunoblotting image with the bands corresponding to PRA (~94 kDa) and PRB (~114 kDa) is presented.

**Statistical analysis**

Values are reported as means ± SEM. Repeated-measures analysis of variance test was applied for comparisons of means, and then Student’s t-test was performed. P < 0.05 was considered significant.

**Results**

The first step of the present work was to ensure the presence of PR on HUVEC. Western blot analysis confirmed the presence of PR with the use of an antibody that recognizes both forms of PR, PRA and PRB. Cells clearly exhibited a 94 kDa band, corresponding to PRA, and a less evident 114 kDa band, corresponding to PRB (Figure 1). There were no significant differences between both PR protein expressions after the different treatments.

To investigate the effects of progesterone and MPA on prostacyclin production, HUVEC were first exposed to either 10 nmol/l of progesterone or 10 nmol/l MPA during different times of incubation, up to 48 h (Figure 2). There was a sustained, spontaneous production of prostacyclin in control, non-stimulated endothelial cells (exposed only to vehicle), for 48 h. Control prostacyclin production at 16, 24 and 48 h was significantly higher (P < 0.05) than those of shorter incubation times. Exposure to 10 nmol/l progesterone or 10 nmol/l MPA increased prostacyclin production, compared to control values, after 24 h (P < 0.05), and remained augmented up to 48 h. Therefore, the remaining experiments were performed at 24 h of incubation with different compounds. In overall experiments, prostacyclin production in control endothelial cells was 1.53 ± 0.12 ng/mg protein.

**Figure 2.** Time-course of progesterone and medroxyprogesterone acetate effects on 6-keto-prostaglandin F1α production by cultured endothelial cells. Human umbilical vein endothelial cells were exposed to 10 nmol/l of either progesterone or medroxyprogesterone acetate (MPA) for the indicated time periods (8–48 h), culture medium was then collected and 6-keto-prostaglandin F1α concentration was measured as described in Materials and methods. Data are expressed as ng of 6-keto-prostaglandin F1α/mg of protein, and are mean ± SEM of six to nine duplicated determinations corresponding to two different experiments performed in cells from different cultures. At each time-point, control values were higher (P < 0.05) than previous. *P < 0.05 versus control values for both progesterone and medroxyprogesterone acetate (MPA) treatments at the same time-point.
The three tested concentrations. These effects are mediated through PR activation, since treatment of cells with 10 μmol/l RU-486 completely abolished both progesterone (P < 0.005)- and MPA (P < 0.02)-increased prostacyclin production (Figure 4).

To examine the role of COX-1 or COX-2 in the observed effects, experiments were conducted to study COX mRNA expression, protein content as well as COX-1 and COX-2 activities. Both progesterone as well as MPA treatments resulted in an increased HUVEC expression of COX-1 and COX-2 mRNA (P < 0.05 versus control values) (Figure 5). Protein concentration also reflected the changes in mRNA expression, since not only progesterone, but also MPA increased COX-1 and COX-2 protein concentration (P < 0.05 versus control values) (Figure 6). Treatment of cells with RU-486 alone modified neither mRNA nor protein content of either COX, but completely abolished the effects afforded by progesterone and MPA on mRNA expression and protein content of both COX-1 and COX-2 (P > 0.05 versus control values) (Figures 5 and 6).

Although progesterone- and MPA-increased production of prostacyclin seems to be mediated through increased mRNA and protein expression of both COX-1 and COX-2, experiments were performed to study the role of both enzyme activities on prostacyclin production by using specific COX-1 and COX-2 inhibitors (Figure 7). The use of 0.1 μmol/l SC-560 alone, an inhibitor of COX-1, decreased prostacyclin production to 44% of control values (P < 0.005). When used in combination with progesterone or MPA, SC-560 completely abolished the increased production of prostacyclin induced by both progestogens (P < 0.001), decreasing prostacyclin content to the same level of SC-560 alone. NS-398
The physiological interaction between estradiol and progestogens on prostacyclin production was tested by exposing cells to a physiological concentration of estradiol and either progesterone or MPA (Figure 8). Treatment of cells with 1 nmol/l estradiol alone increased prostacyclin production by 25% (P < 0.005 versus control values). When used in combination with progestogens, the amount of prostacyclin released was the addition of the effects of estradiol (25%) and progestogens (56 and 55% for progesterone and MPA alone respectively). In both cases, prostacyclin production induced by co-exposure to estradiol and progestogens was significantly higher than by exposure to estradiol alone (P < 0.005).

The possible toxic effect on HUVEC of some of the compounds used was discarded by experiments performed in parallel and the measurement of MTT production. Cell viability after all treatments (progesterone, MPA, RU-486, SC-560, NS-398, estradiol and their combinations) was the same as control cells maintained without treatments (data not shown).

**Discussion**

In this study we demonstrated that both progesterone and MPA are able to increase HUVEC prostacyclin production within 24 h of incubation, through PR-mediated mechanisms, probably involving both enhanced COX-1 and COX-2 expressions and activities.

Our results confirm previous reports regarding the presence of both forms of PR on cultured endothelial cells (Vazquez et al., 1999). Nevertheless, PRB expression is much less detectable than PRA expression, which is in contrast with data from previous studies. For instance, some authors have obtained similar levels of protein expression for PRA and PRB in other types of human endothelial cells (Vazquez et al., 1999). Also, mRNA expression studies demonstrated

![Graph](image1.png)

**Figure 6.** Progesterone and medroxyprogesterone acetate (MPA) increase both COX-1 and COX-2 protein content in human endothelial cells through progesterone receptor activation. Human umbilical vein endothelial cells were exposed to different combinations of progesterone (10 nmol/l) or MPA (10 nmol/l) with 10 μmol/l RU-486 (mifepristone, a progesterone receptor antagonist) for 24 h. Equal amounts of protein (range: 40–80 μg) were subjected to 10% acrylamide gels electrophoresis and immunoblotted with specific antibodies anti-COX-1 or anti-COX-2, as described in Materials and methods. Typical immunoblots and relative levels assessed by densitometry of bands of 70 kDa (COX-1) and of 72 kDa (COX-2) are presented. Data are expressed as percentage of control values, and are mean ± SEM of four to six western blots corresponding to three different experiments performed in cells from different cultures. *P < 0.05 versus respective control values.

![Graph](image2.png)

**Figure 7.** Role of COX-1 or COX-2 inhibition on progesterone- and medroxyprogesterone acetate (MPA)-stimulated prostacyclin production by human endothelial cells. Human umbilical vein endothelial cells were exposed to different combinations of progesterone (10 nmol/l) or MPA (10 nmol/l) with 0.1 μmol/l SC-560 (a COX-1 antagonist) or with 1 μmol/l NS-398 (a COX-2 antagonist) for 24 h. Culture medium was then collected and 6-keto-prostaglandin F1α concentration was measured as described in Materials and methods. Data are expressed as percentage of control values, and are mean ± SEM of nine to 16 duplicated determinations corresponding to five different experiments performed in cells from different cultures. Average control values for all experiments were 1.48 ± 0.12 ng/mg protein (range: 0.32−2.37 ng/mg protein). *P < 0.005 versus control values, and †P < 0.001 versus either progesterone or MPA values.
a high expression of the PRB isoform in HUVEC (Tatsumi et al., 2002). The relative roles of PRA and PRB tend to support the view that PRB is the active PR, whereas PRA is either inactive or acts as an inhibitor of PRB activity. However, both isoforms are often co-expressed in normal physiology and exert both cooperative actions and distinct activities (Graham and Clarke, 2002).

Progesterone increased prostacyclin production in a dose-dependent manner. Physiological concentrations of progesterone were used in the present study, since in the follicular phase, plasma progesterone levels are 0.15–0.70 ng/ml (corresponding to 0.5–2.2 nmol/l) and in the luteal phase are 2.00–25.0 ng/ml (corresponding to 6.4–79.5 nmol/l). Nevertheless, the use of the same concentrations of MPA resulted in an increased, although not dose-dependent, prostacyclin production. The explanation for that difference remains elusive, although the wide range of values could be the main reason, since there is a tendency to increase prostacyclin production with increased MPA concentrations.

Increased prostacyclin production induced by progestogens supports an active role for these hormones on endothelial function, thus discarding previous reports in which it has been denied (Lewis et al., 1986; Mueck et al., 2002). Furthermore, the production of prostacyclin by HUVEC after exposure to progesterone or MPA is comparable to that afforded by physiological concentrations of other steroid hormones such as estradiol (Mikkola et al., 1995, 1996b; Akarasereenont et al., 2000).

Time-course analysis reveals a considerable latency required for the increased prostacyclin production, as progestosterone and MPA effects are evident only after 24 h of incubation, thus suggesting a genomic effect. The use of the antiprogestogen RU-486 at a dose within the adequate range to ensure PR antagonism (Xu et al., 2002) permits the ascription of the observed effects to PR. RU-486 alone had no effect on basal prostacyclin synthesis by HUVEC, as has already been demonstrated in pulmonary artery endothelial cells (Jun et al., 1999). When used in combination with progesterone or MPA, RU-486 completely abolished their effects on prostacyclin production. Taken together, data from Figures 2 and 4 indicate that progesterone and MPA enhanced prostacyclin release which is, at least in part, a genomic action mediated through PR activation.

Nevertheless, other mechanisms cannot be completely refuted. For instance, a rapid (within 5 min) stimulation of prostacyclin production by physiological concentrations of progesterone (1–100 nmol/l) in rat aorta strips has recently been reported, suggesting a non-genomic mechanism. The possible contribution of PR, however, has not been studied (Selles et al., 2002).

It is generally believed that COX-1 is ubiquitously expressed (termed as constitutive isoform), whereas COX-2 is induced by mitogens, growth factors, bacterial endotoxin, and cytokines (inducible COX) (Parente and Perretti, 2003). Nevertheless, in endothelium [and other tissues, such as uterus (Kim et al., 1999)], things may not be so simple, and both COX shared characteristics of constitutive and inducible enzymes.

Data from Figures 5 and 6 demonstrated a basal expression of COX-1 and COX-2, suggesting a constitutive component of COX-dependent prostacyclin production. A careful consideration should be done, however, since other factors, including cytokines, growth factors, etc., may be regulating this level and have not been identified in the present work.

Our results demonstrated a PR-mediated induction of both enzymes after exposure to progesterone or MPA, measured as protein content and as mRNA expression (Figures 5 and 6). These results suggest that COX mRNA in endothelium after exposure to progesterone is highly correlated with protein expression levels, indicating a regulation at the mRNA level (Habermehl et al., 2000). Moreover, the use of COX-1 and COX-2 inhibitors (Figure 7) revealed not only that basal production of prostacyclin by HUVEC was equally mediated through both enzymes, but also that both inhibitors similarly abolished progestogen-increased prostacyclin production. Taken together, results from Figures 5–7 support a role for COX-1 and COX-2 in both basal- and progestogen-induced prostacyclin production.

Therefore, it seems that COX-2 is, at least in part, a constitutive enzyme in HUVEC, and COX-1 an inducible one. Consistent with this role of COX-2, clinical studies with celecoxib, a selective inhibitor of COX-2, have shown that this enzyme exerts control of most systemic prostacyclin production in healthy humans (McAdam et al., 1999).

In addition, COX-1 induction in cultured endothelial cells has previously been reported. As stated in the Introduction, estradiol augments the protein content of both COX-1 (Jun et al., 1998) and COX-2 (Akarasereenont et al., 2000). Agreeing with our data, 10 day treatment with progesterone increases COX-1 expression in ovine renal artery endothelium (Rupnow et al., 2002). Also, pregnancy (a state of...
both high estrogen and progesterone) increases both COX-1 protein and mRNA expression in endothelial cells from sheep (Janiowiak et al., 1998). The dependence of COX-1 on progesterone and PR activity has been shown in baboon endometrium, where COX-1 expression was completely inhibited by the use of an antiprogestogen (Kim et al., 1999).

Our data could have important clinical and therapeutic implications, taking into account that prostogestogens are currently and frequently used as a second component of hormonal substitution in post-menopausal women. On the one hand, progestogens are thought to have a neutral effect or even to counteract the estradiol effects on several cardiovascular and haemostatic parameters, such as blood pressure, vascular tone, lipid profile, fibrinogen plasma concentration or the fibrinolytic system (Winkler, 1999; Kawano et al., 2001; Dubey et al., 2002; Ganz, 2002). Related to prostanoids, progestogens counteract estrogen-induced prostacyclin release in endothelial cultured cells exposed to serum or plasma from post-menopausal women in some studies (Mikkola et al., 2000), but not others (Mikkola et al., 1996a). Results of the present study support an active role for progestogens per se on endothelial-dependent vasodilator production. Moreover, progestogens do not counteract estradiol-induced prostacyclin production and in fact both hormones have an additional effect (Figure 8). Reported differences in the progestogen used or in the design of the experiments seem crucial to an adequate understanding of those results.

On the other hand, and related to the foregoing, our study reveals an equal action for progesterone and MPA. Despite the differences between them on lipid metabolism (Writing Group for the PEPI Trial, 1995), blood pressure (Dubey et al., 2002), vascular tone (Minshall et al., 1998), experimental atherosclerosis (Adams et al., 1997), or HUVEC expression of vascular cell adhesion molecule-1 (VCAM-1) (Otsuki et al., 2001), with a more prejudicial profile for MPA, both progestogens behaved similarly in every parameter assayed in our study.

Moreover, results of the present work cannot explain the differences observed in the Women’s Health Initiative (WHI) clinical trial, which showed that there were no cardioprotective effects of estradiol + MPA, as judged by comparing the number of adverse cardiovascular events in the estradiol + MPA group with that in the placebo group (Rossouw et al., 2002). As stated before, there were no differences between MPA and MPA + estradiol-induced prostacyclin production (Figure 8).

Nevertheless, the possible benefits of increased prostacyclin production by progestogens should be carefully considered. It seems clear that the primary effects of prosta-
cyclin production should be to induce vasodilatation, but enhanced expression and activity of both COX2 could also increase the production of other prostanoids with proaggregatory and vasoconstrictor activity, such as thromboxane A2 or prosta-
glandin F2α (Ospina et al., 2003). In that case, vasodilator and vasoconstrictor effects should be unbalanced, and possible pathological mechanisms promoted.

The reported COX stimulation by progestogens could have other consequences. For instance, it could have an important role on embryo implantation, since estradiol promotes uterine vascular permeability whereas progesterone stimulates uterine angiogenesis during pregnancy (Ma et al., 2001), probably through COX-2-derived prostaglandins (Matsumoto et al., 2002).

In conclusion, our results demonstrate that progesterone and MPA stimulate prostacyclin production by HUVEC through PR-mediated mechanisms, probably involving both COX-1 and COX-2 enzymes, without counteracting the effects of estradiol.

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