Progesterone Regulates Proliferation of Endothelial Cells*

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Francisca Vázquez‡§, Juan Carlos Rodríguez-Manzaneque‡§, John P. Lydon¶, Dean P. Edwards‡, Bert W. O’Malley‡, and M. Luisa Iruela-Arispe‡**

From the ‡Department of Pathology, Beth Israel-Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02215, the ¶Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030, and the §Department of Pathology, University of Colorado Health Sciences Center, Denver, Colorado 80262

The use of steroid hormones in postmenopausal replacement therapy has been associated with prevention of cardiovascular disease. Although the contribution of estradiol to endothelial cell function has been addressed, little information is available on the effect of progestins on this cell type. Here, we provide direct evidence for the presence of functional nuclear progesterone receptor in endothelial cells and demonstrate that physiological levels of progestin inhibit proliferation through a nuclear receptor-mediated mechanism. The effects of progestin were blocked by pretreatment with a progesterone receptor antagonist, and progesterone receptor-deficient endothelial cells failed to respond to the hormone. We evaluated the effect of progestin by analysis of aorta re-endothelialization experiments in wild-type and progesterone receptor knockout mice. The rate of re-endothelialization was significantly decreased in wild-type mice when in the presence of progesterone, whereas there was no difference between control and progesterone-treated progesterone receptor knockout mice. FACS analysis showed that progestins arrest endothelial cell cycle in G1. The lag in cell cycle progression involved reduction in cyclin-dependent kinase activity, as shown by down-regulation in retinoblastoma protein phosphorylation. In addition, treatment of endothelial cells with progestins altered the expression of cyclin E and A in accordance with G1 arrest. These results have important implications to our current knowledge of the effect of steroids on endothelial cell function and to the overall contribution of progesterone to vascular repair.

Progesterone receptor (PR)1 is a member of a family of nuclear receptors capable of regulating gene expression upon binding to the appropriate hormones (1–4). As such, PR is considered a transcription factor with known ability to influence development and morphogenesis (5). Aside from its expression in several cell types of the mammary gland, uterus, and ovary, PR has also been identified in brain and vascular tissue (6–8). The presence of PR in blood vessels has relevance because of the increase use of progestins in hormone replacement therapy (HRT).

HRT with estrogen and progestins has become a well accepted treatment regimen for postmenopausal women, particularly those with an absence of familial breast cancer history. A large body of literature has demonstrated that the benefits of HRT extend beyond the amelioration of symptoms associated with menopause. HRT also aids in the prevention of osteoporosis and in the reduction of cardiovascular disease (9–11). Most of these effects have been attributed to estradiol, as demonstrated by several experimental and epidemiological studies. In fact, estradiol has been shown to diminish the incidence of cardiovascular disease up to 45% (12–14). The contribution of progestins to the overall effect of HRT is less clear. Historically, progestins were included to counteract endometrial dysplasia caused by estradiol (11, 15). Although the inclusion of progestins to HRT has recognized value, dosage levels and frequency have been the object of much controversy, mostly due to collateral effects (16–18).

In blood vessels, PR has been localized in smooth muscle cells of the tunica media (7, 19, 20) and has been shown to suppress smooth muscle cell proliferation in vitro (8). Nevertheless, neither the presence of functional PR nor the effect of progestrene have been addressed on endothelial cells. Because constant levels of exogenous progestins can be associated with the breakdown of vessels and irregular endometrial bleedings (21), we hypothesized that this hormone might play a direct role in endothelial function. Because progesterone mediates signals through its receptor (2–4), we investigated the presence of PR in endothelial cells from several organs and addressed the effect of the ligand on several aspects of endothelial function.

**EXPERIMENTAL PROCEDURES**

Immunohistochemistry—A mouse monoclonal antibody (1294) was produced against purified full-length recombinant human progesterone receptor. Monoclonal antibody 1294 recognizes an epitope in the amino terminus located between amino acids 165 and 534 (22) and specifically reacts with both the A and B isoforms of human PR as determined by immunoprecipitation and Western blot of receptor positive (T47D) and negative cells (MDA-231). Nuclear localization of receptor has been observed by immunocytochemistry in female reproductive tissues and in tumors of reproductive glands.2 Specimens from normal and atherosclerotic human arteries were obtained from the Division of Surgical Pathology at Beth Israel Deaconess Medical Center, Boston, MA. Tissue was fixed in 4% paraformaldehyde, embedded in paraffin, and then sectioned at 5 µm. Sections were cleared, hydrated, and blocked as described previously (23), and subsequently incubated with anti-progesterone receptor monoclonal antibody 1294 (8.5 µg/ml) and polyclonal

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1 The abbreviations used are: PR, progesterone receptor; PRKO, PR knockout; FCS, fetal calf serum; GFG-2, basic fibroblast growth factor; HDEC, human dermal endothelial cell; HRT, hormone replacement therapy; VEGF, vascular endothelial growth factor; pRb, retinoblastoma protein; Cdk, cyclin-dependent kinase; FACS, fluorescence-activated cell sorter; PKE, progesterone-responsive element.

2 D. Edwards, unpublished data.
antibody to vWF (Dako, Carpinteria, CA) followed by Texas Red-conjugated anti-mouse IgG (1:200; Vector Laboratories, Burlingame, CA) and anti-rabbit biotinylated (3 μg/ml) (Vector Laboratories). Finally, avidin-fluorescein isothiocyanate was applied, and tissues were incubated with Hoechst 33258 (10 μg/ml in phosphate-buffered saline) (Molecular Probes; ORI for control experiments included incubation with mouse IgG instead of primary antibody.

**Cell Culture**—Human dermal endothelial cells (HDECs) and human endometrial endothelial cells were isolated as described previously (24, 25). Mouse brain endothelial cells were harvested from adult mice by enzymatic dissociation and further purified using anti-PECAM (Pharmingen, San Diego, CA)–linked to magnetic beads (Dynal, Lake Success, NY). The purity and homogeneity of cultures isolated in our laboratory was evaluated by uptake of acetylated low density lipoprotein, presence of PECAM, CD-34, up-regulation of E-selectin and proliferation in response to VEGF (25). Human coronary endothelial cells and human lung endothelial cells were obtained from Clonetics (San Diego, CA). All cell types were grown in dishes precoated with Vitrogen (Collagen Corp., Palo Alto, CA) and cultured in EBM (Clonetics) supplemented with 15% fetal calf serum (FCS), 25 μg/ml MAMP, and 1 μg/ml hydrocortisone-21-acetate and were used for passages 3–6. Cells were made quiescent by incubation of confluent monolayers with phenol red and serum-free EBM containing 0.2% bovine serum albumin for 48 h. Experiments in which steroid function was assessed were also performed in phenol red and serum-free EBM containing the buffer filtered serum. Progesterone was obtained from Sigma; R5020 (17α,21-dimethyl-19-norgestrel-4,9-diene-3,20-one) from NEN Life Science Products; and RU486 (17α-hydroxy-11-[4-dimethyl-aminophenyl] 17-dimethyl-19-norpregna-4,9-diene-3,20-one) from NEN Life Science Products; and RU486 (17α-hydroxy-11-[4-dimethyl-aminophenyl] 17-dimethyl-19-norpregna-4,9-diene-3,20-one) from NEN Life Science Products. Incubation was allowed to take place in the presence or absence of 100-fold molar excess of R5020 or estradiol (as a negative control competitor). Binding was assessed by liquid scintillation, and Scatchard plots were used to evaluate the data.

**Proliferation Assays**—Quiescent HDECs were trypsinized and plated onto 24-well dishes in phenol red-free EBM supplemented with 1% FCS (Hyclone) and 1% FBS in the presence of either progesterone, R5020, RU486, or vehicle control. During the last 4–8 h of each time point, cells were pulsed with 1 μC/well of [3H]thymidine (NEN Life Science Products). Incorporated [3H]thymidine was measured as described previously (28).

**Endo-endothelialization Assays**—Aortae from wild-type and PRKO mice were dissected and fixed with 4% paraformaldehyde. Tissue sections were incubated with primary antibody to vWF (Dako, Carpinteria, CA) followed by Texas Red-conjugated anti-mouse IgG (1:50; Vector Laboratories) and a–c arteries. Significance was evaluated by standard t test analysis prior to conversion to percentages and within each experimental group.

**Flow Cytometry**—Quiescent HDECs were plated in phenol red-free EBM supplemented with 0.1% charcoal-filtered serum, 50 ng/ml VEGF, and 2 ng/ml FGF-2. Cells were treated with 1 μM progesterone, 10 μM R5020, or vehicle. At the indicated time points, cells were removed by incubation with trypsin, fixed with 70% ethanol, and incubated with 0.1% RNAase (Sigma) for 1 h at 4 °C. DNA and 50 ng/ml propidium iodide (Sigma) containing 1 μg/ml RNaseA. Dead cells were excluded from analysis by gating in FL3. DNA fluorescence of nuclei was measured with a FACScan flow cytometer (Becton Dickinson, Bedford, MA), and percentages of cells in G1, S, and G2/M phases of cell cycle were analyzed using FACScan software programs.

**Blot Analysis**—An equal number of quiescent HDECs were passed to 6-well plates in phenol red-free EBM supplemented with 1% FCS and 50 ng/ml VEGF, in the presence of either progesterone, R5020, or vehicle control. At the times indicated, monolayers were processed, separated by SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes (Schleicher & Schuell). After incubation with primary antibodies, immune complexes were visualized by ECL system (Amersham Pharmacia Biotech). Antibodies included cyclin A (Biocytex), p21, P27, p53, pRb (G1–245) from Pharmingen, and cyclin E (HE12) from Santa Cruz Biotechnology Inc. (Santa Cruz, CA), and pRb (G3–245) from Pharmingen.

**Statistics**—Statistical analysis were done using In-Stat software (Graph Pad Software) for Macintosh. All categorical data (scanning densitometry) is presented as mean ± S.D. when repeated measures were done. Assuming normal distributions, data were analyzed by one-way analysis of variance, followed by either the t test with Dunnett test for comparisons between groups or the Student-Newman-Kles test for multiple comparisons between groups.

**RESULTS**

**Progesterone Receptor Is Expressed by Several Types of Endothelial Cells**—Expression of progesterone receptor was evaluated by three independent assays: (a) immunocytochemistry on tissue sections, (b) Northern blots of purified endothelial cell cultures, and (c) immunoprecipitation of cell culture extracts. Immunohistochemical analysis with an PR–specific monoclonal antibody revealed expression of PR in the endothelium of normal arteries (Fig. 1A, a–c) and atherosclerotic (Fig. 1A, d–f) arteries. Double immunolabelling with von Willebrand’s factor was performed to verify the endothelial nature of these cells. Expression of PR in the endothelium was patchy, with about 25–30% of the total number of endothelial cells lining the lumen positive for PR (Fig. 1A). At present, it is not clear whether this
indicates endothelial heterogeneity or a particular physiological state. Nevertheless, the proportion of PR-positive cells in the endothelium of vessels varied in different organs, with cycling endometrium showing the higher proportion of endothelial PR-positive cells (25). As described previously, we also observed PR expression in the smooth muscle layer (8). In our preparations, the expression was scant and distributed center and lower half of the tunica media, an area not seen in Fig. 1A due to the magnification.

The relative levels of PR mRNA were assessed by Northern analysis (Fig. 1B). Several purified endothelial cell cultures isolated from skin, lung, endometrium, and coronary vessels expressed PR mRNA. However, the relative levels of transcript varied significantly among the cell types examined. In addition, whereas expression of the receptor was maintained in vitro in most endothelial cells tested, levels decreased with increase passage number. The presence of PR protein was evaluated by immunoprecipitation of cell extracts (Fig. 1C). The assays were performed with the same antibody used on immunocytocchemistry. Both forms of the PR receptor, PR-B (120 kDa) and PR-A (94 kDa) were detected at a similar ratio. The relative levels of PR on endothelial cells, however, were about 1/100 of the levels of PR on T47D.

Further characterization of PR on endothelial cells was provided by direct binding assays (Fig. 2A). Cell extracts of endothelial cell cultures were subjected to incubation with [3H]R5020, a progestin agonist. Scatchard analysis showed a unique high affinity binding protein with $K_d = 6.1 \times 10^{-9}$ M, which could be competed with cold R5020 but was unaffected by 17β-estradiol. In addition, radioligand binding assays revealed that early passage endothelial cultures (HDECs) had as many as $10^5$ receptors/cell, but a reduction of up to 100-fold was seen after three passages in vitro.

Functional evaluation of PR activity was determined by transactivation assays (Fig. 2B). Presence of intracellular progestins lead to dimerization of PR and binding to specific cis-acting sequences (PREs). We transfected several endothelial cell types with a construct containing 6 tandem PRE repeats linked to the luciferase gene. In the absence of progestins, there was little to no detectable luciferase; however, treatment of transfected cultures with progestins induced transactivation and expression of luciferase. These experiments were confirmed with at least three independent strains of each cell type. Interestingly, we detected consistent trends in the transactivation assay. Endothelial cells derived from lung and umbilical cord gave the lowest luciferase levels, and coronary and dermal endothelial cells showed the highest. In these assays, low passage number was an essential requirement for a positive transactivation response and correlated with higher expression of PR.

To determine the effect of progestins on endothelial cell function, we evaluated attachment, spreading, migration, in-
vasion, and proliferation of endothelial cells in the presence of progestins. Although a clear effect was detected on proliferation assays, no significant alterations were seen in any of the other endothelial cell functions tested (data not shown).

**Progesterone Is an Effective Suppressor of Endothelial Cell Proliferation**—Inhibition of \[^{3}H\]thymidine incorporation by progesterone was dose-dependent (Fig. 3A). Significant growth suppression was seen with levels as low as 10 nM. At 100 nM, progesterone inhibited endothelial cell proliferation by 30–35% and up to 45–50% at 1 \( \mu M \) after 48 h of treatment. This effect was confirmed with several independent strains of human dermal endothelial cells (17 strains), human coronary endothelial cells (3 strains), bovine aortic endothelial cells (5 strains), and human endometrial endothelial cells (9 strains). The reduction of \[^{3}H\]thymidine incorporation mediated by progesterone reflected changes in cell number, as confirmed in parallel experiments for which cell counts were performed at the end of 4 days of treatment (Fig. 3B). Cells incubated with progestins were not permanently suppressed, because withdrawal of progestins enabled endothelial cultures to respond to mitogens at equal intensity as controls.

The specificity of the response mediated by progesterone was further evaluated with the use of R5020, a progesterone agonist more stable than progesterone. Synchronized endothelial cultures treated with R5020 showed inhibition that was sustained through the entire cell cycle. Fig. 3C shows the effect of R5020 on cell cycle, as determined by thymidine incorporation. A significant reduction (72–75%) in thymidine incorporation was seen at the peak in S phase (48 h). It should be noted that this effect is cumulative over time, an observation that is consistent with cell cycle arrest. A relatively short but reproducible delay of 8–12 h was also noted during the peak of S phase on treated cultures.

To determine whether reduction in total number of cells was due to toxicity, apoptosis, or cell cycle arrest, we counted the number of cells in several time points during the entire assay (48 h), finding no change with progesterone treatment prior to or during S phase. Furthermore, progesterone did not promote changes in cell shape or spreading, nor did it induce apoptotic bodies, as evaluated by microscopic inspection of the cultures.
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Fig. 4. Inhibition of endothelial cell proliferation is mediated by PR. A, HDECs were plated on 24-well plates in EBM containing 0.1% charcoal-filtered serum and 2 ng/ml FGF-2. Treatments as indicated in the figure included progesterone (1 μM) and RU486 (0.1, 0.5, and 1 μM). Incubations were performed for 48 h with an 8-h pulse of [3H]thymidine at the end of the treatment. Each assay was done in triplicate. B, brain endothelial cells were isolated from wild-type and PRKO mice and cultured in EBM containing 0.1% FCS. Cells were then treated with progesterone (5 or 0.5 μM) or R5020 (5 μM). Cells were allowed to grow for 2 days, and a pulse of [3H]thymidine was added during the last 8 h. Bars in all panels indicate S.E.

The data are consistent with a specific cell cycle arrest rather than cell death.

The Effect of Progesterone on Cell Cycle Arrest Is PR-mediated—Two experiments added to the specificity of the effect mediated by progesterone and provided support that the suppression on cell cycle required and was dependent on the presence of PR. Fig. 4A shows that the effect of progesterone was blocked by preincubation of endothelial cultures with increasing concentrations of RU486, a potent antagonist of progesterone that specifically binds to PR (29, 30). Similar levels of RU486 alone had no significant effect on thymidine incorporation.

The role of PR on the progesterone-mediated inhibition was further confirmed by experiments in which endothelial cells derived from PRKO mice were treated with the hormone (5). Proliferation of endothelial cells isolated from wild-type animals were inhibited by both progesterone and R5020 at kinetics similar to those described for HDECs. In contrast, endothelial cells from PRKO mice were not affected by the same treatment (Fig. 4B). These findings also confirmed the specificity and lack of toxicity of progesterone on endothelial cells and demonstrate the requirement of PR for cell cycle arrest.

The inhibitory effect of progesterone was applicable to a variety of mitogenic signals, including basic fibroblast growth factor, vascular permeability/endothelial growth factor, and fetal calf serum, alone or in combination. The relative levels of inhibition were equivalent with each of these factors. These results indicated that the effect mediated by progesterone was mostly downstream from these effectors. The biological significance of our findings was tested in ex vivo models of vascular injury.

Progesterone Affects Re-endothelialization of Injured Aorta—The role of progesterone on inhibition of endothelial cell growth could have relevant implications in the regulation of angiogenesis and vascular repair. To evaluate the possible role of progestins in the repair of endothelial denuded areas, we performed intima injury experiments in aortae of wild-type and PRKO mice, the latter as experimental controls. Following dissection of aortae, a 2–4-mm endothelial area was removed. The extension of the injury was assessed by staining with Evans blue and recorded at time 0. Exposed aortae were treated in vitro with endothelial growth factors in the presence or absence of R5020 or progesterone for 4 consecutive days and were then re-stained with Evans blue to evaluate endothelial regrowth (Fig. 5A). Evans blue is a vital dye that binds to albumin and to other proteins in the extracellular matrix (31). Intact endothelium is impermeable to this dye, remaining white (unstained), whereas areas that lack endothelium stain in blue. This method has been extensively used to reveal luminal areas that expose the smooth muscle layer of the media as a consequence of endothelial injury (32–34). In our assays, the initial extent of blue staining was compared with the same re-stained specimen after treatment as indicated. Only the re-growth of the endothelial layer blocked binding of the dye. Significant re-endothelialization was evident in the absence of progestins in both wild-type and PRKO mice. However, presence of progestins greatly suppressed (77%) endothelial repair in aortae from wild-type animals (Fig. 5B). No difference from controls were observed in the rate of endothelial repair in PRKO aortae treated with progesterone. The experiments were performed in 10 wild-type and 12 PRKO mice and included both males and females. These data provided physiological relevance to our findings and confirmed the suppressive effects of progestins on the endothelium.

To further understand the mechanism of action of progestins on endothelial proliferation, we performed a series of cell cycle analysis in the presence and absence of the hormone.

Progesterone Arrests Endothelial Cells in G1—FACS analysis of endothelial cultures treated with progesterone or R5020 showed that these compounds promoted cell cycle arrest in G1/G0 (Fig. 6A). The arrest was cumulative at the expense of cells in S, G2, and M phases. Retention of cells in G1 increased over time, with 38% of the cells at 24 h, 47% at 48 h, and near 80% at 72 h. These experiments also confirmed absence of apoptosis upon R5020 treatment. In control cultures (cells seeded on bovine serum albumin-coated plates (50 μg/ml)), apoptosis was revealed as a peak of cells with DNA content lower than G0/G1. In contrast, cultures treated with R5020, progesterone, or vehicle did not show such a population of cells.

Cell cycle progression from G0/G1 to S is controlled by changes in activity of G1 phase cyclin-dependent kinases (Cdks) that regulate the phosphorylation state of pRb, a key protein for entry in S phase (35). We analyzed pRb phosphorylation to determine whether changes in Cdk activity might result from progesterone treatment (Fig. 6B). Untreated cells showed maximum levels of pRb phosphorylation by 24 h, with decreased phosphorylation thereafter. In contrast, cultures exposed to R5020 showed a 12–24-h delay in pRb phosphorylation when compared with controls. These results are in agreement with the reduction in the S phase fraction seen by FACS analysis and thymidine incorporation. Interestingly, we have observed a hypophosphorylated form of pRb upon R5020 treatment (doublet in Fig. 6B, R5020, 4–24 h). At least three Cdk complexes mediate pRb phosphorylation, specifically Cdk2-cyclin A, Cdk2-cyclin E, and Cdk4-cyclin D1 (36). These pRb inactivating kinases, however, phosphorylate pRb differently, providing several electrophoretic mobility forms of the protein (36). It appears that treatment with R5020 alters the profile of pRb phosphorylation in addition of delaying hyperphosphorylation. Also, these alterations might relate to the direct effect...
of progesterone on cyclins expression, as mentioned below.

To further support the effects of progesterone on cell cycle progression, we examined the relative protein levels of cyclins E and A. Expression of these proteins is associated with checkpoints and progression through early and late G1, respectively. The pattern of expression for cyclin E and cyclin A is altered upon treatment with R5020 (Fig. 6C). Whereas vehicle-treated cells showed a peak of cyclin E and cyclin A expression at 24–36 h...
and 36–48 h, respectively, the levels of these proteins on R5020 treated cells was sustained as long as 60 h for both. This pattern reflects a temporal extension of G1 phase. The experiments did not distinguish whether decrease in protein levels of cyclins E and A are a cause or a consequence of pRb regulation, and these cyclins have been shown to act both upstream and downstream of pRb. Nevertheless, the results demonstrate that progesterone affects expression of several proteins involved in cell cycle progression, and as cause or consequence, Cdk activity is also affected, resulting in inhibition and delay of pRb phosphorylation. We also evaluated p27 and p21 levels upon treatment with progesterone, and no significant alteration was detected in either of these proteins.

Further analysis is required to clarify the specific mechanism by which progestins mediate endothelial cell cycle arrest. However, this discrete analysis provided support to our findings and indicated that the target of progestins is likely upstream pRb phosphorylation during G1.

**DISCUSSION**

In this study, we have provided functional support to the presence of progesterone receptor on vascular endothelium. Although expression of progesterone receptor has been previously reported in the intima of blood vessels using biochemical and immunocytochemical approaches (19, 20), to our knowledge, this is the first study that performs a broad-based analysis on several endothelial types and that provides a functional relevance to this receptor on endothelial biology.

The use of steroid hormones in postmenopausal replacement therapy has been associated with prevention of cardiovascular disease. However, the contribution of progesterone to the replacement regime has been controversial. Although experimental studies evaluating intimal thickness (37) and bromodeoxyuridine incorporation (38) conclude that progesterone directly inhibits the athero-protective effect of estrogen, several epidemiological and in vitro studies demonstrate otherwise (39, 40). The finding that progesterone inhibits re-endothelialization of denuded aortae suggests that this hormone could have an opposite effect on estradiol in endothelial repair of denuded atherosclerotic lesions (41).

Atherosclerosis is a multifactorial process and the principal contributor to myocardial and cerebral infarction (42). Response to injury is one of the favored hypotheses for development of atherogenesis. It postulates that an alteration of the intima by various risk factors (such as mechanical injury, chemically altered low density lipoprotein, viruses, or toxins) initiates a primary endothelial cell dysfunction that leads to subsequent vascular changes, giving rise to the initial atherosclerotic lesion. The plaque progresses by the accumulation of layers of smooth muscle, macrophages, and foam cells; further deposition of extracellular matrix; and possibly neovascularization. Progression or rupture of the plaque is frequently associated with partial physical disruption of the endothelium. Because of the role of the endothelium in providing an antithrombotic and anticoagulant surface, rapid endothelial repair is of importance to the containment of atherosclerotic lesions. Although progesterone has shown suppressive effects on smooth muscle cell proliferation in vitro (8), its participation in intimal endothelial repair, according to this study, appears to be equally inhibitory and, by extension, possibly deleterious to the endothelial healing of an exposed vessel.

The inhibitory effect of progesterone on endothelial proliferation also provides an explanation for the episodes of vessel breakdown and irregular bleeding associated with progestin-based contraceptives (21). Physiological, but constant, levels of circulating progestins most likely affect the high mitotic rate associated with the endometrial vasculature, leading to capillary rupture. Furthermore, progesterone has been implicated in the suppression of tumor-induced neovascularization (43).

In this study, we showed that progesterone partially blocks and delays endothelial cell cycle progression through a receptor-mediated mechanism that involves changes in expression of cell cycle proteins, cyclins E and A, and changes in pRb phosphorylation. The participation of PR in the regulation of cell cycle has been controversial, with studies indicating that progesterone stimulates, inhibits, or does not alter cell cycle progression even on the same cell type (8, 44–46). When inhibitory in mammary epithelial cells, the mechanism by which progesterone mediates cell cycle arrest has been shown to implicate both suppression of Cdk activity (47) and up-regulation of p21 (48). In endothelial cells, however, it is likely that progesterone acts by regulation of Cdk activity only, because we have not detected changes in either p21 nor p27 expression levels (data not shown). It has now become increasingly clear that the functional contribution of PR as a transcription factor is also dependent on the expression of several binding proteins that modulate PR activity (49–52). Whether or not these binding proteins play a role in the regulation of endothelial cell cycle or any other cell type is, at this point, not clear and deserves further investigation. Nevertheless, we found that endothelial cell proliferation was suppressed by physiological levels of 17a-hydroxy-progesterone in every endothelial cell type examined.

In conclusion, the presence of progesterone receptor in endothelial cells and the direct effect of progesterone described here can be of physiological relevance to the balance between potentiation of endothelial growth, mediated by estradiol, and suppressive signals, mediated by progesterone. This balance could play an important role in the regulation of angiogenesis in the endometrium and corpus luteus during the menstrual cycle. Nonetheless, constant levels of progestins in HRT could be counter-productive. Although the benefits of progestins, as first or second line therapy in the treatment of breast and endometrial carcinoma, have been widely acknowledged, the long-term use of progestins as contraceptives or for the prophylaxis of menopause should be under closer scrutiny. It is clear from these and other data that progestins may have direct growth-inhibitory actions in nonreproductive sites, apart from their ability to inhibit estrogen-mediated cell proliferation in such classical reproductive targets as the endometrium.

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