Progesterone withdrawal causes endothelin release from cultured human uterine microvascular endothelial cells

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**BACKGROUND:** Current theories on the physiology of menstrual bleeding in humans offers an explanation for the shedding of the endometrium as a result of a breakdown of the extracellular matrix due to an inflammatory reaction. The link between the fall in progesterone levels and these events is not clear. Neither has an explanation been presented for the vasoconstriction in the coiled arteries occurring during menses. We have hypothesized a chain of events where the fall in progesterone levels induces an upregulation of the thrombin receptor in the small uterine arteries leading to an increased thrombin response and subsequent endothelin release. METHODS: Endothelial cells from human umbilical cord (HUVECs) and from human small uterine arteries (UtMVECs) were cultured under conditions partly resembling the female hormonal cycle with progesterone withdrawal. RESULTS: Following progesterone increase and subsequent withdrawal, we found an increased production of thrombin receptor and an increased release of endothelin from UtMVECs compared with HUVECs. CONCLUSION: Endothelin release in response to progesterone withdrawal in UtMVECs can offer an explanation for the vasoconstriction seen in the coiled arteries during menses in humans.

**Key words:** coiled artery/endothelin/menstrual bleeding/progesterone/thrombin receptor

**Introduction**

The monthly human menstrual bleed is an intricately and highly regulated event involving the endocrine, cardiovascular and haemostatic systems. Despite enormous advances in molecular biology during the last decades, details of the regulation of menstrual bleeding are still not known. The shedding of the endometrium follows in the absence of or at failed implantation. It is preceded by the demise of the corpus luteum and, as a consequence thereof, a fall in circulating progesterone. The progesterone withdrawal initiates a sequence of events that ultimately lead to the shedding of the endometrium and menstrual bleeding.

Past and present theories regarding the mechanisms of menstruation involve, to some extent, vasoconstriction in the coiled arteries (also called spiral arteries) of the endometrium. In the early works by Markee, this constriction was suggested to precede menstrual bleeding (Markee, 1940). For >60 years, scientists have searched with increasingly advanced methods for a link between progesterone withdrawal and the regulation of menstrual bleeding. However, the link between progesterone withdrawal and the subsequent coiled artery vasoconstriction has been poorly studied and is not at all clear. Many factors have been suggested to be involved; hormones, enzymes, inflammatory mediators, haemostatic factors and growth factors. We have focused our interest on the 21 amino acid polypeptide endothelin (ET). Endothelins consist of a group of three peptides, ET-1, ET-2 and ET-3, with potent biological properties. ET-1 is produced mainly by endothelial cells and is the most potent vasoconstrictor peptide known to date (Yanagisawa et al., 1988). Other tissues produce ET-2 and ET-3.

The ETs appear to act primarily by a direct effect on vascular smooth muscle cells (Gude et al., 1991). Thus, the main site of action of ET in the endometrium is likely to be the vascular smooth muscle cells of the small endometrial vessels. Normally, circulating ET levels, as well as production of the peptide in isolated blood vessels, are rather low due to the absence of stimuli and the presence of potent clearance mechanisms. Important stimulators of ET production are thrombin, angiotensin, arginine, vasopressin and transforming growth factor-β, as well as certain cytokines and physico-chemical factors such as hypoxia (Luscher et al., 1993).

We hypothesized that uterine microvascular endothelial cells might release endothelin in response to progesterone withdrawal, and that ET therefore could be one agent that contributes to endometrial vessel contraction. The resulting endometrial ischaemia could then be one of the regulators of menstrual bleeding.

Menstrual bleeding also intimately involves the coagulation system. As a candidate for the link between progesterone withdrawal, ET release and the coagulation system, we have studied thrombin. Progesterone has been found to regulate
synthesis of the G protein-coupled protease-activated receptor-1 (PAR-1), the receptor for thrombin, in the human endometrium (Hague et al., 2002) and in the arterial wall of the aorta (Herbert et al., 2001). Thrombin is known to upregulate endothelin-converting enzyme (ECE-1) in human endothelial cells (Eto et al., 2001). Furthermore, thrombin is one factor that stimulates ET release, as shown by several authors (Yanagisawa and Masaki, 1989; Ohlstein and Storer, 1992). One endothelial cell response to stimulation by thrombin through the thrombin receptor is release of the contents of the Weibel–Palade bodies. These contain several locally effective proteins, including von Willebrand factor (Wagner et al., 1982) and ET (Russell et al., 1998). Thrombin also induces secretion of another storage particle containing tissue plasminogen activator (tPA) (Eimes et al., 1997).

Following progesterone withdrawal, there is an increase of tPA (Lockwood and Schatz, 1996) possibly as a response to an increased thrombin response.

To test the hypothesis that endothelial cells in the uterine small arteries might upregulate thrombin receptors and release ET in response to progesterone withdrawal, we have cultured uterine microvascular endothelial cells (UtMVEC-Myos) in the presence of estrogen and progesterone, and analysed ET levels in the culture supernatants as well as thrombin receptor occurrence in the cells under conditions simulating progesterone withdrawal. In order to compare the reactions to progesterone withdrawal in UtMVECs with another endothelial cell type commonly used for studies of endothelial production of vasoactive substance, we also performed the same experiment in human umbilical vein endothelial cells (HUVECs). There are documented differences between the two endothelial cell types (Lang et al., 2003).

**Materials and Methods**

**Cells and cell culture**

HUVECs (passage 243) and UtMVEC-Myos (passage three) were from BioWhittaker (Cambrex, Walkersville, MD). Verification of endothelial cell purity was determined by immunoreactivity for CD-31 and lack of immunoreactivity for human smooth muscle actin. The culture media used were Endothelial Cell Growth Medium BulletKit-2 (EGM-2) and Microvascular Endothelial Cell Growth Medium-2 (EGM-2 MV), respectively (BioWhittaker, Cambrex, Walkersville, MD). These media contain endothelial cell growth medium with human recombinant epidermal growth factor, human fibroblast growth factor, vascular endothelial growth factor, ascorbic acid, hydrocortisone, human recombinant insulin-like growth factor, heparin, gentamicin, amphotericin-B and fetal bovine serum (FBS; 2% for HUVECs, 5% for UtMVECs). Trypsinization was performed with Reagent Pack containing trypsin/EDTA, trypsin neutralizing solution and HEPES-buffered saline solution (BioWhittaker). Culture was performed in 25 cm² cell culture flasks from Corning (New York) and cultures were seeded with 62 500 (HUVEC) to 140 000 (UtMVEC-Myo) cells. After 4 days, the cells were confluent to ~75% and each flask contained 0.23–1.35 × 10⁸ cells/ml.

**Treatment of cells**

Each culture was done in three flasks simultaneously with the same batch of medium and with treatments in parallel. The cultures were grown for 4 days, the medium volume was 5 ml. The experiment was repeated once for each cell line. The three treatments used were as follows: (i) control in medium without hormone addition for 4 days; (ii) treatment with estrogen 500 pmol/l for 4 days and progesterone 50 nmol/l for 4 days; and (iii) treatment with estrogen 500 pmol/l for 4 days and progesterone 50 nmol/l for the first 2 days only (progesterone withdrawal). After 4 days, the cells were harvested, centrifuged at 500 g for 10 minutes in room temperature to a pellet, counted and frozen at ~80°C.

Drugs used for treatment were as follows: estrogen in the culture medium was added as a 17β-estradiol:HBC (2-hydroxypropyl-ß-cyclodextrin) complex (catalogue no. E-126), and progesterone was added as 4 pregnene-3,20 dione (catalogue no. P-8783) from RBI Sigma Chemical Co (St Louis, MO). The estrogen preparation is a water-soluble complex, whereas the progesterone was dissolved in dimethylsulphoxide (DMSO) (<0.0005% in final solution) (Merck, Darmstadt, Germany). DMSO was also added in a corresponding amount in sham solution when progesterone was not added.

**Biochemical analyses**

The medium in each flask was changed each day and kept frozen at -18°C until analysed for levels of ET, thrombin and added hormones.

**Endothelin.** ET concentration was measured with Quantiglo Chemiluminescent Immunoassay from R&D Systems. With antibodies against human ET-1, the sensitivity limit is <0.16 pg/ml, and cross-reactivity with ET-2 and ET-3 is 27 and 8%, respectively.

**Thrombin.** The level of thrombin in culture medium was determined by use of the chromogenic substrate S-2238 (Hemochrom Diagnostica AB, Mölndal, Sweden) (Axelsson et al., 1976). Optical densities were measured in a spectrophotometer at 405 nm.

**Estrogen and progesterone.** Estrogen and progesterone concentrations were analysed using a competitive immunoassay, Immulite, from Diagnostic Products Corporation (LA, USA).

**Immunoblotting**

After solubilization in 1% SDS (RBI Sigma), thrombin receptors in the cell pellets were analysed by immunoblotting using a mouse monoclonal IgG antibody raised against amino acids 42–55 of thrombin receptor of human origin (Santa Cruz Biotechnology, Santa Cruz, CA). The semi-quantitative analysis of the amount of thrombin receptor was done using a ChemiDoc Gel Documentation System with the software Quantity One version 4.4.0 from Bio-Rad (Hercules, CA).

**Immunohistochemistry**

We analysed cells cultured on Falcon culture slides (Becton Dickinson Labware, NY). The slides were fixed using 4% paraformaldehyde and 0.03% picric acid, and kept in phosphate buffer pH 7.4 (PBS) in a refrigerator until analysed. Monoclonal mouse antibodies specific for endothelial cells were anti-human CD31 (clone JCT70A code no. M 0823 lot 110, Dako, Denmark). As negative control, we used anti-human smooth muscle actin (clone 1A4 code no. M 0851 lot 050, Dako) and anti-human fibroblast prolyl 4-hydroxylase (clone 5B5 code no. M 0877 lot 029, Dako). For analysis of the thrombin receptor (PAR), the cells were labelled with mouse monoclonal IgG antibody raised against amino acids 42–55 of thrombin receptor of human origin (ATAP2 sc-13503, Santa Cruz Biotechnology, Inc.). For analysis of the progesterone receptor, we used mouse monoclonal IgG antibodies raised against the N-terminal region of the A form of the human progesterone receptor (NCL-PGR-312; Novocastra, Newcastle Upon Tyne, UK).
upon Tyne, UK). These antibodies are specific for the human progesterone receptor of both the A and the B form. Secondary antibodies used were goat anti-mouse IgG labelled with green fluorescent Alexa 488 dye (Molecular Probes, Eugene, OR). Negative controls of the thrombin and the progesterone receptors for these endothelial cells were carried out with secondary antibodies only. The cells were observed and photographed using a fluorescence microscope (Axioskop, Zeiss, Göttingen, Germany) with the appropriate filters.

Coiled arteries response to endothelin

In order to verify that the small uterine vessels (the coiled arteries specifically) contract in response to ET exposure, we performed in vitro studies of cannulated blood vessels. We used a video dimension analyser, a vessel chamber and perfusion equipment from Living Systems Instrumentation (Burlington, VT).

Uterine coiled arteries, 100–120 μm in inner diameter, were obtained from the myometrial–endometrial border of the uterus in connection with hysterectomy for benign disease in pre-menopausal women. The local medical ethics committee approved the study. A segment of the artery (5–10 mm long) was carefully dissected from surrounding muscle, endometrium and connective tissues and transferred to the experimental chamber filled with oxygenated Krebs buffer (NaCl 120 mmol/l, KCl 5.9 mmol/l, MgSO4 1.2 mmol/l, NaHCO3 15.4 mmol/l, KH2PO4 1.2 mmol/l, CaCl2 2.5 mmol/l and glucose 0.2%) at 37°C. The proximal end of the artery was fed onto a glass microcannula (tip diameter 100–120 μm) and tied with a single strand (20 μm) of braided nylon thread. After the artery was flushed with Krebs buffer to remove intraluminal blood, the distal end of the vessel was similarly cannulated and tied. With the use of a pressure servo and peristaltic pump instrument, the intraluminal pressure was kept constant at 70 mmHg (Halpern et al., 1984).

The vessel in the experimental chamber was perfused continuously with Krebs buffer, passing through an external water bath at 37°C. A conductive glass in the base of the vessel chamber, a thermocouple probe and a heat controller maintained the temperature at 37°C. A gassing stone was inserted into the well for superfusate solution pH regulation.

The arteriograph containing the pressurized vessel was placed on the stage of an inverted microscope (Nikon TSM-F, Kanagawa, Japan) with a monochrome video camera attached to a viewing tube. Arterial dimensions were measured using a video system that provides automatic continuous read-out measurements of luminal diameter (Halpern et al., 1984). The output of the video dimension analyser was sent to an IBM-compatible computer by means of a serial data acquisition system (DATAQ Instruments, OH, USA) for visualization of dynamic responses of the diameter.

Data analysis and statistics

All data analyses were run on a Dell latitude laptop computer and the calculations were performed using GraphPad Prism version 3.03 for Windows (GraphPad Software, San Diego CA). Statistical analysis of ET changes was carried out using the Mann–Whitney test and considered significant at the level of \( P < 0.05 \).

Results

Endothelin levels

Basal ET levels were 0.6 to 6.5 pg/ml culture medium from both cell types before treatments were commenced. During the 4 days of treatment, ET production increased daily, but at different rates in the three treatments studied and in the two cell types. In the control group without treatment, there was an approximately linear increase in ET content during the 4 days of culture (Figure 1a), whereas with the two types of hormone treatment, ET changed differentially (Figure 1b and c). In order to quantitate and compare changes in ET production in the cultures, we defined the change in ET production over time (ΔET) as the difference in ET production between one day and the previous day, i.e. the ET production at the observed time point as compared with the ET production 24 h earlier. Using the ΔET index, it is clearly seen that there was no difference in ET production in the control group without added hormones (Figure 2a) or in the group where progesterone was added for all 4 days (Figure 2b). However, in the group subjected to progesterone withdrawal, we found an increased ET production 24 h after progesterone was withdrawn in UtMVECs as compared with HUVECs, as clearly seen by a significantly increased ΔET (Figure 2c). In HUVECs, the opposite was seen,

Figure 1. Total ET content in culture medium during the 4 days of culture. The y-axis shows the ET content (pg/ml) and the x-axis shows the days of culture. Values given are mean and SEM. (a) Control without added hormones. (b) Treatment with estrogen + progesterone for 4 days. (c) Treatment with estrogen for 4 days + progesterone for the first 2 days (progesterone withdrawal).
a decreased ET production (Figure 2c). Thus, the only culture condition where we found an increased rate of production of ET during the last 24 h of culture was in UtMVECs, where ET production was increased in the period 24±48 h after progesterone withdrawal.

**Cell origin control**

We checked that we had endothelial cells in culture by immunohistochemistry using endothelial cell-specific markers (Figure 3). Cells from the first set of controls for each cell line were cultured on a Falcon culture slide and showed positive staining for the endothelial cell marker CD-31 in UtMVECs (Figure 3a) and HUVECs (Figure 3b). As a negative control, we stained for smooth muscle actin, which was absent in both cell types (shown only for HUVECs, Figure 3c) and with secondary antibody only (not shown). Cells were also documented by photography through light microscopy (not shown).

**Thrombin receptors**

The presence and the relative occurrence of thrombin receptors in the cultured endothelial cells were studied by immunohistochemistry and by immunoblotting. We found evidence for the presence of thrombin receptors in both cell types by immunofluorescence staining (Figure 4). In UtMVECs, there was a strong perinuclear and granular staining pattern, with occasional staining also more peripherally in the cytoplasm (Figure 4a). In HUVECs, staining was weaker, but the localization was similar to that seen in UtMVECs (Figure 4b). The negative control gave no staining (not shown).

A semi-quantitative analysis of thrombin receptor content by immunoblotting extracts of the two different types of cells showed an increased amount of thrombin receptor in HUVECs and a decreased amount in UtMVECs after 4 days of exposure to progesterone. In the cells subjected to progesterone withdrawal, this difference was reduced (Figure 5). After 4 days of progesterone, the relative density of the thrombin receptor band in HUVECs was 1.4 (±0.7) and in UtMVECs 0.5 (±0.2).

**Thrombin**

We could not detect any production of thrombin in the cell lines with our treatment regimens. All the thrombin content in the culture medium originated from the FBS (not shown).

**Progesterone receptors**

We found, by immunohistochemistry, the presence of progesterone receptors in the nuclei of both UtMVECs (Figure 6a) and HUVECs (Figure 6b). Cells exhibited a strong nuclear staining as compared with controls incubated with only secondary antibody (not shown).

**Endometrial vessel responses to ET**

The analysis of vessel contractility showed that, like other arterial vessels, the coiled artery contracts as a result of exposure to ET. After addition of ET (10⁻⁷ mol/l), we obtained a reversible and reproducible contraction, from 300 to 140 μm external diameter. This contraction was blocked by the specific ET receptor blocker BQ-123 (10⁻⁷ mol/l) (Figure 7).

**Discussion**

Early theories on the physiology of menstruation originate from the work of Markee (1940) who postulated that the tissue destruction initiating the shedding of the endometrium resulted from anoxia. This was concluded from the finding that vessels in autologous endometrium transplanted to the anterior chamber of the eye of rhesus monkeys reacted to progesterone withdrawal with vasoconstriction. Current theories suggest an inflammatory response with cytokine upregulation with effects on numerous systems including the matrix metalloproteinases (MMPs) (Henriet et al., 2002). This in turn initiates menstruation by tissue destruction and breakdown of the extracellular matrix, preceding as well as following vasoconstriction (Salamonsen et al., 1999; Critchley et al., 2001b; Kelly et al., 2002).

ET is one of the most potent endogenous vasoconstrictors known. Previous work has shown that ET may be involved, but...
compelling evidence for the role of ET in regulation of menstruation is lacking. It has been shown previously that ET has a crucial role in the regulation of vessel contractility (Yanagisawa et al., 1988) in several organ systems including the urogenital tract. ET contracts vascular and myometrial smooth muscle in the uterus (Fried and Samuelson, 1991) and has been shown to be more potent in contracting small branches of the uterine artery than in contracting the main stem (Ekström et al., 1991). Furthermore, previous studies have demonstrated cyclical changes in the endometrial content of ET (Economos et al., 1992; Marsh et al., 1994) and ET receptors (O’Reilly et al., 1992). Marsh et al. (1997) also showed a correlation between menorrhagia and reduced ET levels as well as lack of cyclic variation in the glandular epithelium. Thus several authors postulate a central role for ET in the events preceding and regulating menstrual bleeding. In the present experiment, we show an increase in ET secretion from UtMVECs in response to progesterone withdrawal.

The ET that would reach the coiled arteries and act as a vasoconstrictr is likely to be released from vessels preceding the coiled arteries in the blood flow, from radial artery endothelial cells. It is known that endothelial cells have different properties in different types of vessels and in different organ systems. Earlier studies have shown changes in the expression of endothelial function markers after treatment with the progestins medroxyprogesterone acetate (MPA) and norethisterone (NET) (Mueck et al., 2002). To our knowledge,
no investigations exist on the difference in ET production from uterine microvasculature when exposed to progesterone and subsequent withdrawal.

The present work supports our theory that there is an increased level of ET in the smallest uterine arteries as a response to progesterone withdrawal. The reason for the relative increase of ET in our experimental set-up may be due to a number of factors. It may be due to increased synthesis of the precursor peptide pre-proendothelin, increased processing of the precursor peptide, decreased degradation of ET, changes in turnover not related to synthesis or degradation, i.e. transport and/or clearance, or it may even be due to factors related to cell culture such as cell density, passage number or changes in cell adhesion properties. It is known that ET production in HUVECs is strongly dependent on culture conditions (Ranta et al., 1997). In the two types of cells that we have studied, UtMVECs and HUVECs, we saw a diametrically opposed response when exposed to progesterone withdrawal. The HUVECs are derived from a vein that has the properties of an artery, and transports oxygenated blood from the placenta to the fetus. Thus, physiologically, it would be deleterious if a drop in progesterone levels should induce increased production of ET and constriction in the umbilical vein. Growth requirements and growth condition for UtMVECs and HUVECs differ, therefore culture conditions may be a reason for differences in ET synthesis pattern between the two cell types. However, our experimental set-up was such that we believe that factors related to culture conditions are controlled.

**Figure 4.** Immunofluorescence images showing (a) UtMVECs and (b) HUVECs stained with mouse monoclonal IgG antibody against the thrombin receptor. Note the granular cytoplasmic staining in the immediate perinuclear region, often asymmetrically localized predominantly adjacent to one nuclear hemisphere. (Magnification ×400.)

**Figure 5.** Thrombin receptor content in the two cell types quantitated by relative optical density after immunoblot. Values given are mean and range (n = 4, SD). Control: no hormones added, Prog 4 days: addition of estrogen for 4 days and progesterone for 4 days. Prog 2 days: addition of estrogen for 4 days and progesterone for the first 2 days.
for, and thus would not be the reason for the increase in ET in UtMVECs after progesterone withdrawal.

Our protocol with 4 days of hormone treatment partly resembles the female hormonal cycle during the last 4 days of a normal menstrual cycle. The longer the treatment period continues, the higher is the risk of confounding factors such as differing density of cells. Even if it would be beneficial to prolong the treatment period, not only to better resemble the direct effects of hormone influence but also to achieve a possible estrogen-induced priming of the progesterone receptors, we considered the disadvantages to outweigh the advantages. We also found that previous work presenting similar studies of hormone effect on cells in culture report results after 24 h (Mueck et al., 2002) to 3 or 5 days (Casey et al., 1991). A decreased degradation of ET after progesterone withdrawal is one possibility for the observed results. This would then imply a link between progesterone and enkephalinase, the main ET-degrading enzyme [neutral endopeptidase (NEP) EC 3.4.24.11] (Vijayaraghavan et al., 1990). The activity of enkephalinase, localized predominantly to stromal cells in the human endometrium (Casey et al., 1991; Imai et al., 1992; Head et al., 1993), changes through the menstrual cycle, with an increase between early proliferative and mid secretory phases. The subsequent fall in enkephalinase activity coincides with the fall in progesterone concentrations after luteolysis (Casey et al., 1991). During menstruation, enkephalinase activity is low, and this is a possible explanation for the increased ET activity seen during menstruation (Casey et al., 1991; Imai et al., 1992; Head et al., 1993). The mechanism regulating enkephalinase activity fluctuation during the menstrual cycle is not known.

We believe that another likely explanation for the increase in ET may be an increase in synthesis or processing. ET is processed from its precursor peptide pre-proendothelin by the enzyme ECE-1 (Eto et al., 2001). One factor that both upregulates ECE-1 and stimulates ET release is thrombin (Ohlstein and Storer, 1992). Thrombin is a serine protease and one of the most important enzymes in the blood clotting cascade. In addition to its action in haemostasis, thrombin has been found to act as a cofactor in response to sex steroids (Henrikson et al., 1990, 1994; Henrikson, 1992). Treatment with progesterone increased the levels of mRNA for the proteolytically activatable thrombin receptor (PAR-1) in vascular smooth muscle cells (Herkert et al., 2001). We did not find evidence for production of thrombin in our cultured endothelial cells in any of the hormone treatments. We do, however, find indications of an increased expression of the thrombin receptor in HUVECs and, interestingly, a decrease in UtMVECs. This effect is not seen after progesterone withdrawal, which gives the effect of a relative thrombin receptor upregulation in UtMVECs after progesterone withdrawal. This would be in agreement with the observation that progesterone withdrawal decreases ET in HUVECs and increases ET in UtMVECs, assuming that thrombin receptors mediate ET

Figure 6. Immunofluorescence staining of (a) UtMVECs and (b) HUVECs showing progesterone receptors in the cell nuclei. Note the granular staining throughout the cell nuclei and absence of staining in the cytoplasm. (Magnification ×400.)

Figure 7. Contraction of a coiled artery from 300 to 140 μm external diameter as a response to ET exposure. The specific endothelin blocker BQ-123 blocks the contraction.
release and increased activity of ECE-1. One part of the difference in ET release after progesterone withdrawal could then be explained by the increased expression of thrombin receptor.

In order to respond directly to progesterone withdrawal, it is likely that the UtMVECs express progesterone receptors. There are reports of the absence of progesterone receptors in the vascular endothelium of the animal coiled arteries (Perrot-Applanat et al., 1994; Kohen et al., 2000; Critchley et al., 2001a). However, cells in the vascular tree immediately preceding the spiral arteries must also be considered in this regard. We found in our cultured UtMVECs consisting of endothelial cells from small uterine vessels including both spiral arteries and vessels preceding the spiral arteries, a positive staining for progesterone receptors (Figure 6), explaining the observed reactivity to changes in progesterone exposure.

The effects of estrogen and progesterone have also been reported in other organ systems. For example, Mueck et al. (2002) found a reduced ET production in human female coronary endothelial cell cultures when treated with estrogen alone or with estrogen and a progestin (MPA or NET). It may be hypothesized that a similar inhibitory effect exists in the endothelial cells in our study. Thus the special property of the endothelium from the small uterine arteries is to ‘rebound’ and increase the production of ET when the progesterone inhibition is removed. Interestingly, it has also been shown in vivo that high circulating levels of estrogen in women undergoing hormone stimulation for IVF were accompanied by a decrease in circulating levels of ET-1 (Cacciatoire et al., 1997).

The events that take place in the endometrium following progesterone withdrawal, leading to menstruation and endometrial repair, are complex. Several cell types and several subpopulations of each cell type are involved. In this report, we present the results of a study on one of these cell types that, at least in vivo, interacts with adjacent and more distal cells through processes and paracrine actions. We have, however, in this isolated cell type and as a result of progesterone withdrawal, been able to detect an increased production of the potent vasoconstrictor ET, by many postulated to be pivotal in the constriction of the spiral arteries during menses. These findings can add another piece to the jigsaw of understanding menstrual physiology.

References


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