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Expression and cyclic variations of catechol-O-methyl transferase in human endometrial stroma

Sana M. Salih, M.D.^a, Salama A. Salama, Ph.D.^a, Amin A. Fadl, Ph.D.^b, Manubai Nagamani, M.D.^a, and Ayman Al-Hendy, M.D., Ph.D.^a

^aDepartment of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas

^bDepartment of Microbiology & Immunology, Tuskegee University School of Veterinary Medicine, Tuskegee, Alabama

Abstract

Objective—To investigate the role of catechol-O-methyl transferase (COMT) in the regulation of estrogen metabolism in human endometrium.

Design—Laboratory study.

Setting—Academic research laboratory.

Intervention(s)—Immunohistochemistry was used to localize COMT protein in human endometrial tissues. Catechol-O-methyl transferase promoter–luciferase reporter gene transactivation assay was used to assess COMT promoter activity in response to estrogen and progesterone treatment in primary human endometrial stroma (pHES) cells. Catechol-O-methyl transferase protein and mRNA expression were determined by Western blot and/or real-time polymerase chain reaction. The effect of 2-methoxy estrogen treatment on DNA proliferation, B-cell lymphoma 2, and vascular epithelial growth factor protein expression were assessed by Hoechst and Western blot analyses, respectively.

Main Outcome Measure(s)—Catechol-O-methyl transferase protein and mRNA subcellular localization and expression in human endometrial tissues and pHES cells.

Result(s)—Catechol-O-methyl transferase protein expression in human endometrial tissues was up-regulated in the proliferative phase and down-regulated in the midsecretory phase of the menstrual cycle. Estrogen induced a dose-dependent increase in COMT proximal promoter–luciferase transactivation in pHES cells whereas progesterone inhibited it. Estrogen up-regulated soluble COMT protein isoform expression whereas the addition of progesterone down-regulated it in pHES cells. High doses of 2-methoxy estrogen inhibited endometrial stroma cell proliferation, and down-regulated B-cell lymphoma 2 and vascular epithelial growth factor protein expression.

Conclusion(s)—Catechol-O-methyl transferase expression is hormonally regulated in human endometrial stroma. Catechol-O-methyl transferase product, 2-methoxy estrogen, inhibited endometrial stroma cell proliferation and decreased vascular epithelial growth factor and B-cell lymphoma 2 protein expression.

Keywords

Catechol-O-methyl transferase; human endometrial stroma; catechol estrogen; 2-methoxy estrogen

The human endometrium is a metabolically active tissue that undergoes monthly cell proliferation, differentiation, and apoptosis during the female reproductive cycle. The molecular mechanisms that control synchronized endometrial development during each menstrual cycle are still poorly understood. Estrogen and progesterone are known to modulate the endometrium in a variety of ways. Estrogen stimulates endometrial cell proliferation whereas progesterone induces endometrial cell decidualization and prepares the endometrium for blastocyst implantation. Genes that are modified by estrogen and/or progesterone are likely to be important players in the monthly cyclic development of the endometrium and possible culprits in the pathogenesis of endometrial disorders. Catechol-O-methyl transferase (COMT) is an enzyme that catalyses O-methylation and inactivation of a variety of metabolically active catechol compounds such as epinephrine, norepinephrine, and dopamine. Catechol-O-methyl transferase is also involved in the final stages of estrogen metabolism.

Estrogen is oxidized by the cytochrome P450 group of enzymes (1A1 and 1B1) to catechol estrogens (2-hydroxy and 4-hydroxy estrogens, respectively). Catechol-O-methyl transferase converts 2-hydroxy estrogens (2-OHE) to their respective 2-methoxy estrogens (2-ME) by catalyzing the transfer of O-methyl group to one of the hydroxyl groups of the catechol substrate in the presence of MG^{+2} (1). 2-Hydroxy estrogens bind to estrogen receptors and works as an antiestrogen, while 2-ME has a 50% estrogen receptor-independent estrogenic effect at physiologic concentrations (1–4). 2-Methoxy estradiol (2-ME2) has a number of effects on its own, unrelated to estradiol, 2-hydroxy estradiol, or other methoxy estrogen derivatives (5). 2-Methoxy estradiol is one of the most potent endogenous inhibitors of angiogenesis known to man (6). It is a potent inhibitor of endothelial cell proliferation and migration as well as angiogenesis in vitro. It disturbs the function of the microtubules, leading to failure of microtubular polymerization (7, 8). It also plays a crucial role in cellular proliferation and differentiation through the induction of ERK mitogen-activated protein kinase (9). 2-Methoxy estradiol is exclusively produced by O-methylation of 2-hydroxy estradiol by the COMT enzyme in extrahepatic tissues (1).

The regulation of COMT expression and the role of perturbed COMT metabolites in human endometrium has not been well characterized. In this report we study the regulation and the potential role of COMT expression in human endometrium. We evaluated COMT mRNA and protein expression in human endometrial tissues and investigated the in vitro regulation of COMT using pHES cells. Furthermore, we assessed the effect of increased COMT product, 2-ME2, on the proliferation and expression of cell cycle regulatory proteins in primary endometrial stroma cells.

MATERIALS AND METHODS

Patient Selection and Human Tissues Processing

Endometrial tissue samples were collected from 16 women presenting with a benign gynecologic disorder such as uterine fibroids, endometriosis, and cervical dysphasia, and requesting definitive treatment in the form of hysterectomy. Patients' age ranged from 35 to 45 years. All patients have COMT^{Met/Val} genotype, which has an intermediate COMT enzyme activity, compared with the high activity allele (COMT^{Val/Val}) and to the low activity COMT allele (COMT^{Met/Met}) as described below. Eight patients were in the secretory phase of their cycle and eight patients were in the proliferative phase of their cycle. Patients did not receive hormonal treatment for at least 6 weeks before sample collection. Endometrial samples were collected from the upper lateral anterior and posterior uterine walls after the completion of hysterectomy surgery. Tissue samples were submersed immediately in liquid nitrogen or formalin and stored until use. Part of the specimen was collected in sterile Hank's balanced salt solution (containing 25 mM of HEPES and antibiotics), and was used to establish primary endometrial stromal cell culture. Fixed formalin tissue blocks were used for immunohistochemistry, while protein was extracted from the frozen endometrial tissues for Western blot analysis. This study protocol was approved by the institutional review board of the University of Texas Medical Branch.

Genotyping of the COMT Gene

DNA was extracted from peripheral blood samples (10). The following primers 5'-CTC ATC ACC ATC GAG ATC AA-3' (forward) and 5'-CCA GGT CTG ACA ACG GGT CA-3' (reverse) were used in polymerase chain reactions (PCRs) using a DNA thermocycler Perkin-Elmer Cetus, GeneAmp PCR system 9600 (Perkin Elmer Cetus, Norwalk, CT) as described previously (11). Polymerase chain reaction product size was 109 bp. The PCR product was digested with 2 U of *Nla*III (New England BioLabs, Beverly, MA) at 37°C overnight, followed by 4.5% agarose gel electrophoresis. Catechol-O-methyl transferase genotypes (Val and Met alleles) were discriminated by the size of the restriction fragments. The Val/Val homozygotes (86 and 23 bp), Met/Met homozygotes (68 and 18 bp), and Val/Met heterozygotes (86, 68, 23, and 18 bp) were visualized by ethidium bromide staining.

Immunohistochemistry

Human endometrial tissue specimens were collected from representative areas. Tissues were fixed in phosphate-buffered saline (PBS) solution containing 10% formalin, embedded in paraffin, then sectioned. Immunohistochemistry was conducted using standard techniques (12). Paraffin-embedded tissue sections (5 µm) were deparaffinized and dehydrated by passage through xylene and graded ethanol solutions. Highly specific primary antihuman COMT polyclonal antibodies raised in the guinea pig (kind gift from Tenhunen, Orion Corporation, Orion-Farmos, Research Center, Helsinki, Finland) was applied to sections at a 1:250 dilution as described earlier (13). Diaminobenzidine served as the chromagen to detect COMT protein, and tissue sections were counterstained with hematoxylin. Negative controls included omission of the primary antibody or substitution of the COMT antiserum with normal serum from the guinea pig, the species used to generate the COMT antiserum.

Catechol-O-methyl transferase immunoreactivity in endometrial tissues was visually assessed by assessing the intensity and counting the frequency of the brown staining (Fig. 1).

Cell Culture

Primary human endometrial stromal (pHES) cells were used to study the in vitro regulation of COMT expression in response to estrogen, progesterone, or 2-ME2 treatment. The cells were established from fresh endometrial tissues collected from hysterectomy samples, as have been described previously (14). Endometrial tissue was finely minced, and cells were dispersed by incubation in Hank's balanced salt solution containing HEPES (25 mM), penicillin (200 U/mL), streptomycin (200 µg/mL), collagenase (1 mg/mL, 15 U/mg), and DNase (0.1 mg/mL, 1,500 U/mg) for 20–30 minutes at 37°C with agitation. Stroma cells were separated from glandular cells by filtration through a wire sieve with 73-µ diameter pores. The stroma cells that were found in the filtrate portion were pelleted, washed, and suspended at 37°C in 5% CO₂/air in phenol red-free Dulbecco's modified Eagle's medium (DMEM), containing antibiotics, 2-mM L-glutamine, and 10% fetal bovine serum. The cells were passaged once and grown to confluence. Confluent mono-layers were maintained in media containing 10% charcoal-stripped fetal bovine serum for 48 hours, and subsequently treated with different concentrations of 17-β-estradiol (10⁻¹⁰–10⁻⁶ M), progesterone (10⁻⁸–10⁻⁶ M) with or without 17-β-estradiol (10⁻⁸), 2-ME2 (10⁻¹⁰–10⁻⁶ M) in 0.01% (v/v) ethanol, or with only 0.01% ethanol as a negative control. Cells were harvested 24 hours after treatment for RNA isolation or 48 hours after treatment for protein extraction.

Construction of Chimeric COMT Proximal Promoter (COMTP1) and COMT Distal Promoter (COMTP2)–Luciferase Reporters

Catechol-O-methyl transferase proximal promoter (COMTP1) and COMT distal Promoter (COMTP2) were amplified and cloned in pGL3–luciferase reporter vector as we have previously described (13) using genomic DNA isolated from normal human myometrial tissues as a template. Briefly, the promoter regions were amplified using promoter-specific forward and reverse primers. The PCR products were purified, cloned into a pGEM-T Easy cloning vector, and subsequently subcloned into a linearized PGL3 luciferase reporter vector. Colonies containing plasmids with the correct inserts were cultured and plasmid DNA was isolated using QIAFilter (Qiagen Inc., Valencia, CA).

Mammalian Cells Transfection with pCOMT Promoter–Reporter Plasmids

The activities of COMTP1–luciferase and COMTP2–luciferase reporter constructs were determined in transiently transfected logarithmically growing pHES cells. Cells (60%–70% confluent) were transfected with COMT promoter–luciferase reporter constructs (10 µg), using the calcium phosphate transfection method as we have described earlier (13). Briefly, after DNA transfection, pHES cells were fed fresh medium and incubated for 16 hours at 37°C. The medium was then removed, and the cells were incubated in fresh medium with the addition of different concentrations of 17-β-estradiol (10⁻⁷–10⁻¹⁰ M), progesterone (10⁻⁶–10⁻⁷ M) in 0.01% (v/v) ethanol, or with only 0.01% ethanol as a control. After 48 hours of incubation of the transfected cells with different treatments, luciferase activity was determined using luciferase assay Kits (Promega, Madison, WI). Protein concentrations

were determined by the BCA protein assay kit (Pierce Co., Rockford, IL). Luciferase activity was expressed after normalization against protein concentration.

RNA Isolation and Reverse-Transcribed-PCR

Total RNA was extracted from the cultured cells by the RNA STAT-60 reagent (ISO-TEX Diagnostics, TX), according to the manufacturer's instructions. Total RNA (2 µg) was reverse-transcribed using TaqMan Reverse Transcription Reagents (ABI, Branchburg, NJ) in a final volume of 50 µL. The reaction mixture contained 1 × RT buffer (500 mM each dNTP, 3 mM MgCl₂, 75 mM KCl, 50 mM Tris-HCl [pH 8.3]), 10 units RNase inhibitor, 10 mM DTT, 50 units MultiScribe Reverse Transcriptase (ABI), and 1.5 mM random hexamers. The thermal cycling parameters for reverse transcription reactions were as follows: 25°C for 10 minutes, 48°C for 30 minutes, and 95°C for 5 minutes. The RT-generated cDNA samples were stored at -20°C until PCR amplification. Real-time quantitative RT-PCR was performed using an ABI Prism 7700 sequence detection system. Catechol-O-methyl transferase TaqMan probe and primers spanning exons 5–6 were purchased from Applied Biosystems (Bedford, MA; Assay-on-Demand [catalog number Hs_00241349m1]). The primers and probe for GAPDH were designed and manufactured by Applied Biosystems.

Simultaneous detection of both COMT and the GAPDH internal reference was performed in the same tube. This allows standardization of the amount of the target gene to that of the reference gene to control for the different amounts of cDNA used. All cDNA samples and controls were assayed in triplicate. For each sample, the *C_t* values were determined by subtracting the average of triplicate *C_t* values of the target gene from the average of triplicate *C_t* values of the reference gene (GAPDH). The result of the quantification was transformed into an exponential value, 2^{-*C_t*}. The final results were expressed as n-fold differences in COMT expression in cells treated with estrogen or combination of estrogen and progesterone compared with vehicle-treated cells.

Western Blot

Western blot analyses were performed using whole tissue or cell homogenate (10 µg per lane). Endometrial tissues were collected from different phases of the menstrual cycle, while cell lysate was prepared from control (ethanol only), 17-β-estradiol, progesterone, or 2-ME2-treated pHES cells as described previously (13). Western blot with purified COMT antibody (1:10,000) raised in sheep against a monopeptide was used to detect COMT protein (a generous gift from J. T Xia, Division of Neurology, University Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong). Antibodies to B-cell lymphoma 2 (Bcl2; 1:500) or vascular endothelial growth factor (VEGF; 1:200) were purchased from Santa Cruz Biotechnologies (Santa Cruz, CA). Membranes were developed using HRP-conjugated sheep or mouse IgG with ECL Western Blotting Detecting Reagents (Amersham Biosciences, UK). The intensity of each band was determined using a scanning densitometer (Epson 4870, Epson America, Long Beach, CA). β-Actin was used as an internal control for normalization of the protein concentrations in different samples (Sigma, St. Louis, MO).

Proliferation Assay

Primary human endometrial stromal cells were plated into six-well Corning plastic cell culture dishes (Sigma-Aldrich, St. Louis, MO) at 50,000 cells per well in DMEM medium 10% fetal bovine serum. The media was then aspirated and the wells were washed twice with 1× PBS followed by the addition of DMEM 10% charcoal-stripped serum (Hyclone, Logan, UT) for 48 hours and were allowed to grow to 70% confluence. The media was then aspirated and the wells were washed twice with 1× PBS, followed by the addition of DMEM 10% charcoal-stripped serum containing 2-ME2 (10^{-10} – 10^{-6} M) (Sigma). Ethanol was used as the vehicle control at 0.01% concentration. The cells were treated for 48 hours. The medium was aspirated and the wells rinsed twice with 1× PBS. The number of cells was counted using the Coulter counter (Beckmann Coulter Co., Fullerton, CA). Cells were disrupted by keeping the cells at -20°C overnight after adding 1 mL of H_2O to each well. Cells were then pipetted up and down and centrifuged at $10,000 \times g$ for 15 minutes. Hoechst dye was added to 100 μL of the cell lysate of each sample. Cell count was confirmed by measuring the fluorescence of Hoechst 33258 dye, as we described previously (15). Experiments were done in triplicate and the mean value was normalized to a standard curve.

Statistical Analysis

All experiments were performed in triplicate, and wherever appropriate, the data were analyzed using the Student's *t* test. A *P* value of .05 was considered significant. The data are presented as an arithmetic mean $\pm$ standard deviation.

RESULTS

Studies on Human Endometrial Tissues Revealed Cyclic Variations of COMT Expression in Human Endometrium

Human endometrial tissues from different phases of the menstrual cycle were examined with immunohistochemistry and Western blot analyses. Immunohistochemistry showed that COMT protein was expressed in human endometrium, both in the endometrial stroma and endometrial glands. Catechol-O-methyl transferase expression in the endometrial glands was cytoplasmic and has an apical and supranuclear distribution. In stroma, there was more intense COMT immune staining in the proliferative phase compared with the secretory phase endometrium (Fig. 1). Western blot quantification of human endometrial tissues showed that the two COMT protein isoforms, membrane bound (MB-COMT, 32 kDa) and soluble (S-COMT, 25 kDa), were expressed in the human endometrium (Fig. 2). COMT showed significant changes through different phases of the menstrual cycle. Western blot analysis showed increased COMT protein abundance in the proliferative phase (lanes 1 and 2, labeled P, Fig. 2), and early secretory phase—cycle days 16 to 20 (lanes 3–5, labeled S16, S18, and S20). There was marked decrease in COMT protein expression in the mid and late secretory phases—cycle days 21 to 24 (lanes 6–9, labeled S21 to S24, Fig. 2). The decrease in MB-COMT and S-COMT were most significant on cycle day 22 and 23 (*P* 6.2E-5 and 8.7E-6, respectively).

Studies in pHES Cells Showed That Estrogen Increased While Progesterone Decreased COMTP1–Luciferase Transactivation

Catechol-O-methyl transferase proximal promoter–luciferase transactivation experiments showed that 17- β -estradiol (100 pM–100nM) induced a dose-dependent increase in COMT P1–luciferase transactivation in pHES cells; there was a 54% ($P=.04$) increase at 100 nM concentration (Fig. 3A). Addition of progesterone to stroma cells that were pretreated with estrogen (10 nM), on the other hand, induced an inhibition in COMT P1–luciferase transactivation in pHES cells. There was a 24% ($P=.045$) decrease at 1 μ M progesterone when compared with 10 nM estrogen alone (Fig. 3A). Estrogen treatment did not have consistent effects on COMTP2 promoter–luciferase reporter gene transactivation in pHES cells, while the addition of progesterone increased COMTP2–luciferase activity at 10 nM (23%) (Fig. 3B). This increase, however, was not statistically significant ($P=.057$).

Catechol-O-methyl transferase mRNA and Protein Expression Is Up-regulated by Estrogen and Down-regulated by Progesterone in pHES Cells

Real-time PCR assays of COMT-specific RNA derived from pHES cells treated with progesterone concurred with luciferase studies. Although treatment with estrogen modestly increased COMT mRNA expression because of the high baseline COMT levels in the control sample, treatment with progesterone, however, showed a marked decrease in COMT mRNA expression with a 30% and 37% ($P=.03$ and $.04$) decrease at 100 nM and 1 μ M progesterone, respectively, when compared with untreated controls (Fig. 4). A Western blot of COMT protein showed that both COMT isoforms were expressed in pHES cells. Soluble-COMT protein was hormonally regulated in pHES cells. Soluble-COMT protein expression was up-regulated with estrogen treatment compared with control, and downregulated with progesterone treatment in a dose-dependent fashion (Fig. 5).

2-Methoxy Estradiol Treatment Decreased Bcl-2 and VEGF Protein Expression in pHES Cells

To gain insights in the possible consequences of the physiologic variations in COMT expression in the endometrium, we studied the effects of its product, 2-ME2, on cell proliferation and expression of VEGF (angiogenesis growth factor) and Bcl-2 (cell cycle regulatory protein) in pHES cells. We selected these biologic processes to investigate because of their obvious involvement in endometrium preparedness for implantation as well as the menstruation phenomenon (16–19). 2-Methoxy estradiol had an inhibitory effect on the proliferation of pHES cells. As shown in Figure 6, 2-ME2 treatment inhibited pHES cells proliferation at higher doses: 1 μ M led to a 13% ($P=.01$) decrease in cell numbers. Furthermore, 2-ME2 downregulated Bcl-2 and VEGF protein expression levels at 1 μ M, indicating that 2-ME has proapoptotic and antiangiogenic effects on pHES cell (Fig. 7).

DISCUSSION

Here we report for the first time that S-COMT protein isoform is expressed in the human endometrial tissues in a menstrual cycle-dependent, phase-specific manner. Catechol-O-methyl transferase protein expression was upregulated in the human endometrial tissues in the proliferative phase of the menstrual cycle and was down-regulated in the midsecretory

phase of the menstrual cycle. We used pHES cells to further investigate the in vitro regulation of COMT expression. We confirmed that S-COMT expression is hormonally regulated in pHES cells. Catechol-O-methyl transferase proximal promoter, which regulates the expression of the S-COMT isoform, was tightly regulated by estrogen and progesterone as demonstrated by the COMTP1–luciferase reporter gene transactivation studies. Furthermore, S-COMT protein and mRNA transcripts expression were up-regulated in response to estrogen and down-regulated in response to progesterone treatment.

The effect of estrogen on COMT expression is cell type specific. The estrogen mediated up-regulation of S-COMT expression in pHES cells in this study was similar to the effect of estrogen on COMT expression in hamster kidney cells where subchronic estrogen treatment increased COMT immunostaining (20). It contrasted, however, with the effects of estrogen on S-COMT expression in MCF-7 and in human myometrial cells where estrogen treatment down-regulated S-COMT expression levels (13, 21, 22). The variable cell-type specific response of S-COMT expression to estrogen is likely because of the presence of different levels of estrogen receptor isoforms as well as different receptor coactivators and corepressors in different cell types (23, 24). Xie and colleagues (21) reported that estrogen down-regulated S-COMT expression in MCF-7 breast cancer cells that synthesize high levels of estrogen receptor α , but not in a glial cell line (U138MG), which produces only modest levels of the receptor (21). The phenomenon of differential hormonal regulation of genes in the uterus is a well-known phenomenon. Tamoxifen, for example, increases human endometrial cell proliferation and causes endometrial hyperplasia, whereas it decreases human myometrial cell proliferation and causes myometrial atrophy (25).

Menstrual cycle-dependent phase-specific regulation of human S-COMT isoform expression by estrogen and progesterone in the human endometrial stroma may be essential for endometrial stroma function. To gain insights into the possible effects of COMT overexpression, we studied the effects of high levels of 2-ME2 on proliferation, and expression of angiogenesis growth factor and cell cycle regulatory proteins in pHES cells. We chose 2-ME2 excess to simulate the situation of COMT overexpression because 2-ME2 is exclusively produced by the COMT enzyme (26). 2-Methoxy estradiol is known to inhibit angiogenesis and endothelial cell proliferation in breast cancer cells (7, 27). In vitro treatment of pHES cells with high doses of 2-ME2 significantly decreased cell proliferation. Furthermore, 2-ME2 down-regulated Bcl2 and VEGF protein expression in pHES cells. This establishes the proapoptotic and antiangiogenic effects of 2-ME2 on endometrial stroma. It also implies that high COMT expression and the consequent production of high levels of 2-ME2 may interfere with endometrial stroma function.

The general function of COMT is the elimination of biologically active or toxic catechols and their hydroxylated metabolites. Catechol-O-methyl transferase is overexpressed in the placenta during the first trimester of pregnancy to protect the developing embryo from the activated hydroxylated compounds. Catechol-O-methyl transferase acts as an enzymatic detoxicating barrier between the blood and the placenta, shielding against the detrimental effect of xenobiotics in prgenanacy (28). Estrogen mediated up-regulation of COMT in endometrial stroma may play a role in maintaining orderly endometrial stroma growth through affecting angiogenesis and apoptosis. Angiogenesis is an important process that

supports endometrial growth after menstruation and provide a vascular receptive endometrium for implantation and placentation (29). Estrogen profoundly inhibits angiogenesis in the endometrium by affecting angiogenesis growth factor production (30). The vascular antimitogenic effects of estradiol are estrogen receptor independent, and involve the sequential conversion of estradiol to hydroxy estradiols and then to methoxy estradiols (27). This further explains the importance of the estrogen-mediated regulation of COMT in endometrial stroma.

In the present study we provided evidence that progesterone decreased S-COMT protein expression in pHES cells. Furthermore, COMT expression was down-regulated in the endometrial stroma in the midsecretory phase, which is consistent with the implantation window that is limited to days 21 to 24 of the menstrual cycle. Catechol-O-methyl transferase down-regulation is possibly mediated by progesterone as well as other unidentified factors. Decreased COMT expression would increase catechol estrogens and decrease methoxy estrogens production in the endometrial stroma. Catechol estrogens are essential for blastocyst implantation (31), and have been proposed to play a role in blastocyst activation and implantation (32, 33). Estrogen prepares the progesterone-primed uterus to the receptive state via interaction with the classic estrogen receptor, whereas 4-hydroxy estrogen makes the blastocyst competent for implantation via generating prostaglandins (33). This explains the relevance of our findings of decreased COMT expression in the midsecretory phase in the human endometrial stroma.

Absent COMT expression and 2-ME2 deficiency does not appear to adversely affect fertility or embryogenesis, as is evident by COMT knock-out mice, which were normally fertile (34). The effects of increased COMT expression, however, are not yet known. Here, we have shown that COMT product, 2-ME2, inhibited angiogenesis and promoted apoptosis in endometrial stroma cell. Attenuation of 2-ME2 will be conducive of augmented angiogenesis and endometrial stromal cell proliferation and remodeling, all processes crucial for successful implantation (31, 35, 36). High levels of 2-ME2 may thus interfere with blastocyst implantation and folliculogenesis (37). Klauber et al. (38) have recently shown that synthetic compounds with broad antiangiogenesis action such as O-chloroacetylcamoyl fagagillol inhibit implantation in the mouse. Furthermore, COMT catalyzes the O-methylation of both catecholamine and catechol estrogens, and it could provide a link between stress and infertility (39).

In summary we have reported for the first time that COMT is expressed in human endometrium in a menstrual cycle-dependent, phase-specific manner. Regulation of human COMT expression by estrogen and progesterone is essential for normal endometrial function. Perturbed COMT expression in the endometrium leading to increase 2-ME2 may result in reproductive failure by affecting endometrial stroma cell proliferation and angiogenesis.

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FIGURE 1.

Catechol-O-methyl transferase immunohistochemistry in human endometrium. Proliferative phase: (A) COMT immunostaining, (C) negative control, (E) hematoxylin and eosin stain. Secretory phase: (B) COMT immunostaining, (D) negative control, (F) hematoxylin and eosin stain. The immunoreactivity of catechol-O-methyl transferase (COMT) antibody was evaluated. There was COMT protein immunoreactivity in both endometrial stroma and endometrial glands (A, B). Catechol-O-methyl transferase expression in the endometrial glands was cytoplasmic and has an apical and supranuclear distribution (*arrowhead*). Cytoplasmic COMT expression in the endometrial stroma (*arrow*) was higher in the proliferative phase (A) compared with the secretory phase endometrium (B) ($\times 200$).

FIGURE 2.

A representative Western blot of COMT in human endometrial tissues showing marked down-regulation of COMT protein in the midsecretory phase (* $P < .05$). Each COMT band was normalized to the actin band, and the average results from all samples were displayed as histograms. The cycle dates of the tissues are listed on the X axis, and the ratio of COMT band density to actin is shown on the Y axis in arbitrary densitometry units. Error bars are SEM. The *asterisk* indicates statistically significant difference. Human endometrial tissues were collected from patients undergoing hysterectomy at different phases of the menstrual

cycle. Polyclonal COMT antibodies were used to detect COMT immunoreactivity. P indicates proliferative phase endometrium, S indicates secretory phase endometrium.

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FIGURE 3.

(A) Effect of estrogen and progesterone on COMTP1–luciferase reporter gene transactivation in pHES cells. Catechol-O-methyl transferase P1–luciferase activity increases with estrogen and decreases with progesterone (* $P < .05$). (B) Catechol-O-methyl transferase P2–luciferase reporter gene transactivation was not affected by estrogen, while the addition of progesterone (100 nM) to estrogen (10 nM) increased COMTP2–luciferase activity (23%). This increase, however, was not statistically significant ($P = .057$). Data were normalized to total protein contents. Each value is the mean $\pm$ SEM of triplicate wells in two independent experiments. * $P < .05$ compared with estrogen stimulation at 10 nM. Lane 1 (C) indicates vehicle control, lanes 2–5 indicate estrogen treatment, lanes 6–7 indicate progesterone and 10-nM estrogen treatment, and lane 8 indicates untransfected cells.

FIGURE 4.

Real-time RT-PCR analyses of the effects of estrogen and progesterone on COMT mRNA transcripts in pHES cells. Progesterone treatment induced marked reduction in COMT mRNA expression. Each value is the mean $\pm$ SEM of duplicate wells in two independent experiments. GAPDH is the internal loading control. Results represent standardized mRNA quantification transformed into an exponential value, 2^{-Ct} . * $P < .05$ compared with control. Lane 1 (C) indicates vehicle control, lanes 2–3 indicate estrogen treatment, and lanes 4–5 indicate progesterone treatment.

FIGURE 5.

Effects of estrogen and progesterone on COMT protein expression in pHES cells. Soluble-COMT protein expression was up-regulated with estrogen treatment and down-regulated with progesterone treatment. β -actin is the internal loading control. *Lane 1* (C) indicates vehicle control, *lane 2* indicates estrogen treatment, and *lanes 3 and 4* indicate progesterone treatment.

FIGURE 6.

Effects of 2-methoxy estrogen (2-ME2) on pHES cell proliferation. Primary human endometrial stroma cells were treated with increasing concentration of 2-ME. Cell numbers were examined by measuring DNA contents using the Hoechst method 48 hours later, and were compared with cells exposed to medium alone. Each value is the mean $\pm$ SEM of triplicate wells in two independent experiments. * $P < .05$ compared with untreated control. C indicates vehicle control.

FIGURE 7.

Effect of 2-ME2 on Bcl-2 and VEGF protein expression in pHES. 2-Methoxy estradiol markedly down-regulated Bcl-2 and VEGF protein expression at 1 μ M. β -actin is the internal loading control. +C indicates positive control, C indicates vehicle control.