Endometrial and Endometriotic Concentrations of Estrone and Estradiol Are Determined by Local Metabolism Rather than Circulating Levels

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Context: Aberrant estrogen synthesis and metabolism have been suggested to increase local estradiol (E2) concentration in endometriosis and thus to promote the growth of the lesions. However, tissue estrogen concentrations within the endometrium and different types of endometriosis lesions have not been described.

Objective: The aim of the study was to evaluate local E2 and estrone (E1) concentrations in the endometrium and different types of endometriosis lesions, and to correlate them with the expression of estrogen-metabolizing enzymes.

Patients: Patients with endometriosis (n = 60) and healthy controls (n = 16) participated in the study.

Main Outcome Measures: We measured serum and tissue concentrations of E2 and E1 as well as mRNA expression of the estrogen-metabolizing enzymes.

Results: Endometrial or endometriotic intratissue E2 concentrations did not reflect the corresponding serum levels. In the proliferative phase, endometrial E2 concentration was five to eight times higher than in the serum, whereas in the secretory phase the E2 concentration was about half of that in the serum. Accordingly, a markedly higher E2/E1 ratio was observed in the endometrium at the proliferative phase compared with the secretory phase. In the endometriosis lesions, E2 levels were predominating over those of E1 throughout the menstrual cycle. Among the hydroxysteroid (17β) dehydrogenase (HSD17B) enzymes analyzed, HSD17B2 negatively correlated with the E2 concentration in the endometrium, and HSD17B6 was strongly expressed, especially in the deep lesions.

Conclusions: Endometrial or endometriotic tissue E2 concentrations are actively regulated by local estrogen metabolism in the tissue. Thus, the inhibition of local E2 synthesis is a valid, novel approach to reduce local E2-dependent growth of endometriotic tissue. **(*J Clin Endocrinol Metab* 97: 4228–4235, 2012)**

Endometrium proliferates in response to estrogens during the proliferative phase of the menstrual cycle, whereas during the secretory phase the tissue differentiates in response to progesterone secreted by the corpus luteum. According to the current hypothesis, in addition to the systemic estrogens of ovarian origin, estrogen action is

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Abbreviations: CE, Control endometrium; DEEP, deep endometriosis; E1, estrone; E2, estradiol; ESR1, estrogen receptor α; ESR2, estrogen receptor β; FC, fold change; HSD, hydroxysteroid dehydrogenase; OV, ovarian endometriosis; PE, eutopic endometrium; PERIT, peritoneal endometriosis; qRT-PCR, quantitative reverse transcriptase-PCR; RPL19, ribosomal protein L19.

also regulated by steroid metabolism at the target tissues. This is especially true for postmenopausal women with low circulating estrogen concentrations. However, steroid-metabolizing enzymes are also expressed in the endometrium of premenopausal women (1).

Endometriosis is an estrogen-dependent disease characterized by endometrial-like tissue growing outside the uterine cavity, typically on the pelvic peritoneum, in the ovaries, and in the rectovaginal septum (for review, see Ref. 2). Similar to the eutopic endometrium, endometriotic tissue proliferates in response to estrogens. Hence, the appearance and growth of the lesions is related to estrogen action, whereas the reduced estrogen effect, *e.g.* after the menopause, during pregnancy, or by pharmacological suppression of endogenous estrogen secretion, typically diminishes the disease. Accordingly, the current hormonal therapies for endometriosis are based on the inhibition of systemic estrogen action.

The expression of the estrogen-metabolizing enzymes in the endometrium (1) as well as in ovarian, peritoneal, and deep endometriosis lesions (for review, see Ref. 3) has been observed. Among the estrogen-metabolizing enzymes, those regulating the balance between highly active estradiol (E2) and a weak estrone (E1), namely the hydroxysteroid (17β) dehydrogenases (HSD17Bs), have been suggested to be essential for the regulation of intratissue estrogen concentration in the endometrium and endometriosis lesions (4). A role for aromatase enzyme (CYP19A1) and the steroid sulfatase in local estrogen synthesis in the endometriosis has also been proposed (5–14). The studies with animal models support the suggested role of these enzymes in the regulation of estrogen concentration within the target tissues (7, 15, 16). Accordingly, inhibition of aromatase, steroid sulfatase, and HSD17Bs has been suggested as potential treatment options to reduce the estrogen driven growth of endometriosis (6, 7, 17–20).

Knowledge of the possible differences in endometrial and endometriotic tissue concentrations of estrogens is essential for understanding the regulation of estrogen action in both of the tissue types and understanding the possible difference in the response of the various lesion types to systemic estrogens and estrogen-suppressing medication. In this study, we have evaluated the tissue concentrations of E2 and E1 in peritoneal, deep and ovarian endometriosis, as well as in eutopic endometrium of women with and without endometriosis. The tissue hormone concentrations were also compared with the circulating hormone levels of the same patient and were correlated with the expression of the HSD17B enzymes.

Patients and Methods

Patients and samples

Samples of 60 patients with endometriosis and 16 healthy controls were included into the study. The study subjects and sample collection have been described previously (21). A written informed consent was required from all patients before sampling, and the study protocol was approved by the Joint Ethics Committee of University of Turku and Turku University Central Hospital, Turku, Finland. A prior decision of surgical treatment preceded the surgery in all patients taking part in the study. Therefore, all endometriotic tissue was surgically removed as a part of the treatment intervention. Endometrial biopsies were taken solely for research purposes. Definitive diagnosis of endometriosis was reached during operation by laparoscopy or laparotomy and confirmed by histopathological evaluation. Control subjects were verified to be free from endometriosis by laparoscopy during the tubal sterilization. The phase of the menstrual cycle was determined by evaluating the endometrial histology and by comparing the data to the expected day of the menstrual cycle provided by the women. Proliferative and secretory phase samples of healthy (control) endometrium (CE, n = 8 and 7), eutopic endometrium of endometriosis patients (PE, n = 9 and 9), peritoneal (PERIT, n = 3 and 3), ovarian (OV, n = 9 and 9), and deep endometriosis (DEEP, n = 8 and 9) and the corresponding serum samples were analyzed for E1 and E2 concentrations. In addition, the effect of hormonal medication was studied in 21 serum samples, and in the tissue specimens of nine PE, three PERIT, eight OV, and eight DEEP specimens from endometriosis patients. Of the 21 women, 17 used combined hormonal contraceptive methods, three used progestin only, and one used GnRH agonist.

Measuring E1 and E2 concentrations

The concentrations of E2 and E1 were measured in matched endometrium and endometriosis tissues and in serum samples of the same individuals using a liquid chromatography–tandem mass spectrometry. Measurements of E2 and E1 concentrations were extensively validated in human serum (22) and for mouse serum and tissues (23). In addition, we validated E2 and E1 measurements in human endometriotic tissue by showing dilutional linearity (E2, 103–104%; E1, 101–103% accuracy) and consistently high levels of quantitative recovery of E2 and E1 spiked (as a range of concentrations 5–400 pg) into pools of endometriotic tissue homogenates (E2, 90% accuracy; 8.9% coefficient of variation; E1, 96% accuracy; 11.5% coefficient of variation) as well as in individual endometriotic tissue samples (n = 6; E2, 89% accuracy; E1, 74% accuracy). Twenty milligrams of frozen tissues were homogenized in 200 μl of sterile water using ultra-turrax (IKA-Werke GmbH & Co. KG, Staufen, Germany), centrifuged (at 3000 rpm for 10 min at 4 C), and extracted with hexane:ethyl acetate (3:2) mixture containing d4-E2, 0.15 ng/ml). The organic phase with lipophilic steroids was evaporated and redissolved in 500 μl of 20% methanol in PBS (pH 7.4) for analysis. The lower limit of quantification was defined as the lowest amount of the analyte detected with a precision of less than 20% and accuracy of 80–120%. The limit of quantification was 5.0 pg/ml for E2 and 2.5 pg/ml for E1. To obtain comparable local and systemic estrogen concentrations, we considered that 1 g of tissue corresponds to 1 ml of serum.

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Expression of steroid-metabolizing enzymes

The mRNA expressions of certain HSD17B enzymes were evaluated in the proliferative and secretory phase samples by quantitative reverse transcriptase PCR (qRT-PCR). For CYP19A1, HSD17B1, HSD17B2, HSD17B6, HSD17B10, and HSD17B14 as well as estrogen receptor α (ESR1) and β (ESR2), the analysis was performed in six to 10 samples of proliferative and secretory phases CE, PE, PERIT, and OV and for four DEEP lesions in both cycle phases. For the other HSD17Bs, the qRT-PCR analysis was performed for four samples in each sample group. The analysis was performed using DyNAmo HS SYBR Green qPCR Kit (Finnzymes, Espoo, Finland) according to the manufacturer's instructions. The data were normalized by ribosomal protein L19 (RPL19) and analyzed using the Plaffl method developed for relative quantification of the expression for reactions with diverse amplification efficiencies (24). The primers used for qRT-PCR analysis are presented in Supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

The correlations between tissue estrogen concentrations and mRNA expression of the estrogen-metabolizing enzymes and the related receptors (Supplemental Table 2) were also evaluated by comparing the mRNA expression in 11 CE, 14 PE, 4 PERIT, 9 OV, and 20 DEEP observed by the microarray with the E1 and E2 concentrations detected in the same specimens. All steps of the microarray analysis were carried out at the Finnish DNA-Microarray Centre using the Sentrix Human Illumina 6 V2 Expression BeadChips (Illumina, San Diego, CA), as previously described (25). Normalization of the microarray data were performed using the statistical software R package limma (http://www.R-project.org).

Statistical analyses

Intratissue and serum E2 and E1 concentrations and E2/E1 ratios were compared between sample and hormonal status groups using two-way ANOVA with multiple comparisons with *post hoc* Tukey-Kramer test on log-transformed data. CE and PE as well as serum samples of women with and without endometriosis were compared separately using Student's *t* test because the lack of a medication group in healthy control groups prevented their inclusion in the two-way ANOVA. Pearson Product Moment Correlations were examined between log-transformed intratissue and serum E1 and E2 concentrations. Similar correlation analysis was performed for log-transformed E2 concentrations and the mRNA expression of the estrogen-metabolizing enzymes analyzed in the same tissue specimen by the microarray analysis. The mRNA expressions of the selected genes in subgroups of endometriosis were analyzed using two-way ANOVA with multiple comparisons with *post hoc* Tukey-Kramer test on log-transformed data. Statistical analyses were performed using statistical software Sigma Stat 3.11 (Systat Software Inc., Chicago, IL) and NCSS (Kaysville, UT).

Results

Tissue E2 and E1 concentrations and the ratio of local to systemic E2 and E1

Endometrium

Endometrial intratissue E2 concentration was up to 10-fold higher in the proliferative phase compared with the secretory phase ($P = 0.003$; Table 1). No difference in tissue E2 concentrations was observed between healthy and eutopic

TABLE 1. Systemic and local estrogen concentrations in women with and without endometriosis

Sample type	Hormonal status	n	E2 (pg/ml) Median	E2 (pg/ml) 25/75%	P	E1 (pg/ml) Median	E1 (pg/ml) 25/75%	P	E2/E1 ratio Median	E2/E1 ratio 25/75%	P
Serum											
Healthy	Proliferative	9	77.1	48.0/110.0		59.3	48.1/77.0		1.37	0.97/1.81	
	Secretory	7	157.3	117.2/174.7	#	78.4	68.3/96.0		1.76	1.55/2.02	
Endometriosis	Proliferative	19	68.7	52.1/195.0		63.4	49.1/90.8		1.46	0.89/1.98	
	Secretory	20	109.8	76.8/176.0		74.9	51.7/97.5		1.50	1.16/2.14	
	Medication	21	2.5	2.5/58.6	†	21.3	16.6/96.1	†	0.13	0.12/0.80	†
Tissue											
Healthy endometrium	Proliferative	9	532.0	334.7/736.0		55.6	41.7/67.1		8.34	7.33/9.27	
	Secretory	7	66.0	52.5/100.8	##	85.1	72.1/106.0	#	0.69	0.50/1.40	###
Patient endometrium	Proliferative	9	649.3	404.0/1168.7		57.3	44.0/152.8		8.64	4.72/13.11	
	Secretory	9	68.5	25.0/157.3	##	117.6	61.1/194.7		0.80	0.69/0.99	###
	Medication	9	25.0	25.0/115.5		12.5	12.5/96.3		2.00	2.00/3.50	†
Peritoneal endometrium	Proliferative	3	238.0	78.9/397.0	†	55.1	43.3/96.5		5.50	0.82/7.21	
	Secretory	3	176.0	49.4/355.0		37.5	12.5/129.0		3.95	1.36/9.47	
	Medication	3	25.0	25.0/25.0		12.5	12.5/32.8		2.00	0.76/2.00	
Ovarian endometrium	Proliferative	9	3430.0	1809.7/21600.0	*	1380.0	631.5/4280.0	*	4.35	1.69/5.53	
	Secretory	9	305.0	199.0/758.0	##	262.0	164.1/441.5	##	1.34	1.04/3.28	#
	Medication	8	25.0	25.0/564.8	†	75.2	12.5/1709.0	†	2.00	0.62/2.58	
Deep endometrium	Proliferative	8	112.0	53.9/162.0		74.3	36.7/194.0		1.06	0.60/2.60	*
	Secretory	9	25.0	25.0/117.3		12.5	12.5/34.4	##	2.00	1.44/4.77	
	Medication	8	38.5	25.0/115.6		23.4	12.5/128.8		2.00	0.65/2.00	

*, $P < 0.05$ *vs.* PE within cycle phase. †, $P < 0.05$ *vs.* proliferative phase. #, #, ###, $P < 0.05$, < 0.01, and < 0.001, respectively, *vs.* serum within the cycle phase.

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endometrium. Interestingly, a similar strong cycle dependent change was not observed in the E1 levels ($P = 0.751$ for PE; Table 1). As a result, the ratio of E2 to E1 in the endometrium tissue decreased markedly from proliferative to secretory phase without a difference between women with and without endometriosis ($P < 0.001$ for PE; Table 1).

In the proliferative phase, the median for the E2/E1 ratio in the tissue concentrations was 8.3 and 8.6 in healthy and eutopic endometrium, respectively, whereas the ratio was 0.7 and 0.8 in the secretory phase. Thus, the E2/E1 ratio was 10 to 12 times higher in the proliferative phase compared with the secretory phase (Table 1). This shows that the balance between tissue E2 and E1 concentrations changes dramatically during the cycle, with E2 being the dominant steroid in the proliferative phase and E1 in the secretory phase. The endometrial intratissue E2 concentration was markedly higher ($P = 0.003$) than that observed in the serum during the proliferative phase (the median being eight and five times higher for women with and without endometriosis, respectively), whereas in the secretory phase no significant difference was observed between endometrial and serum E2 concentration (Table 1). A strong correlation ($r = 0.746$; $P < 10^{-5}$) was observed between paired endometrial intratissue E1 and serum E1 concentrations, whereas a remarkably weaker correlation was observed between the corresponding E2 concentrations ($r = 0.452$; $P = 0.021$), which provides further evidence for the observation that intratissue E2 in the endometrium is more strongly regulated compared with that of E1.

Endometriosis

The intratissue E2 and E1 concentrations in the ovarian endometriosis tissue were far higher than those measured in the other types of endometriosis lesions or in eutopic endometrium, and they were four to five times higher than in eutopic endometrium depending on the cycle phase ($P < 0.05$). The local E2 and E1 concentrations as well as E2/E1 ratio in the ovarian endometriosis tissue were also significantly higher than the corresponding serum concentrations in the proliferative phase of the menstrual cycle ($P < 0.001$). Similar to that observed in the endometrium, a cycle-dependent change in intratissue E2 concentration was detected in ovarian endometriosis lesions ($P < 0.001$), the value being 11 times higher in the proliferative phase than in the secretory phase. Thus, between the proliferative and secretory phase, a higher drop was observed for E2 than for E1, and especially in the proliferative phase the ovarian endometriosis is under a very strong E2 stimulus (Table 1).

Of the different lesion types, the intratissue E2 concentration was lowest in the deep lesions, and the concentrations did not vary significantly between the cycle phases, whereas the E1 concentration in the secretory phase was lower than that in the proliferative phase specimens ($P = 0.026$). Furthermore, in the proliferative phase, the intratissue E2/E1 ratio was significantly ($P < 0.001$) lower in deep lesions compared with eutopic endometrium of women with endometriosis. In the peritoneal lesions, we did not observe any significant cycle-dependent variation in E2 or E1 concentrations (Table 1).

Effect of hormonal treatment on local and systemic E2 and E1 concentrations

Expectedly, suppression of ovarian steroid synthesis by hormonal contraceptives significantly decreased serum E2 [fold change (FC) = −43.9; $P < 0.001$] and E1 (FC = −3.5; $P = 0.021$) concentrations (Table 1). Interestingly, the E2 concentration was more strongly altered, and the serum E2/E1 ratio was 0.13 in the patients on hormonal medication, whereas it was between 1.46 and 1.50 at the different menstrual phases in the women without medication. Accordingly, the medication also decreased both E2 and E1 concentrations in the eutopic endometrium to concentrations being lower than those measured in the proliferative or secretory phase. The strong suppression of ovarian steroidogenesis reflected into a dramatic drop also in the intratissue concentration of E2 and E1 in ovarian endometriosis lesions (FC = −137.2 and −18.4, compared with proliferative phase specimens; Table 1).

Comparison of the gene expression of estrogen-metabolizing enzymes in endometrium and different types of endometriosis

The expression profiles of AKR1C3, CYP19A1, and HSD17B1, -2, -4, -6, -7, -10, -12, and -14 were analyzed using both the microarray and qRT-PCR analyses (Fig. 1). The qRT-PCR data demonstrated that among the HSD17B genes, the most striking difference between the healthy or eutopic endometrium and different types of endometriosis specimens was observed for HSD17B2 and HSD17B6.

The level of expression of HSD17B2 was significantly higher in healthy (FC = 17.1; $P < 0.001$) and eutopic endometrium (FC = 6.7; $P < 0.001$) during the secretory phase, compared with the proliferative endometrium. However, no cycle-dependent change in the expression of HSD17B2 was observed in the endometriosis lesions. Thus, the level of HSD17B2 mRNA was markedly lower in PERIT, OV, and DEEP endometriosis ($P < 0.05$) compared with that in the CE (FC = 0.11, 0.13, and 0.10, respectively). Furthermore, in the proliferative phase, we did not detect a difference in the HSD17B2 expression between the CE or PE and endometriosis specimens. By comparing the E2 concentration and the mRNA expression analyzed from the same specimen by the microarrays,

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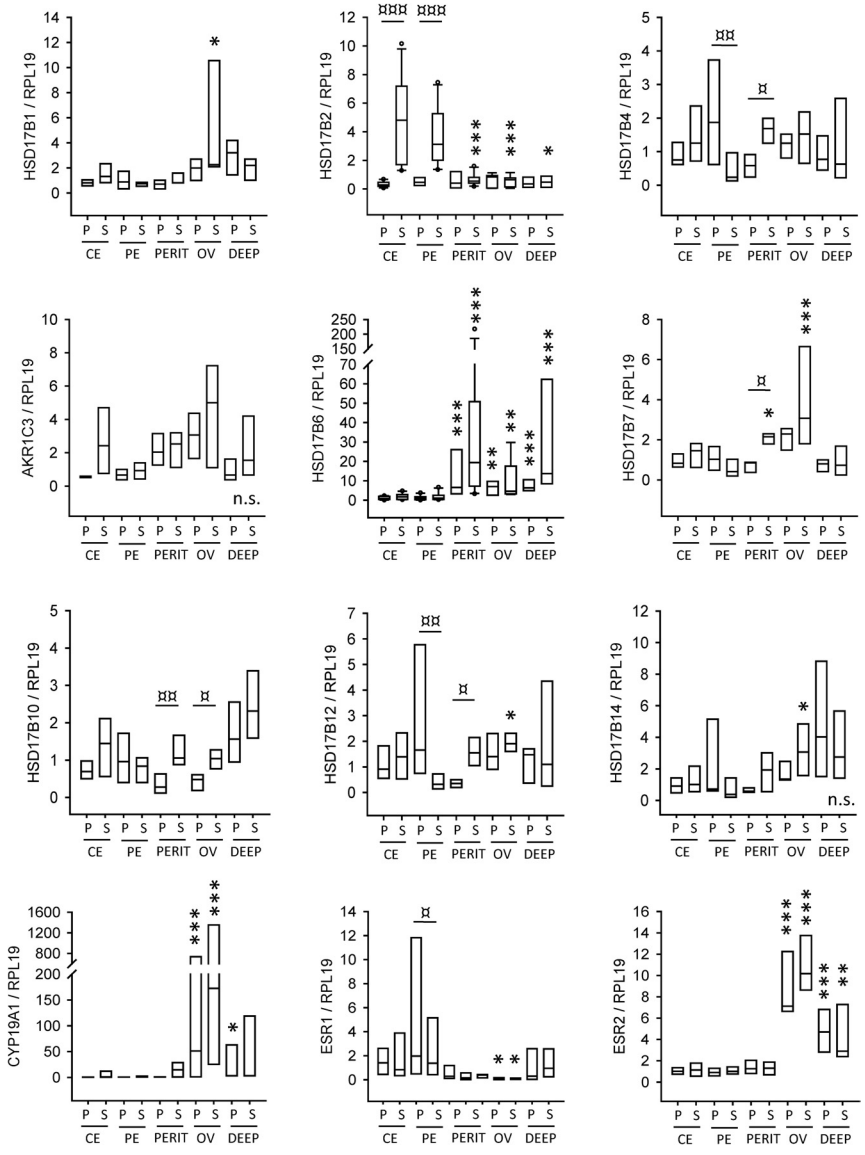

FIG. 1. mRNA expression of AKR1C, HSD17B1, -2, -4, -6, -7, -10, -12, and -14, as well as ESR1 and ESR2 in healthy endometrium (CE) and eutopic endometrium of endometriosis patients (PE), and PERIT, OV, and DEEP endometriosis by qRT-PCR analysis in proliferative and secretory phases of the menstrual cycle (median with 25th/75th percentiles). Statistically significant changes compared with CE within the cycle phase are labeled as: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. The significant difference between proliferative and secretory phases within each sample group is labeled as: ¤, $P \leq 0.05$; ¤¤, $P \leq 0.01$; ¤¤¤, $P \leq 0.001$ (two-way ANOVA with multiple comparisons with *post hoc* Tukey-Kramer test on log-transformed data). P, Proliferative; S, secretory.

we observed a significant negative correlation between the E2 concentration and the HSD17B2 mRNA expression in endometrium of women with and without endometriosis. However, HSD17B2 mRNA and E2 concentration did not correlate in peritoneal, ovarian or deep lesions (Supplemental Table 2). Identical data were obtained by verifying the microarray data by qRT-PCR analysis, whereas the other E2 inactivating HSD17Bs were only weakly regulated in the endometrium or endometriosis.

The expression of HSD17B6 was remarkably higher in endometriosis lesions compared with eutopic endometrium of patients with endometriosis or healthy controls (Fig. 1). Compared with the healthy endometrium, the median expression in different types of endometriosis was 8- to 9-fold higher in the proliferative phase and 3- to 11-fold higher in the secretory phase (FC = 11.2, 2.7, and 7.9 for peritoneal, ovarian, and deep lesions, respectively; $P < 0.01$). Furthermore, the HSD17B6 expression did not vary during the menstrual cycle in any of the sample groups. Of interest, HSD17B6 was strongly but negatively correlated with E2 levels in healthy endometrium, whereas no correlation was observed in endometriosis lesions with high mRNA expression (Supplemental Table 2). Only minor differences in mRNA expression for the E1 activating HSD17Bs, including HSD17B1, -5, -7, and -12, were observed between the endometrium and endometriosis specimens. However, it is worth noting that HSD17B1 and HSD17B7 expression was higher ($P < 0.05$) specifically in the ovarian endometriosis lesions during the secretory phase, but only HSD17B7 expression showed correlation with intratissue E2 and E1. The microarray data were validated by the strong positive correlation observed between the progesterone receptor expression and tissue E2 concentration (Supplemental Table 2), and with its correlation with the intratissue E2/E1 ratio in the healthy endometrium.

The mRNA expression of ESR1 (Fig. 1) was significantly lower specifically in ovarian endometriosis compared with endometrium, whereas no statistical difference was observed between endometrium and other endometriosis lesion types. In contrast, ESR2 mRNA expression was significantly higher in both ovarian and deep endometriosis compared with the endometrium.

Discussion

Estrogen-dependent growth of endometriosis lesions may be promoted by both systemic and locally synthesized E2 (3, 8). Estimating the possible differences in endometrial

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and endometriotic tissue concentrations of estrogens is essential for understanding the regulation of estrogen action in both of the tissue types. Expression of several enzymes involved in estrogen synthesis all the way from cholesterol to E2 has been shown to be present in endometriotic tissue, including aromatase and HSD17Bs (8). In addition, inactivation of E2 may also be diminished in endometriosis by aberrant expression and activity of HSD17B2, possibly due to progesterone resistance (9, 13, 26, 27).

The present data indicate that in the endometrium, E2 is dominating in the proliferative phase, whereas in the secretory phase, the E1 level is higher than that of E2 in the endometrium of women both with and without endometriosis. The intratissue E2 and E1 concentrations were similar in healthy and eutopic endometrium, suggesting that the possible changes in gene expression between healthy women and endometriosis patients do not significantly affect the total intratissue estrogen concentrations in the endometrium. The differential intratissue E2 and E1 concentrations, the variation in the intratissue E2/E1 ratio during the menstrual cycle, and the variation of the local/systemic E2 during the cycle indicate a central role for local metabolism in the regulation of estrogen action in the endometrial and endometriotic tissues. The data also suggest the importance of the HSD17B enzymes in this process. The present data show that in the proliferative phase, endometrial E2 is five times higher than that in the serum, whereas the local and systemic E1 concentrations were equal. This suggests that a reductive HSD17B activity, converting E1 to E2, is accumulating E2 in the tissue. The observation is in contrast with the recent data suggesting that even in the proliferative phase endometrium, the inactivation of E2 predominates over E2 activation (4). However, the observed contribution of local activation of E1 to E2 in the target tissue provides the rationale for the development of HSD17B enzyme inhibitors for endometrial disorders. In the secretory phase, the ratio of tissue E2 to systemic E2 is reduced, E2 concentration being two times higher in serum, whereas E1 level does not change similarly. Thus, intratissue E2 concentration is actively reduced in the secretory phase endometrium, and our data support the theory that the induction of HSD17B2 enzyme would be the major enzyme contributing in the E2 inactivation (26). The negative correlation between E2 concentration and E2/E1 ratio with HSD17B2 expression further supports the hypothesis that HSD17B2 enzyme activity is critical in the regulation of tissue E2 level in the secretory phase endometrium.

Among other HSD17B enzymes catalyzing oxidative reaction, HSD17B6 showed a negative correlation with endometrial intratissue E2 concentration. However, the main enzymatic activity of HSD17B6 is still to be determined. In addition to the 17β-HSD activity, the enzyme has been shown to process both 3β-HSD and 3α-HSD activity *in vitro*, and thereby to metabolize, *e.g.*, 5α-androstane-3α,17β-diol (3α-Adiol) to 5α-dihydrotestosterone (DHT) and DHT to 5α-androstane-3β,17β-diol (3β-Adiol), respectively (28–30). In addition, the enzyme has been shown to metabolize neurosteroids, *e.g.* by converting 3α-hydroxy-5α-androstan-17-one (androsterone) and 5α-pregnane-3α-ol-20-one (allopregnenolone) to their inactive 3β-epimers (28, 29). The 3β-Adiol has been shown to act as a ligand for ESR2. Interestingly, the expressions of both HSD17B6 and ESR2 were remarkably high in ovarian and deep endometriosis. Thus, the data suggest that endometriosis present with both an aberrant ESR2 expression (12, 31–33) and an elevated synthesis of the ligand, further promoting ESR2-mediated signaling in endometriosis. However, the role of an increased ESR2 signaling in endometriosis remains to be resolved.

In contrast to endometrium, there were no cyclical changes in intratissue E2 concentrations in peritoneal and deep endometriosis, and E2 appeared to be the dominating sex steroid in both of the cycle phases. Accordingly, in the endometriosis lesions, the induction of HSD17B2 expression in the secretory phase was diminished, and no correlation between HSD17B2 expression and tissue E2 concentration was observed in any types of endometriotic tissues. Tissue E2/E1 ratio in peritoneal endometriosis remains high, independent of the cycle phase, suggesting a continuously elevated estrogen action in the diseased tissue, whereas in ovarian endometriotic cysts, the cyclical change of E2/E1 ratio mimics that of the endometrium. In deep endometriosis nodules, the E2 and E1 concentrations are close to the lower limit of detection. Noteworthy, deep lesions are poorly vascularized and contain a high amount of extracellular matrix, which may lead to very low estrogen concentrations measured in the tissue homogenates.

Compared with endometrium or peritoneal and deep endometriosis, the ovarian endometriosis lesions presented with markedly higher intratissue E2 and E1 concentrations and high intratissue/systemic E2 and E1 levels. This suggests either high local estrogen synthesis within the ovarian endometriosis lesions or strong contribution by the ovarian E2 production provided by a paracrine manner. Accordingly, we detected markedly higher CYP19A1 expression in the ovarian endometriosis specimens compared with endometrium or other type of lesions, suggesting a key role for P450 aromatase in E2 production, especially in ovarian endometriosis. The observed stronger cyclic changes in estrogen levels in the ovarian endometriosis compared with the serum estrogen

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levels further support the idea of autocrine and paracrine source for the estrogens in ovarian endometriosis.

Suppression of gonadotropin-dependent steroid synthesis in the ovary by hormonal contraceptives decreased both systemic and tissue estrogen concentrations in endometrium and endometriosis lesions, as expected. The medications increased intratissue/systemic E2 in eutopic endometrium and deep lesions, supporting the hypothesis of an aberrant local gonadotropin-independent estrogen metabolism in the tissues. In contrast, the ratio of local to systemic E2 and E1 in ovarian endometriosis decreased, suggesting that tissue estrogen levels in ovarian endometriosis are strongly affected by gonadotropin stimulus. The present data support the idea that the inhibition of the local estrogen synthesis, in addition to systemic estrogen concentrations, *e.g.* with inhibitors of steroid-metabolizing enzymes expressed within the disease tissues, may be essential to further reduce local E2 concentration and E2-dependent growth of endometriosis tissue.

Taken together, the endometrial or endometriotic tissue E2 concentrations did not reflect the corresponding serum levels. In the proliferative phase, the local endometrial E2 concentration was higher than in the corresponding serum samples, suggesting active local E2 synthesis in the tissue. In contrast, the secretory phase E2 concentrations were higher in serum, suggesting active local inactivation of E2 in endometrium changing from E2 dominating proliferative phase to E1 dominating secretory phase. In endometriosis, the E2 levels were predominating over those of E1 throughout the menstrual cycle. Among the different types of endometriosis, the ovarian endometriotic cysts presented with the highest intratissue E2 and E1 concentrations, and in those lesions the major source of estrogens is the intraovarian hormone production. In endometrium and extraovarian endometriosis, the increased ratio of local E2 to systemic E2 during the medication may be due to gonadotropin-independent local estrogen synthesis. Thus, the inhibition of local E2 synthesis is essential in reduction of local E2 concentrations in endometriosis lesions.

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