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Myometrial effects of selective estrogen receptor modulators on estradiol-responsive gene expression are gene and cell-specific☆

Yuhua Z. Farnell, Nancy H. Ing*

Departments of Animal Science, and Veterinary Anatomy and Public Health, Faculties of Genetics and Reproductive Biology, Texas A&M University, College Station, TX 77843-2471, USA

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Abstract

We examined in vivo effects of selective estrogen receptor modulators (SERMs) 4-OH-tamoxifen (Tam), GW 5638 (GW) and EM-800 (EM) on myometrial gene expression. The uteri of ovariectomized ewes were infused with 10^{-7} M of one SERM via indwelling catheters for 24 h preceding hysterectomy. Half of the ewes in each SERM group received an intramuscular injection of 50 μg 17β-estradiol (E2) 18 h prior to hysterectomy. Northern blot analysis and in situ hybridization demonstrated that E2 increased estrogen receptor (ER), progesterone receptor (PR) and cyclophilin (CYC) gene expression in the cells of both inner layer of myometrium (IM) and outer layer of myometrium (OM) as well as glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene expression in OM. Tam also increased ER mRNA levels in OM. EM appeared to increase ER gene expression, but antagonized E2's up-regulation of PR and CYC gene expression in both IM and OM. Tam and GW also antagonized E2 up-regulation of PR gene expression in OM but not IM. No SERM affected GAPDH gene expression with or without E2. Immunohistochemistry indicated that E2 increased nuclear ER and PR protein levels in both IM and OM. EM was unique in up-regulating ER protein levels, opposite to its effects in endometrial cells. All SERMs tested antagonized this increase in PR immunostaining preferentially in OM compared to the IM layer. These results illustrate gene and cell layer-specific effects of SERMs in sheep myometrium.

Keywords: Selective estrogen receptor modulators (SERMs); Estradiol; Myometrium; Sheep; Gene expression

1. Introduction

Estrogen receptor (ER) and progesterone receptor (PR), members of the nuclear receptor superfamily, are crucial to development and function of reproductive tissues [1]. In sheep and other mammals, the high concentrations of estrogens produced from the preovulatory ovarian follicles contribute to the high levels of ER and PR mRNAs in the myometrium that occur at day 1 of the estrous cycle [2,3]. Effects of estrogen are further illustrated in ovariectomized animal models, where 17β-estradiol (E2) treatment up-regulates ER or PR protein levels in myometrium of the rat [4], mouse [5,6] and sheep [7,8]. We have seen some of the greatest induction of PR gene expression by E2 on in the innermost layer of myometrium and the adjacent deep endometrium [9]. This affects subsequent estrogen and progesterone responses in the cyclic preparation of the endometrium for implantation and in the cyclically regulated peristalsis contraction of inner layer of myometrium (IM) for uterine sperm transport [10,11].

Antiestrogen drugs, like tamoxifen (Tam), have been developed to inhibit estrogen-dependent growth of breast cancer [12]. Similar to its effect in mammary tissue, Tam antagonizes of E2-dependent cell proliferation in myometrial leiomyomas (benign tumors of myometrium) and derived cells [13,14]. In addition, Tam inhibits E2-induced PR gene expression in leiomyoma-derived cell lines [15]. However, Tam shows some effects as an estrogen agonist (or mimic) in other tissues, such as endometrium [16]. New drugs were designed as tissue-specific agonists or antagonists of estrogen action, and join Tam in being classified as "selective estrogen receptor modulators" (SERMs). Two new SERMs, GW 5638 (GW) and EM-800 (EM), have been developed to exhibit estrogen-antagonist activities in mammary tissue and growth of mammary tumors. GW showed minimal E2-agonist activity in the growth of rat uterus [17], and EM displayed pure antiestrogen activities in the uterus of mouse [17–19]. However, little information was available on their effects on myometrial gene regulation.

☆ This is the second paper in a set, with the first concerning endometrial effects in the same ewes.

* Corresponding author. Tel.: +1-979-862-2790; fax: +1-979-862-3399.
E-mail address: ning@cvm.tamu.edu (N.H. Ing).

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Here, we examined the acute effects of Tam, GW, and EM to see whether they can block acute E2 effects on the myometrial expression of ER, PR, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and cyclophilin (CYC) genes. These four genes have been previously demonstrated to be upregulated by E2 treatment in sheep myometrium and endometrium [8,9,20]. Comparisons of gene expression profiles in ovariectomized sheep treated with or without E2 identified the antagonist and agonist effects of these SERMs. Histochemical techniques were used to distinguish changes in gene expression in the inner and outer layers of myometrium (OM) in response to E2 and/or SERMs.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma, unless otherwise indicated. EM-800 and GW 5638 were obtained from Dr. Fernand Labrie (Laval University; Que., Canada), and Dr. David C. Morris (Glaxo Wellcome Research and Development; Durham, NC), respectively. Tam, GW and EM were dissolved in ethanol to make 10^{-3} M stock solutions. These solutions were diluted to 10^{-7} M in 0.1% ovine serum albumin in phosphate-buffered saline for infusion. E2 was dissolved in ethanol and diluted in charcoal-stripped corn oil (Kodak; Rochester, NY). Chemical structures of the SERMs are shown in the companion paper [44].

2.2. Animals, treatments and sample collection

After confirmation of estrous cycles of normal duration (16–18 days), ewes were ovariectomized, and indwelling catheters were placed into the tips of their uterine horns [21]. Fifteen days following ovariectomy, ewes were randomly separated into control (Con), Tam, GW and EM groups (10 ewes per group). Uterine horns were infused continuously with 10^{-7} M SERMs or drug vehicle (0.1% ovine serum albumin in phosphate-buffered saline) at a rate of 3 ml/h for 24 h. Six hours after the beginning of the infusion and 18 h prior to hysterectomy, half of the ewes from each treatment group were injected intramuscularly with E2 (50 μg) or drug vehicle (0.5 ml charcoal-stripped corn oil).

After hysterectomy, a 1 cm long cross-section was removed from each uterine horn for fixation in 4% paraformaldehyde and subsequent in situ hybridization and immunohistochemistry [22]. The myometrium was dissected from the endometrium, minced and snap-frozen in liquid nitrogen and stored at −80 °C. All animal procedures were approved by the Laboratory Animal Care and Use Committee at Texas A&M University.

2.3. Total RNA preparation and Northern blot analysis

Total cellular RNA was extracted from a 0.5 g sample of frozen myometrium from each ewe using Tripure Reagent (Boehringer Mannheim; Indianapolis, IN). Northern blot analysis of total RNA (8 μg per lane) was performed as previously described [22]. For detecting ER, PR, GAPDH, and CYC mRNAs and 18S rRNA on Northern blots, antisense cRNA probes were generated and used as previously described [9,22]. Hybridization signals were quantitated by directly scanning blots on InstantImager (Packard; Meriden, CT).

2.4. In situ hybridization to identify which cells alter expression of E2-responsive genes

Adjacent 7 mm cross-sections from each uterus were placed on Superfrost Plus slides (Curtin Matheson Scientific, Houston, TX) less than 1 week prior to histochemical development. In situ hybridization studies using [^{35}S] UTP-labeled antisense and sense ER, PR, GAPDH and CYC cRNA probes on cross-sections were performed to identify responses of specific uterine cells to SERMs in the presence or absence of E2 challenge as described previously [9]. NTB-2 autoradiography emulsion (Eastman, Kodak) was exposed for 5 weeks for slides with ER and PR cRNA probes, but 8 weeks for slides with GAPDH and CYC cRNA probes. Sections were counterstained with hematoxylin. Quantitative analysis of pixel densities was performed to count the silver grains (relating to amount of mRNAs) using Reichert MicroStar IV Microscope (Diagnostic Instrument; Michigan) & NIH image 1.61 software as described (reference [9] and accompanying paper).

2.5. Immunohistochemistry to localize changes in ER and PR protein levels

Immunohistochemistry for detection of ER and PR protein levels was performed with monoclonal antibodies: rat anti-human ER antibody H222 and mouse MA1-411 anti-human PR antibody (Afinity BioReagents, Golden, CO), respectively [8]. Immunostaining was developed with peroxidase and 3,3′-diaminobenzidine tetrahydrochloride reagent [8]. Nonimmune rat IgG (for ER) or mouse IgG (for PR) was used on adjacent sections as negative controls.

2.6. Statistical analysis

All quantitative data were analyzed by least-squares ANOVA using the general linear model procedure of Statistical Analysis version 8.1 for Windows (SAS Institute, Cary, NC). Data analysis of Northern blots used 18S rRNA levels to correct for unequal loading of RNA between lanes. Data are presented as least squares means with standard errors for treatment groups. The results for treatment groups were compared as follows: (1) results from the E2 group were compared to those of Con group for E2 effects; (2) data from groups with SERM treatment alone were compared to those from the Con group to identify SERM effects as estrogen agonists; (3) data from ewes treated with SERM

in the presence of E2 were compared to those of the E2 group to identify antagonistic effects of SERM. The level of statistical significance is a P value of less than or equal to 0.05, unless otherwise indicated.

3. Results

3.1. Northern analysis identified gene-specific E2-agonist actions of SERMs in myometrium

We examined here effects of E2 ± SERM on expression of ER, PR, GAPDH and CYC genes in entire myometrium. Hybridization signals on Northern blots for the mRNAs are shown in Fig. 1 (panel A), along with that of the 18S rRNA. The last was used to correct for RNA loading differences between lanes in the quantitiative analyses of the Northern results for the ewes in each treatment group (panel B). E2 increased myometrial ER mRNA levels 30% compared to the values from Con ewes. ER gene expression was also up-regulated 40, 40 and 49% by Tam, GW and EM treatment, respectively. In the EM + E2 treatment group, the effects of both agents were additive in up-regulating myometrial levels of ER mRNA. In terms of PR gene expression, E2 enhanced PR mRNA concentrations in myometrium 123% relative to values from Con group. No SERMs tested affected PR gene expression as an E2 agonist, except that EM appeared to slightly enhance PR mRNA levels ($P = 0.09$). There was also no significant regulation of GAPDH mRNA levels by E2 and/or SERM treatment in RNA samples from myometrium analyzed on Northern blots. However, CYC mRNA levels increased in response to E2 and Tam by an average of 97% and 88%, respectively, relative to values from Con ewes. None of the SERMs tested demonstrated

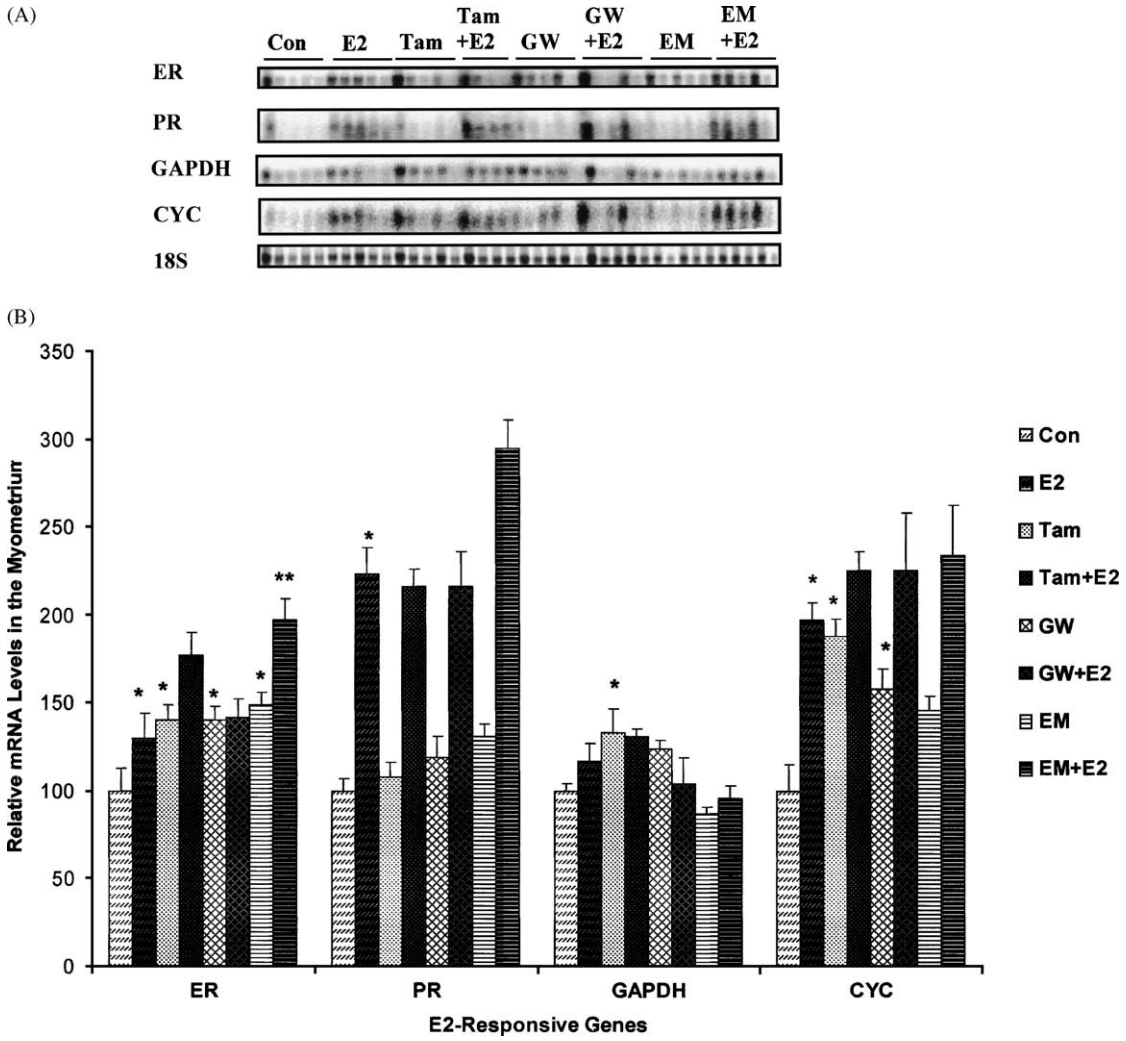

Fig. 1. The SERMs 4-OH-tamoxifen (Tam), GW 5638 (GW) and EM-800 (EM) act as E2 agonists by up-regulating myometrial ER mRNA levels. Northern analysis was performed on myometrial RNA from each ewe. Quantitative results are presented as least square means ± standard error of the mean with values for Con group set at 100. Results of E2 or SERM treatments alone were compared to those of Con treatment (significant difference was designed by asterisk (∗)). Double asterisks (∗∗) over ER mRNA for "EM + E2" treatment indicates additive effects of the two treatments. The level of statistical significance was a P value less than or equal to 0.05.

antagonistic effects on the E2-induced increases of ER, PR and CYC gene expression in myometrium.

3.2. *In situ hybridization identified the myometrial cells that respond to E2 and/or SERMs*

In situ hybridization was performed to distinguish changes in the expression of ER, PR, GAPDH and CYC genes in cells within the two smooth muscle layers of myometrium: the inner circular layer and the outer longitudinal layer [23]. The outermost perimetrium, composed of loose connective tissues with vessels and nerves, showed weak and unregulated hybridization signals for all gene products assayed (data not shown). Hybridization signals for ER mRNA levels in IM and OM were moderate in control ewes and were increased by E2. No SERMs tested showed E2-agonist or antagonist effects on ER mRNA regulation except that Tam tested alone enhanced ER gene expression in OM. Since these results are similar to those from previous paper about E2's increase in ER mRNA levels in sheep myometrium [9], and others of Tam's increase in ER gene expression in rat myometrium [24], the raw data are not shown here, but are quantitatively analyzed in the following section.

Representative data describing the response of PR gene expression to SERM ± E2 treatments in OM are shown in Fig. 2. Brightfield images show black, punctate, silver grains, which represent hybridization signals of PR mRNA on cross-sections of OM from Con, Tam, GW, and EM-treated ewes on the left, and E2, Tam + E2, GW + E2, and EM + E2 ewes on the right. Nuclei stained with hematoxylin are large gray areas most obvious in the left panels

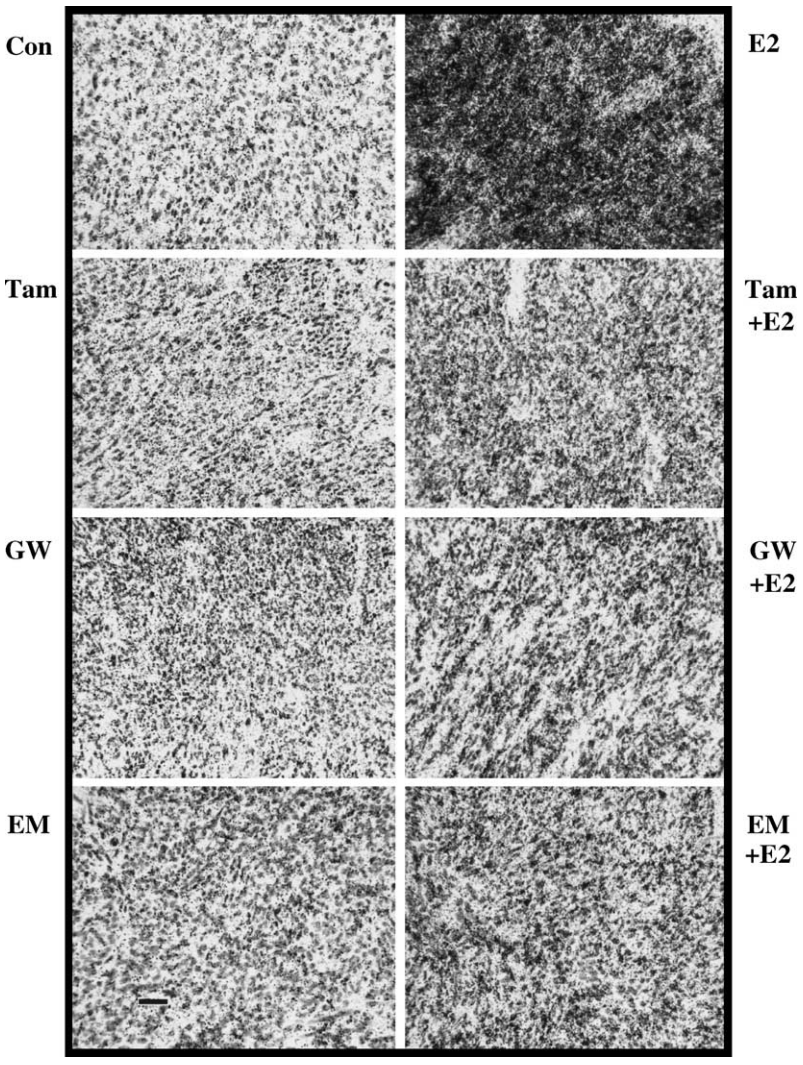

Fig. 2. In situ hybridization demonstrates SERM antagonism of E2 up-regulation of PR mRNA levels in cells of the outer layer of myometrium (OM). Paraformaldehyde-fixed uterine cross-sections were mounted onto glass slides, prehybridized, and hybridized with ^{35}S-labeled antisense PR cRNA probe. After autoradiography, cell nuclei on sections were lightly counterstained with hematoxylin. Black silver grains represent hybridization signals of PR mRNA on brightfield image. Representative images from Con, Tam, GW and EM ewes are shown in panels at left, with comparable images from animals also receiving E2 treatment in panels at right. The bar in the EM panel represents 100 μm.

where hybridization signals are the weak to moderate. The strong up-regulation of PR gene expression by E2 in OM (upper right panel) was antagonized by all three SERMs tested (lower right panels). No SERMs examined alone showed E2-agonist effects on PR gene expression (lower left panel). Therefore, Tam, GW and EM appeared to be pure E2 antagonists on PR gene expression in OM.

Consistent with Northern blot results, in situ hybridization results demonstrated that CYC gene expression in IM and OM showed strong increases in response to E2 treatment (Fig. 3, compare "Con" to "E2" panels). Tam and GW demonstrated no E2-agonist or antagonist effects on CYC gene expression (data not shown). However, EM antagonized E2's up-regulation of CYC mRNA levels in cells from both IM and OM (Fig. 3, compare the "E2" and "EM + E2" panels). Thus, EM appeared to be an E2 antagonist of CYC gene expression in IM and OM.

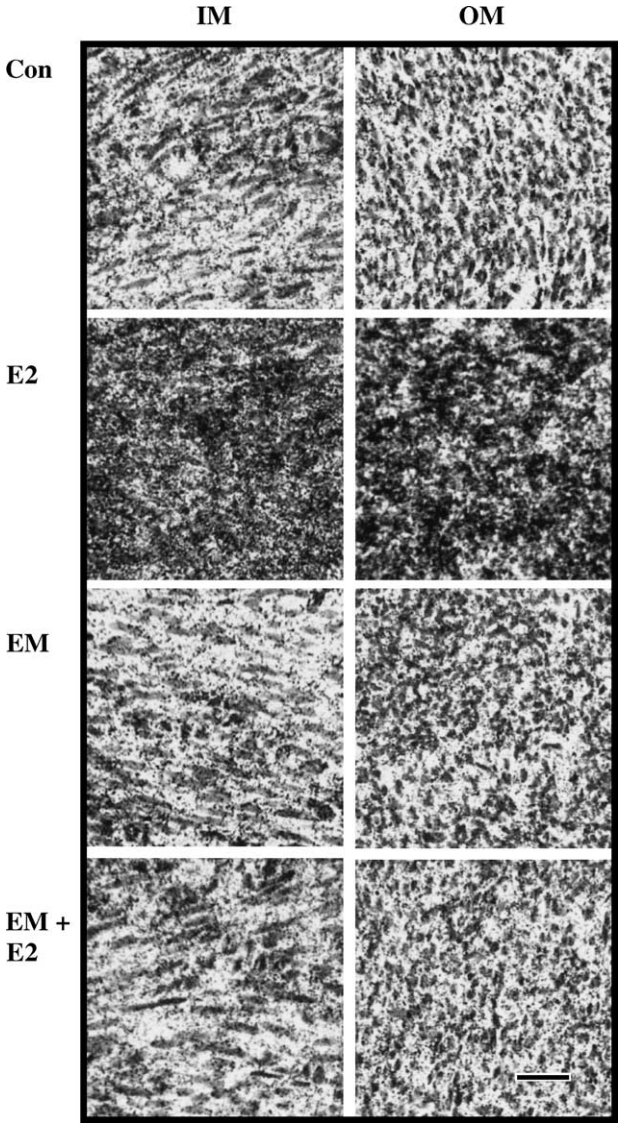

Fig. 3. In situ hybridization demonstrates EM antagonism of E2 up-regulation of CYC mRNA levels in cells from the inner layer of myometrium (IM) and OM. Representative slides for Con, E2, EM and EM + E2 are shown in the panels. In the left (IM) and right (OM) side panels, hybridization signals for CYC mRNA increase with E2 treatment, but this is antagonized by EM treatment. The bar in the OM "EM + E2" panel represents 100 μm.

3.3. Semi-quantitative analyses of in situ hybridization identifies E2-antagonist effects of SERMs on PR and CYC gene expression

To complement Northern blot quantitation of the changes of mRNA levels in whole myometrium, in situ hybridization results were quantified to demonstrate regulation in the different myometrial cell layers. Quantitative data summarizing the in situ hybridization results are shown in Table 1, in which average hybridization signals ± S.E.M. of ER, PR, GAPDH and CYC mRNAs are reported for IM and OM cell compartments from each ewe treatment group. E2 increased ER gene expression about 250% in both IM and OM. Tam weakly up-regulated ER mRNA levels and did so only in OM cell compartment. EM treatment examined alone showed trends of increasing ER gene expression in IM ($P = 0.07$) and OM ($P = 0.09$).

Consistent with data the images of the OM layer in Fig. 2, semiquantitative analyses of in situ hybridization data demonstrated that E2 treatment greatly increased PR mRNA levels in IM and OM layers (Table 1). SERM treatment alone did not have any effects. However, Tam, GW and EM antagonized E2's increase in PR gene expression in the OM. EM also inhibited 74% of the up-regulation of PR mRNA levels by E2 in the IM cells.

In situ hybridization also revealed that E2 weakly up-regulated GAPDH gene expression in OM cells. No SERMs tested, in the presence or absence of E2, altered GAPDH mRNA levels in IM or OM. E2 also increased CYC mRNA concentrations in both IM and OM tissue layers. Tam treatment showed a trend toward E2 agonism by increasing CYC gene expression in the OM cells ($P = 0.08$). Of the SERMs tested, only EM antagonized E2 up-regulation of CYC gene expression, and did so in both IM and OM layers.

3.4. Immunohistochemistry reveals SERM effects as E2 agonists and antagonists at the level of ER and PR protein expression

To identify changes in ER and PR protein levels in response to E2 and/or SERM treatments, we performed immunohistochemistry on uterine cross-sections. Representative results are shown in Figs. 4 and 5, respectively. ER immunostaining is shown for IM (left side panels) and OM (right side panels) for Con, E2, EM, and EM + E2 treatment groups (Fig. 4). Left side panels show the IM layer situated between the deep glandular epithelium ("DGE", at lower left) and the OM layer (at upper right). In the right side panels, OM is pictured with IM at the bottom and

Table 1
Quantitation of in situ hybridization results for ER, PR, GAPDH and CYC mRNAs in inner (IM) and outer (OM) layers of myometrium. Pixel densities are expressed as means ± standard errors

Genes	layers	Treatments							
		Con	E2	Tam	Tam + E2	GW	GW + E2	EM	EM + E2
ER	IM	18.4 ± 3.3	53.6 ± 1.2*	19.6 ± 2.6	49.5 ± 6.7	14.6 ± 3.6	55.4 ± 8.9	33.7 ± 7.0	56.0 ± 2.0
	OM	10.6 ± 2.5	43.2 ± 4.7*	25.6 ± 4.8*	31.8 ± 4.5	16.2 ± 3.2	30.4 ± 4.2	23.0 ± 5.3	33.2 ± 8.6
PR	IM	22.4 ± 2.6	82.8 ± 4.1*	28.4 ± 3.6	69.3 ± 5.0	18.6 ± 4.4	70.8 ± 7.4	13.5 ± 4.0	38.2 ± 5.8**
	OM	10.8 ± 1.1	49.0 ± 5.2*	18.2 ± 3.4	28.0 ± 5.2**	11.0 ± 3.0	32.8 ± 4.3**	9.0 ± 2.9	13.8 ± 2.0**
GAPDH	IM	70.7 ± 6.5	89.8 ± 4.3	80.1 ± 9.4	85.7 ± 9.7	79.5 ± 5.0	94.3 ± 3.0	77.1 ± 7.8	106 ± 5.7
	OM	54.3 ± 8.3	76.8 ± 7.3*	62.0 ± 14.5	74.5 ± 1.4	55.1 ± 6.7	74.5 ± 3.5	57.1 ± 6.4	73.3 ± 3.9
CYC	IM	17.1 ± 5.4	47.0 ± 4.0*	21.2 ± 6.1	32.0 ± 5.5	15.9 ± 4.6	41.1 ± 7.2	12.8 ± 2.7	29.4 ± 3.1**
	OM	11.1 ± 4.4	49.0 ± 4.3*	25.3 ± 4.3	41.2 ± 3.5	10.6 ± 3.5	38.8 ± 12.2	16.1 ± 3.3	26.3 ± 2.4**

Treatment groups are described in the text.
* Indicates the difference of treatment group compared to Con group ($P \leq 0.05$).
** Indicate the difference of treatment group compared to E2 group ($P \leq 0.05$).

perimetrium (“P”) at the top. ER protein levels in Con ewes were very low or undetectable in IM and OM (Fig. 4, the top panels). Their faintest regions were like the staining in the negative control sections incubated with nonimmune IgG (data not shown). E2 treatment strongly induced nuclear staining for ER protein in both IM and OM (Fig. 4, the two top panels). The intensity of ER immunostaining and number of ER-positive cells in IM and OM after Tam treatment were intermediate between Con and E2 group and Tam + E2 treatment did not exhibit additive effects on ER gene expression (data not shown). GW in the absence or presence of E2 did not change ER protein levels in IM and OM from those in Con and E2-treated ewes, respectively (data not shown). However, EM enhanced nuclear ER staining intensity in IM to be between that of Con and E2 ewes (Fig. 4, left “EM” panel). ER nuclear staining was strongly enhanced by EM treatment in OM, similar to E2 treatment (Fig. 4, right “EM” panel). However, EM effects did not add to the E2 up-regulation of ER protein levels in IM or OM in “EM + E2” ewes (Fig. 4, bottom panels). No SERMs antagonized E2’s increase in ER protein levels in myometrium.

Representative results of PR immunostaining in OM are shown in Fig. 5. PR protein was observed primarily in nuclei of IM (data not shown) and OM in Con ewes (Fig. 5, top left panel). In Con ewes, PR immunostaining was very weak in some cell nuclei. Along with the cytoplasm, lack of immunostaining was seen in the negative control sections incubated with nonimmune IgG (data not shown). E2 treatment increased cytoplasmic and nuclear PR immunostaining in both IM and OM (Fig. 5, top right panel). SERMs in absence or presence of E2 did not change levels of PR protein in IM; however, in OM, Tam, GW and EM antagonized the E2-induced increase in nuclear PR staining (Fig. 5, lower right panels). Thus, the PR immunohistochemistry data indicate that all SERMs tested were antagonists of E2 effects on PR gene expression in OM, but not in IM.

4. Discussion

The uterus is one of the main targets of the hormone estrogen. Effects of estrogen on the uterus have been extensively studied in different species, including the cat [25], rat [26,27], mouse [28], sheep [8], pig [29] and rhesus monkey [30]. Myometrium comprises the majority of the uterus and has important functions throughout estrous/menstrual cycles and pregnancy. It is composed of two different layers of smooth muscle: the circular IM and longitudinal OM, with a dense vascular bed between them. Therefore, we examined mRNA levels in whole myometrium by Northern blot analyses, as well as mRNA levels in the individual myometrial layers by in situ hybridization. The fact that we detected many SERM effects in the myometrium, especially the OM, indicates that the intrauterine delivery of SERMs was effective in delivering the drugs throughout the uterus.

Our current results agree with previous reports that E2 increased ER mRNA and protein levels in sheep and rat myometrium [7,8,24,31]. Also, E2 has been reported to increase E2 binding sites in nuclei of ovine myometrial cells, a functional assay of ER protein levels [32]. No SERMs tested here or elsewhere antagonized E2’s up-regulation of ER gene expression in myometrium. On the contrary, they exerted E2-agonist effects on ER gene expression in myometrium. Another group found that Tam increased ER gene expression in the OM layer of rat uteri [24].

In agreement with this data describing E2 up-regulation of PR gene expression in the sheep myometrium, others demonstrated similar results in myometrium of sheep [9,33], rat [24], mouse [5,6], guinea-pig [34] and rhesus monkey [35] as well as in rat uterine leiomyoma-derived cell lines [36]. However, the actions of SERMs as E2 agonists or antagonists depended on the gene involved and cofactors available in the cells [37,38]. Similar to our results, Tam had gene-specific effects: acting as an E2 antagonist of PR gene expression in uterine leiomyoma-derived cells [14,15],

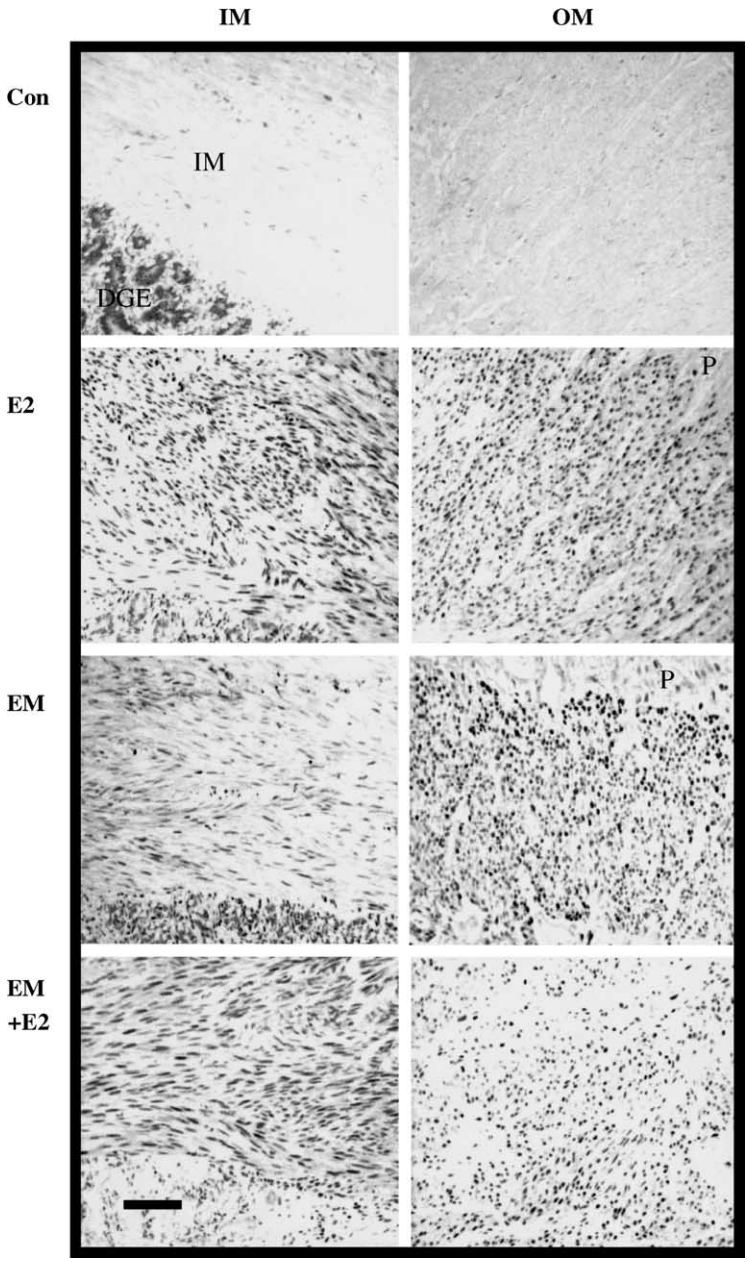

Fig. 4. E2 and EM treatments up-regulate ER protein levels in myometrium. Representative results of immunohistochemistry with an ER antibody on cross-sections of myometrium are shown for ewes from Con, E2, EM and EM + E2 ewes are shown (IM in left side panels, OM in right side panels). The IM is situated between the deep glandular epithelium ("DGE", in the Con panel) at the bottom of the left side panels, and OM beyond the top of each panel. Likewise, OM is between IM (below) and perimetrium (above, "P" in E2 and EM panels). The most striking effects were from E2 and EM treatments, which increased nuclear ER staining in both IM and OM. The bar in the left "EM + E2" panel represents 100 μm.

but as an E2 agonist on estrogen-responsive calbindin-D 9k gene expression in primary cultures of rat myometrial cells [39]. In situ hybridization and immunohistochemistry demonstrated that three SERMs tested here acted as pure estrogen antagonists of the up-regulation of PR mRNA and protein concentrations. However, effects of Tam and GW were limited to the OM, and EM antagonized the E2 effect more completely in OM than in IM. These results contrast with those of ICI 182,780 treatment, which antagonized the up-regulation of ER and PR genes preferentially in IM compared to OM [9]. These OM-specific effects were masked in Northern analyses by the two to three times more predominant IM layer.

Our previous data illustrated the up-regulation of GAPDH and CYC gene expression in sheep endometrium by E2 [9,20]. Here, we examined how E2 and SERMs affect those genes in myometrium. To our knowledge, this is the first report of the regulation of GAPDH and CYC gene expression by EM and GW treatment in myometrium. GAPDH gene expression was only enhanced by E2 in the OM cell

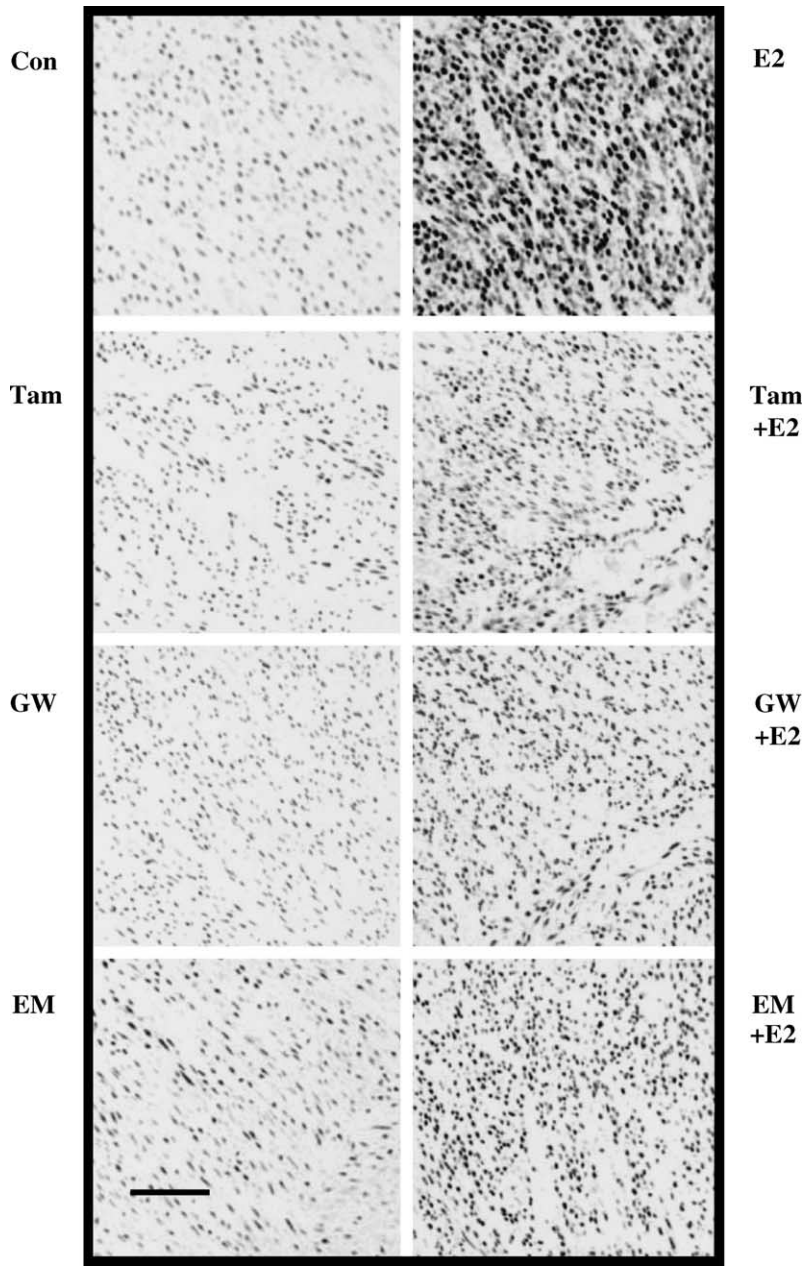

Fig. 5. All of the SERMs inhibit E2's increase in PR protein levels in OM. PR immunohistochemistry demonstrates mainly nuclear staining in myometrial cells. Representative results of OM cross-sections are shown for Con, Tam, GW, and EM-treated ewes in the left side panels, and cognate E2-treated ewes in the right side panels. E2 enhanced PR immunostaining in OM cells and all three SERMs inhibited this effect. The bar in the EM panel represents 100 μm.

compartment. The Tam and GW failed to antagonize E2 up-regulation of either GAPDH or CYC gene expression in myometrium (IM and OM), but EM (data presented here) and ICI 182,780 [9] antagonized E2's up-regulation of CYC (not GAPDH) mRNA levels.

Many lines of evidence have confirmed that the IM and OM layers of myometrium are functionally and morphologically different [40,41]. IM is ontogenetically and functionally distinct from OM in that IM is of paramesonephric origin, like endometrium, and has various functions during estrous/menstrual cycles, pregnancy and parturition. On the other hand, OM is of nonparamesonephric origin with functions primarily confined to parturition. Our data and others' demonstrate distinct patterns of ER and PR gene expression across the myometrial wall layers [11]. Some E2-responsive genes are preferentially up-regulated in OM, including those of GAPDH in sheep (this report) and IGF-II receptor in the monkey [42]. Doualla-Bell et al. [41] also found that EM-139, a drug from the same family as EM-800, antagonized E2 up-regulation of connexin-43 gene expression in myocyte cultures from OM of bovine myometrium, but not those from IM [43]. This is similar to the OM-specific

antagonism of PR gene regulation by SERMs reported here.

Like the data from endometrium, all of the SERMs increased ER mRNA levels in myometrium. However, the combination of EM and E2 increased ER mRNA levels in an additive manner in Northern blot analyses of myometrium in contrast to a return to basal levels in endometrium. In addition, EM used alone mimicked E2's up-regulation of ER protein levels in the IM and OM. This contrasts our data that E2 and EM decreased ER protein levels in glandular epithelium throughout the endometrium, respectively, in these same ewes (accompanying paper). Another difference was that the strong up-regulation of GAPDH gene expression by Tam and EM in endometrium was absent in myometrium. However, in both endometrium and the OM layer of myometrium, all three SERMs tested showed estrogen-antagonist effects on PR gene expression. EM also antagonized the E2-induced up-regulation of CYC gene expression in endometrium and both layers of myometrium. Our observations suggest that responses to E2 and SERM treatments are gene-specific, as well as cell-specific, depending upon whether smooth muscle cells are a part of the inner or outer layers of the myometrium. This indicates different molecular mechanisms of E2 up-regulation. For PR and cyclophilin gene expression, antagonism by SERMs in OM indicates that the ER protein in involved. In endometrium, ER gene expression also appears ER protein-dependent because of SERM antagonism. In myometrium, however, the lack of SERM antagonism of ER mRNA up-regulation by E2 indicates a distinct mechanism of regulation that appears independent of the action of the ER protein.

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