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Regulation of estrogen activity by sulfation in human Ishikawa endometrial adenocarcinoma cells

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Abstract

Sulfation is an important conjugation reaction in the metabolism of steroids. Steroids sulfates do not interact with the appropriate hormone receptors; additionally, the presence of the charged sulfate moiety increases the aqueous solubility and excretion of most steroids. Estrogen sulfotransferase (EST) is the major form of human cytosolic ST involved in the conjugation of estrogens. EST is important in the inactivation of β-estradiol (E2) during the luteal phase of the menstrual cycle. EST has a significantly higher affinity for the sulfation of E2 and 17α-ethinylestradiol (EE2) than for other potent estrogens such as diethylstilbestrol (DES) and equine estrogens. The ability of EST to sulfate these estrogenic compounds at physiologic concentrations is important in regulating their activation of the ER in estrogen responsive cells. Human Ishikawa endometrial adenocarcinoma (ISH) cells possess an estrogen receptor (ER)-regulated alkaline phosphatase (AlkPhos) which is used to assay ER activation. To study the effects of EST activity on the ER activation of different estrogenic compounds, ISH cells were stably transformed with an EST expression vector. Dose–response curves for the induction of AlkPhos activity by the different estrogenic compounds were generated with EST/ISH and control pcDNA/ISH cells. EST/ISH cells were 200-fold less sensitive to E2 and EE2 than were control cells. No differences were observed in the dose–response curves for DES between EST/ISH and pcDNA/ISH cells. EST/ISH cells were approximately 3–10-fold less sensitive to the equine estrogens equilin and 17-equilin as compared to control cells. The ability of EST to decrease the ER activation of an estrogen correlates with the sulfation of these compounds at nanomolar concentrations by EST/ISH and pcDNA/ISH ISH cells. These results indicate that EST is capable of efficiently inactivating E2 and EE2 but is significantly less effective in inhibiting the ER binding of other potent estrogenic compounds.

Keywords: Sulfotransferase; Estrogen; Sulfation; Endometrial adenocarcinoma cells; Ishikawa cells; β-estradiol

1. Introduction

In hormone-responsive tissues such as the human endometrium, the sulfation of steroids is an important mechanism in the modulation of steroid activity. The addition of a sulfate moiety to a steroid decreases the binding of the steroid to its receptor, effectively lowering the concentration of the bioactive steroid [1]. The cyclic growth and shedding of normal human endometrium during the menstrual cycle is regulated by the secretion of β-estradiol (E2) and progesterone (PG) from the ovaries. Prior to ovulation, E2 stimulates the proliferation of endometrial tissues whereas after ovulation large quantities of both E2 and PG are secreted; however, the endometrium differentiates in response to PG in preparation for implantation of the fertilized egg [2]. In a previous report, our laboratory has shown that expression of human estrogen sulfotransferase (EST) is increased by PG during the proliferative phase of the menstrual cycle whereby the increased EST levels have a role in decreasing the activity of E2 via sulfation [3].

The conjugation of steroids with sulfate is catalyzed

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by a family of enzymes termed the sulfotransferases (STs); three STs found in human tissues have been reported to be capable of conjugating estrogens. Both dehydroepiandrosterone-ST (DHEA-ST) and the phenol-sulfating form of phenol-ST (P-PST) sulfate E2 and estrone at micromolar concentrations [4]. EST differs from DHEA-ST and P-PST in that it sulfates estrogens optimally at low nanomolar concentrations [5,6]. This is physiologically significant because it is at these nanomolar concentrations that estrogens interact with the estrogen receptor (ER) and initiate a biological response. Although EST has a high affinity for the sulfation of estrone, E2 and EE2, its affinity for the sulfation of environmental and therapeutic estrogens such as diethylstilbestrol (DES) and equilin (EQ) is significantly lower [5].

Our laboratory has previously described the selective induction of EST in human Ishikawa adenocarcinoma (ISH) cells by PG [7] which parallels the situation found in the human endometrium. ISH cells possess both ER and PG receptors and have been used as a model system to study the effects of steroid hormones on human endometrium [8,9]. PG treatment of ISH cells results in a down regulation of ER levels, an increase in 17β-dehydrogenase activity and an increase in E2 sulfation [10]. E2 stimulates ISH cell replication and ER-regulated processes such as the induction of an ER-regulated alkaline phosphatase (AlkPhos) activity [11].

In this report, ISH cells were used as a model system to investigate the effects of increased EST levels on the ER activation activity of different endogenous and exogenous estrogens. To provide a model with which to study the effects of elevated EST activity in the endometrium, ISH cells were stably transformed with EST [5]. The effects of E2 on these EST/ISH cells were evaluated as compared to E2 effects on control cells using the parameters of ER activation and estrogen metabolism. In addition to comparing the effects of EST on ER activation by E2 and E2 metabolism in control and EST/ISH cells, the effects of other potent estrogens such as 17α-ethinylestradiol (EE2), diethylstilbestrol (DES), and the equine estrogens EQ and 17β-equilin (17EQ) on ER activation in these two cell lines were also evaluated.

2. Materials and methods

2.1. Materials

E2, EE2, DES, EQ, dopamine, *p*-nitrophenol (PNP) and DHEA were obtained from Sigma (St. Louis, MO). [6,7-^{3}H]-E2 (45 Ci/mmol), [1,2,6,7-^{3}H]DHEA (79 Ci/mmol), [^{35}S]-3′-phosphoadenosine-5′-phosphosulfate (PAPS) (2 Ci/mmol), [^{3}H]-DES (95 Ci/mmol) and [^{3}H]-EQ (34.5 Ci/mmol) were purchased from New England Nuclear (Boston, MA). 17EQ was synthesized by Dr. P.R. Rao, Southwest Foundation for Biomedical Research, San Antonio, TX. PAPS was purchased from Dr. Sanford Singer, University of Dayton, Dayton, OH. Eagle's minimum essential medium (MEM), fetal bovine serum (FBS), geneticin and Lipofectin were purchased from Life Technologies (Gaithersburg, MD). All other chemicals were of reagent grade quality.

2.2. Generation of stably transfected ISH cells

The ISH cell line was a generous gift from Dr. E. Gurpide, Mt. Sinai Medical Center, New York, NY. Cells were routinely maintained in MEM containing 7% FBS at 37°C in a humidified atmosphere with 5% CO_2.

To isolate cells stably transformed with EST, the EST cDNA in pCRII [5] was isolated as a PstI–XhoI fragment and subcloned into the Pst I–Xho I restriction sites of pBluescript SK+ (Stratagene). The full-length EST cDNA was isolated from pBluescript by a complete digestion with XhoI followed by a partial digestion with BamH I. The BamH I–Xho I EST DNA fragment containing the complete EST open-reading frame was subsequently inserted into the BamH I–Xho I sites of the pcDNA3 mammalian expression vector (Invitrogen). Plasmid DNA for the transformation of ISH cells was isolated from *E. coli* XL1-Blue cells using a Qiagen Endo Free Maxi Kit (Qiagen, Palo Alto, CA). ISH cells were transfected using Lipofectin as per the manufacturer's instructions. Selection of transformed ISH colonies was done with selective medium containing geneticin at a concentration of 400 μg/ml. After approximately two weeks in geneticin-containing medium, single colonies of both pcDNA3/ISH and EST/ISH cells were isolated by trypsinization using cloning cylinders and transferred to 24-well plates. The individual colonies were allowed to grow. Colonies expressing EST were identified by cytosolic EST activity and immunoblot analysis [5]. Both pcDNA/ISH and EST/ISH cells were routinely maintained in 7% FBS/MEM/geneticin (400 μg/ml) at 37°C in a 95% air/5% CO_2 atmosphere.

All cultures including untransformed ISH cells were expanded to have a sufficient number of cells in order to cryopreserve a low passage seed stock. In our experiments we used fresh cultures of cryopreserved cells every three months subsequent to three recovery passages.

2.3. Sulfation assays

ISH cell cytosols were prepared from confluent 100 mm plates of cells by rinsing the attached cells twice

with ice-cold phosphate-buffered saline (PBS), pH 7.4, then scraping the cells from the plate into triethanolamine buffer (10 mM triethanolamine, pH 7.5, 10% glycerol, 1.5 mM dithiothreitol, 10 μM phenylmethylsulfonylfluoride). After cell disruption on ice with a motor-driven Teflon-glass homogenizer, the cell homogenate was centrifuged at $100{,}000 \times g$ for 30 min to obtain the cell cytosolic fraction.

Cell cytosols were assayed for estrogen sulfation activity at a concentration of 20 nM [^{3}H]-E2 as described previously [5] using an alkaline–chloroform extraction procedure [12]. Each reaction contained 50 mM Tris–HCl, pH 7.4, 7 mM $MgCl_2$ and 20 μM PAPS in a final volume of 0.125 ml. DHEA sulfation activity was assayed in the same manner but with 3 μM [^{3}H]-DHEA as substrate [12]. To assay P-PST activity and M-PST activity, 4 μM PNP and 10 μM dopamine were used as substrates, respectively. PNP is a selective substrate for P-PST activity and dopamine is a selective substrate for M-PST [13,14]. The reactions also contained 50 mM Tris–HCl, pH 7.4 and 20 μM [^{35}S]-PAPS in a final volume of 0.125 ml. Control reactions were identical except that substrate was not added. Reactions were run at 37°C and terminated by spotting a 50 μl aliquot of each reaction on a silica gel F-250 TLC plate. The plates were developed in methylene chloride:MeOH:ammonium hydroxide (81:15:5, v/v/v) and the radiolabeled sulfated products were localized by autoradiography. The sulfated products were scraped into scintillation fluid and the radioactivity determined by scintillation spectroscopy.

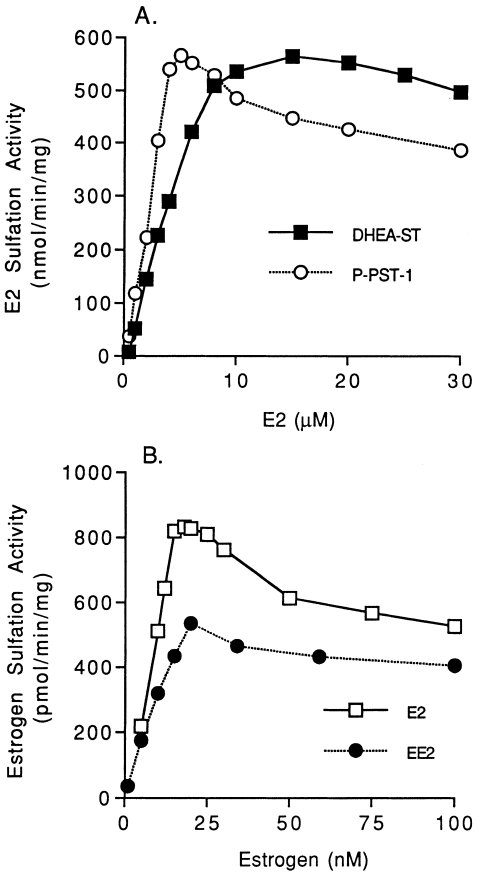

Fig. 1. Estrogen sulfation by EST, P-PST and DHEA-ST. (A) The sulfation of increasing concentrations of E2 (0.25–30 μM) by partially purified preparations of bacterially-expressed P-PST and DHEA-ST [4] was compared. (B) The ability of partially purified bacterially expressed EST [5] to sulfate E2 and EE2 at nanomolar concentrations is shown. Assays were performed using the alkaline–chloroform extraction protocol described in the text. Results are the means of triplicate assays.

2.4. Immunoblot analysis

For immunoblot analysis of EST proteins present in cytosol from both pcDNA/ISH and EST/ISH cells, as well as PG-induced ISH cells for comparative purposes, cytosolic proteins (100 μg protein per lane) were separated on 12.5% polyacrylamide gel and electrophoretically transferred to a nitrocellulose membrane. The membrane was blocked with 0.5% nonfat dry milk for 1 h. Primary antibody incubations with a rabbit anti-EST polyclonal antibody were carried out at room temperature for 1 h at a 1:10,000 dilution [5]. Goat anti-rabbit IgG horseradish peroxidase conjugate was used as the secondary antibody at a 1:60,000 dilution. Immunoconjugates were visualized by chemiluminescence using the Pierce SuperSignal Substrate kit.

2.5. Assay of ER activation

The AlkPhos bioassay which was used as an indicator of ER activation was conducted on quiesced ISH cells [11]. Cells were cultured for 3 days in 7% FBS stripped of endogenous estrogens with dextran coated charcoal [15] in phenol-red free MEM then passed in the same medium into microtiter cell plates. After 24 h, the medium was changed to serum-free MEM and cells were allowed to quiesce for 24 h prior to addition of steroids. The test steroids (E2, EE2, DES, EQ or 17EQ) were added from ethanol stocks so the final concentration of ethanol in the medium was consistently less than 0.1%. Control cells received ethanol alone at the same concentration. The AlkPhos bioassay was performed in microtiter plates as described by Littlefield et al. [11] after 4 days of exposure to steroids. Plates were monitored periodically at 405 nm in an ELISA plate reader during the one to three hour incubation period after addition of the chromogenic substrate until maximally stimulated control ISH cells had an absorbance at 405 nm of about 1.2. This development ensured a linear enzymatic analysis. All experimental conditions were assayed in quadruplicate.

Table 1
ST activities in pcDNA/ISH, EST/ISH and PG-induced ISH cell cytosols. Cytosol was prepared from cells as described in the text. ISH cells were induced with PG as described previously [7]. ST activities were determined from three different experiments for each cell line. Rates are expressed as pmol substrate sulfated/min/mg protein. Values are means ± SD. Substrate concentrations at which ST assays were performed were estrone, 20 nM; *p*-nitrophenol, 4 μM; dopamine, 10 μM; DHEA, 3 μM. N.D. indicates that there was no detectable activity

	ST activity (pmol/min/mg)			
	estrone	*p*-nitrophenol	dopamine	DHEA
pcDNA/ISH	0.089 ± 0.011	15.9 ± 2.8	3.7 ± 0.5	N.D.
EST/ISH	1.006 ± 0.098	16.2 ± 1.9	3.6 ± 0.7	N.D.
PG-induced ISH	0.760 ± 0.157	17.5 ± 1.0	4.2 ± 0.5	N.D.

2.6. Steroid metabolism

For studies of E2 metabolism by pcDNA/ISH and EST/ISH cells, cells for each cell line were plated in 6-well plates in triplicate and allowed to attach for 24 h. At this time, 20 nM [^{3}H]-E2 in phenol-red free MEM was applied to the cells. The medium (50 μl) was sampled at 0, 15, 30, 60, 120 and 180 min then alkalinized by the addition of Tris–HCl (pH 8.7) to a concentration of 120 mM. The samples were then extracted with 3 ml chloroform to remove unsulfated [^{3}H]-E2. An aliquot of the aqueous phase was counted by scintillation spectroscopy to quantify E2-sulfate production. Protein isolated from each well was determined by the Bio-Rad protein assay. To compare the metabolism of E2, EE2, EQ, 17EQ and DES in both pcDNA/ISH and EST/ISH cells, the experiment was performed as described above with triplicate wells receiving the appropriate steroid at a concentration of 20 nM.

3. Results

To provide a background for evaluating and understanding steroid sulfation by the human STs, concentration curves for the sulfation of E2 and EE2 by EST, P-PST and DHEA-ST are shown in Fig. 1. EST is the only human ST that sulfates E2 significantly at low nanomolar concentrations, with a Km of approximately 5 nM [5,6]. Substrate inhibition is a common characteristic of the STs [5,6,16]; with EST maximal

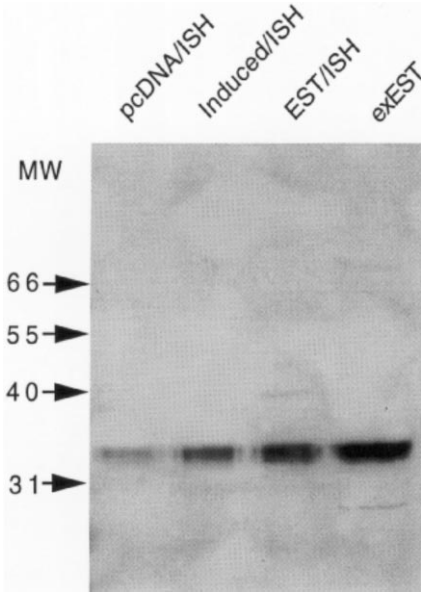

Fig. 2. Immunoblot analysis of pcDNA/ISH, EST/ISH and PG-induced ISH. To confirm that EST was expressed at similar levels in EST/ISH and PG-induced ISH, immunoblot analysis of cell cytosols with an anti-EST polyclonal antibody was performed. Bacterially expressed EST was used as a positive control. The lanes contain 100 μg uninduced ISH cytosol (control), cytosol from PG-induced ISH cells (PG-induced), and cytosol from EST/ISH cells as well as bacterially expressed EST [5].

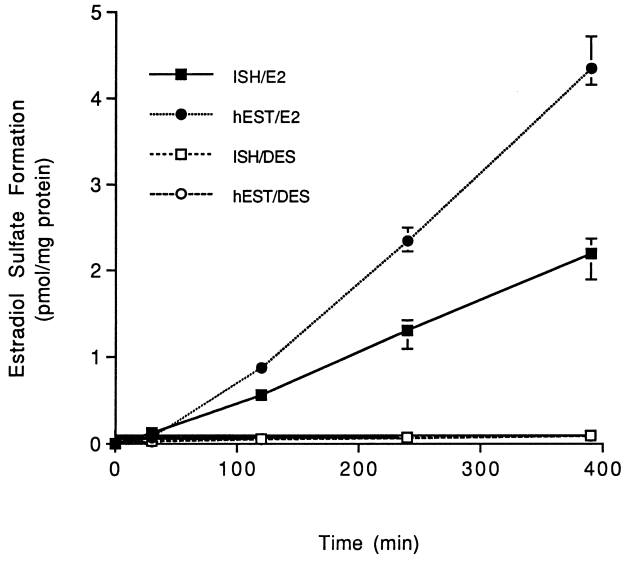

Fig. 3. Metabolism of E2 and DES by pcDNA/ISH and EST/ISH cells. Cells for each cell line were plated in 6-well plates in triplicate and allowed to attach for 24 h. 20 nM [^{3}H]-E2 or [^{3}H]-DES was added to the cells in fresh medium. Aliquots (50 μl) of the medium were removed at different time points and alkalinized with 150 μl of 250 mM Tris–HCl, pH 8.7. The medium was then extracted with 3 ml of chloroform and the [^{3}H]-E2 or [^{3}H]-DES sulfate in the aqueous phase determined by scintillation spectroscopy.

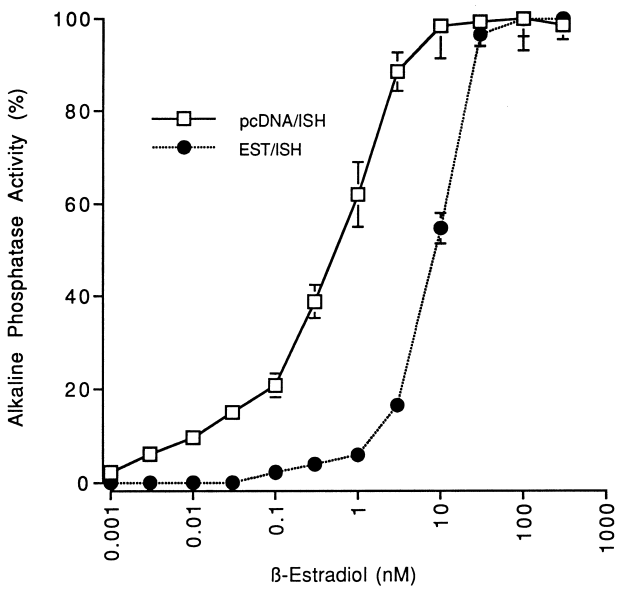

Fig. 4. Induction of AlkPhos activity by E2 in control ISH and EST/ISH cells. Cells were cultured for 3 days in 7% steroid-stripped FBS/phenol-red free MEM then plated in microtiter plates in the same medium. After 24 h, the medium was replaced with serum-free MEM and cells were quiesced for 24 h. E2 was added to the appropriate final concentration for 4 days. The AlkPhos bioassay to monitor ER induction was performed as described by Littlefield et al. [11] after 4 days of exposure to E2.

sulfation was attained at an E2 concentration of approximately 20 nM. Stably transformed ISH cells with elevated levels of EST activity were prepared to use as a model system for the investigation of the regulation of ER receptor activation via sulfation.

Table 1 shows that PG-induced ISH cells possessed levels of EST activity approximately 8-fold greater than control cells [7]. In comparison, stably transformed EST/ISH cells contained EST activity levels approximately ten-fold greater than the EST activity found in pcDNA/ISH cells Table 1 and therefore mimic the situation in endometrial tissue following PG induction of EST activity. Activity levels of the other STs analyzed (P-PST, M-PST and DHEA-ST) were similar in PG-induced/ISH, EST/ISH and pcDNA/ISH cells. Fig. 2 shows that immunoblot blot analysis of EST in the PG-induced ISH, pcDNA/ISH and EST/ISH cells is consistent with the levels of E2 sulfation activity in the different cells. These results indicate that the transformation of these cells with EST was specific and resulted in the elevated sulfation of 20 nM E2 but not of the substrates selective for the other cytosolic STs.

As predicted from the concentration curve for E2 sulfation by EST, cultured EST/ISH cells sulfate E2 more rapidly than do pcDNA/ISH cells. When 20 nM E2 is supplied in the medium to cultured ISH cells, E2 sulfate is produced more rapidly by EST/ISH cells than by pcDNA/ISH cells (Fig. 3). This confirms that the elevated EST level in EST/ISH cells is physiologically effective and alters the milieu of the growing cells. Sulfation of EE2 by the two cell lines is similar to that of E2, as would be expected because EST sulfates EE2 with kinetics similar to those found for E2. DES and the equine estrogens EQ and 17EQ are not significantly sulfated by expressed EST at low nanomolar concentrations [5]. DES is not metabolized via sulfation by either pcDNA/ISH or EST/ISH cells when it is applied to the cells at a concentration of 20 nM (Fig. 3). Similarly, when either 20 nM EQ or 17EQ is applied in the medium to either pcDNA/ISH or EST/ISH cells, detectable sulfation of EQ or 17EQ does not occur (data not shown). Because these estrogens are not sulfated at nanomolar concentrations by the STs found in ISH cells (EST, P-PST, M-PST), it is logical that their sulfation by ISH cells would not be detectable.

Over-expression of EST activity in ISH cells decreases the ability of E2 to stimulate the expression of ER regulated AlkPhos activity. Control pcDNA/ISH and EST/ISH cells were treated with concentrations of E2 from 1 pM to 300 μM and assayed for ER activation using the AlkPhos bioassay. As shown in Fig. 4, pcDNA/ISH cells respond to E2 at a concentration of 3 pM, with maximal response attained at a concentration of approximately 10 nM. In contrast, EST/ISH cells do not begin to respond to E2 until an E2 concentration of about 100 pM, with maximal response attained at a concentration of 30 nM. Thus, EST/ISH cells require higher concentrations of E2 to elicit ER activation than do pcDNA/ISH cells; this effect is more prominent at lower E2 concentrations. We hypothesize that this is due to the increased inactivation of E2 via sulfation by EST/ISH cells, resulting in a lower effective E2 concentration in the cellular environment. Thus, a higher concentration of exogenously applied E2 is necessary to attain cellular concentrations equivalent to those found with control pcDNA/ISH cells because EST/ISH cells are inactivating E2 via sulfation.

ER activation with E2 occurs in both pcDNA/ISH and EST/ISH cells but at significantly different E2 concentrations (Fig. 4). The activation of the ER which occurs with EE2 is similar to that found with E2, as would be expected from the similar kinetics of E2 and EE2 sulfation by EST (Fig. 1). Both E2 and EE2 are high affinity substrates for EST and the presence of EST activity in the EST/ISH cells results in a rapid inactivation of these estrogens (Fig. 5A). In contrast, DES is not sulfated by EST at significant levels at low nanomolar concentrations (Fig. 3). When DES activation of the ER is compared in pcDNA/ISH and EST/ISH cells using the AlkPhos assay, there is no difference in the activation concentration curves for

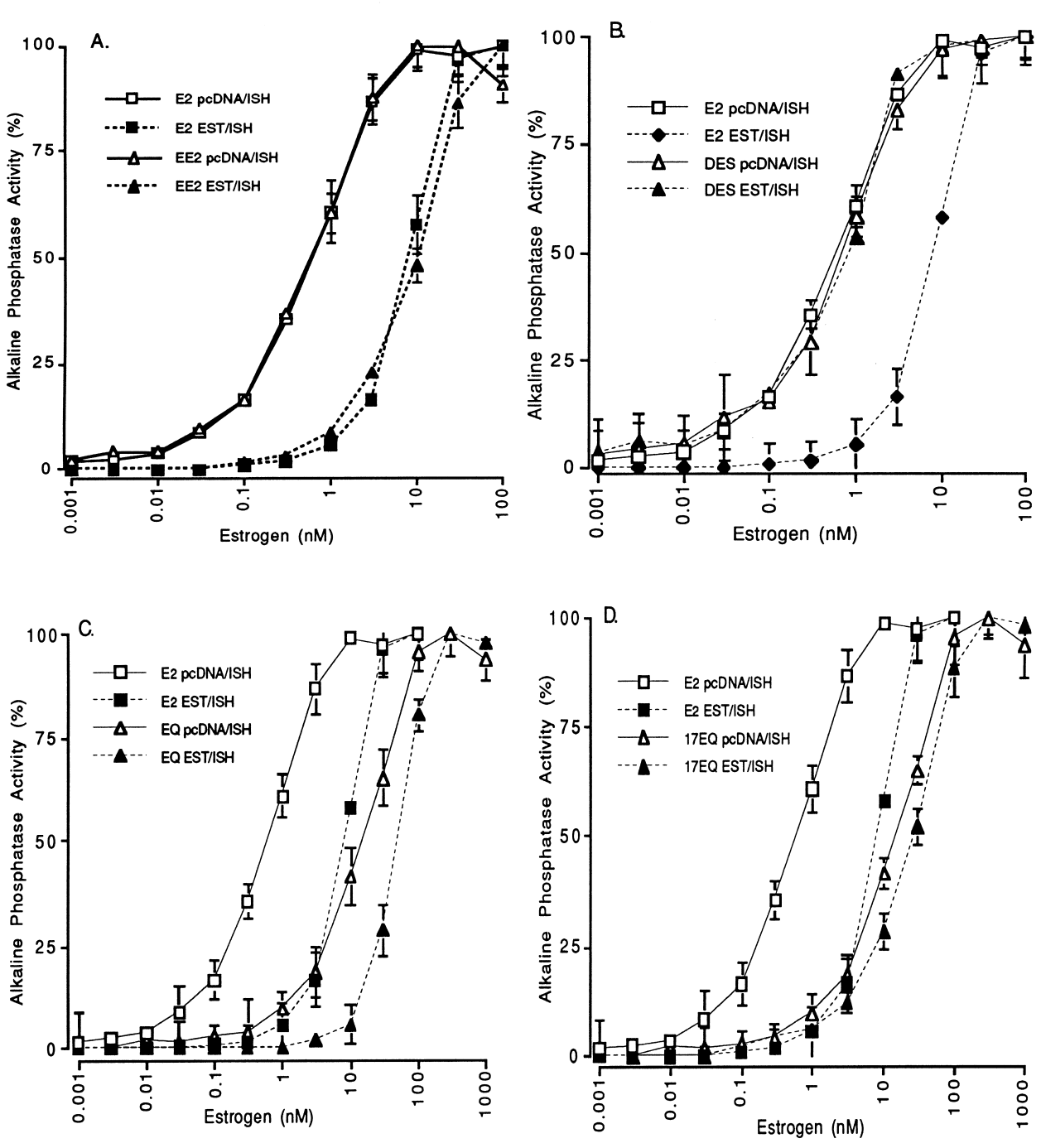

Fig. 5. Comparison of AlkPhos activity induction by E2 and other estrogens in pcDNA/ISH and EST/ISH cells. Cells were cultured for 3 days in 7% steroid-stripped FBS/phenol-red free MEM then plated in microtiter plates in the same medium. After 24 h, the medium was replaced with serum-free MEM and cells were quiesced for 24 h. Either E2 or another steroid was added to the appropriate final concentration for 4 days. The AlkPhos bioassay to monitor ER induction was performed as described by Littlefield et al. [11] after 4 days of exposure to steroids. (A) Comparison of E2 and EE2, (B) comparison of E2 and DES, (C) comparison of E2 and EQ, (D) comparison of E2 and 17EQ.

DES between the two cell lines (Fig. 5B). The pcDNA/ISH and EST/ISH cells differ only in their expression level of EST; because DES is not sulfated by this enzyme, the ER induction concentration curve for DES is not significantly different between the cell lines. Additionally, it is similar to the E2 ER activation concentration curve in pcDNA/ISH cells.

EQ is an important estrogen found in preparations

of conjugated equine estrogens used in hormone replacement therapy [17–19]. When ER activation by EQ in pcDNA/ISH and EST/ISH cells is compared, there is only a small difference for the pattern of ER activation between the two cell lines (Fig. 5C). Slightly higher levels of EQ are needed in the EST/ISH cells to activate the ER as compared to pcDNA/ISH cells. When the pattern of ER activation is compared for E2 and EQ, it is apparent that EQ is required at approximately 15-fold higher concentrations than E2 in pcDNA/ISH cells to be effective in eliciting a cellular response, indicating that EQ is not as effective at activating the ER as is E2.

17EQ was synthesized to determine whether the presence of the 17β-OH group on an equine estrogen is important in altering the activation of the ER or the ability of EST to alter its ER activation activity. Similar to EQ, 17EQ shows no significant differences in ER activation between the pcDNA/ISH and the EST/ISH cell lines, and is required at significantly higher concentrations than E2 to be effective (Fig. 5D). The dose–response curves for EQ and 17EQ are also similar in both cell lines.

4. Discussion

Sulfation is important in the synthesis, metabolism and regulation of the activity of steroids in many human tissues [5,16,20]. Estrogen sulfation has been implicated in the regulation of E2 activity in several tissues including liver, endometrium, breast and testis [20–22]. The kinetic properties of the STs involved in E2 sulfation are important in determining their physiological role in regulating estrogenic activity. DHEA-ST and P-PST sulfate E2 with maximal activity at micromolar concentrations [4] whereas EST maximally sulfates E1, E2 and EE2 at low nanomolar concentrations [5,6]. Therefore, EST readily sulfates E2 at concentrations at which it binds to the ER suggesting that it has a significant physiological role in modulating the response of ER-positive tissues to estrogenic stimulation. High levels of EST in a tissue would render the tissue less responsive to estrogenic stimulation because the sulfated estrogens are not able to bind to the ER and initiate a cellular response [22].

Our laboratory has previously demonstrated that EST is capable of regulating the activity of E2 in human breast cancer MCF-7 cells [23,24]. MCF-7 cells express P-PST but not EST and are responsive to the mitogenic effects of E2. The stable expression of EST in MCF-7 cells results in cells which are significantly less sensitive to the growth-stimulating properties of E2 than control cells [24]. These results indicate that the evaluation of the role of the STs in modulating the activity of estrogens requires careful attention to the specific isoforms of ST expressed in the target tissue.

The cyclic growth and shedding of normal human endometrium during the menstrual cycle is regulated by the secretion of E2 and PG from the ovaries. E2 secreted during the proliferative phase of the cycle prior to ovulation stimulates the proliferation of endometrial tissues. After ovulation, large quantities of both E2 and PG are secreted from the ovaries. During the secretory phase of the cycle, the presence of PG prevents the stimulation of the endometrium by E2 via several mechanisms including down-regulation of the level of ER and an increase in E2 metabolism including an increase in E2 sulfation [9,10]. We have reported previously that EST is present at significant levels in human endometrial tissues during the secretory phase of the menstrual cycle but is not detectable by EST activity assays, immunoblot or Northern blot analysis during the proliferative phase of the menstrual cycle [3]. Additionally, we have shown that the specific induction of EST by PG occurs in human ISH endometrial cells [7]. This supports the hypothesis that PG induction of EST has an important role in the regulation of estrogenic activity in secretory endometrium. Thus, EST/ISH cells which stably express high levels of EST have been used to investigate the effects of increased sulfation on the activity of estrogenic compounds. Stable expression of EST in the ISH cells mimics PG induction of EST activity without the other effects of PG which include the down-regulation of ER levels and the induction of 17B-hydroxysteroid dehydrogenase activity.

Metabolic sulfation of estrogens by pcDNA/ISH cells and EST/ISH cells correlates with the kinetics of their sulfation by EST. Those estrogens such as E2 and EE2, which are sulfated efficiently by EST at low nanomolar concentrations, are sulfated rapidly by EST/ISH cells as compared to pcDNA/ISH cells. This difference in the rate and affinity for the sulfation of estrogens is important in regulating and ameliorating estrogenic effect in ISH cells. Estrogens such as DES which are not sulfated significantly by ISH cells may provide physiologically unchecked stimulation of the ER in ISH cells. If the physiological situation in humans parallels that found in the ISH cell model system, DES may stimulate secretory endometrial cells in an unregulated fashion which may have a role in hyperestrism and in the induction of endometrial and vaginal cancer which has been found in women who have taken DES [18,19,25].

Over-expression of EST activity in ISH cells decreases the ability of E2 and EE2 to stimulate the expression of ER-regulated AlkPhos activity but does not alter the ability of DES, EQ or 17EQ to increase AlkPhos activity. Thus, potent estrogenic compounds which are not high affinity substrates for EST have the

same estrogenic activity in EST/ISH cells as that found with pcDNA/ISH cells. Estrogens such as E2 and EE2 which are high affinity substrates for EST show a differential effect on EST/ISH as compared to pcDNA/ISH cells because their effectiveness is diminished via sulfation in the EST/ISH cells. The responsiveness of ISH cells to those estrogens such as DES and the equine estrogens EQ and 17EQ which are not readily sulfated by EST is unaltered by the presence of elevated EST levels in the cells. These results indicate that EST has a role in the selective inactivation of E2 and estrone but is significantly less effective in the sulfation and inactivation of other therapeutic or environmental estrogenic compounds. In endometrial tissue these estrogenic compounds may still be capable of activating the ER during the luteal phase of the menstrual cycle when the activity of E2 is greatly decreased. Thus, the activity of equine estrogens which are commonly used in hormone replacement therapy and environment estrogens such as DES may not be modulated at the cellular level in the same manner as E2 and estrone.

Acknowledgements

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