Carcinogenic Activities of Various Steroidal and Nonsteroidal Estrogens in the Hamster Kidney: Relation to Hormonal Activity and Cell Proliferation

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ABSTRACT

The therapeutic use of estrogens has been associated with an increased risk of some of the most predominant, as well as less prevalent, cancers in women. The estrogen-induced renal tumor is one of the primary animal models to evaluate the carcinogenic properties of estrogens. Correlations were made with various estrogens by using parameters of estrogenicity end points such as competitive binding, progesterone receptor induction, and alterations in prolactin levels; in vitro renal proximal cell proliferation; and in vivo estrogen-induced carcinogenicity. The most potent estrogens were Moxestrol (MOX), diethylstilbestrol (DES), and 17β-estradiol, followed by indenestrol B, 16α-hydroxyestrone, and 11β-methoxyestradiol with moderate estrogenic activities, whereas 11β-methyl estradiol, 17α-estradiol, indenestrone, and deoxoestrone were all relatively weaker. As expected, hydrolyzed Premarin (unconjugated estradiol) was strongly estrogenic. Of the estrogens tested, MOX was the most potent carcinogenic estrogen in the hamster kidney. Both 16α-hydroxyestrone and 11β-methoxyestradiol induced intermediate tumor incidences with distinctly lower frequencies of renal tumor foci compared to the most potent carcinogenic estrogens. However, hamsters treated for 9.0 months with 11β-methyl estradiol, 17α-estradiol, deoxoestrone, and indenestrone exhibited no tumors. In contrast, treatment with estrone, equilin plus d-equilenin, and hydrolyzed Premarin for the same time period resulted in 100% renal tumor incidences and numerous tumor foci. Cell proliferation studies of cultured hamster kidney proximal tubule cells were carried out at varying estrogen concentrations (0.01-10 nM). Exposure to MOX resulted in consistently high renal cell proliferative response over a concentration range of 0.1-10 nM. Strongly carcinogenic estrogens such as estrone had a maximal renal cell proliferation response (2.4-fold above untreated control levels) between 0.1 and 10 nM, DES and 17β-estradiol responded at 1.0 nM, and 4-hydroxyestradiol responded at 10 nM. Interestingly, exposure to ethinylestradiol, a potent estrogen, at similar or higher doses as those used for DES and 17β-estradiol, yielded only a 10% renal tumor incidence and induced only a 1.7-fold increase in proximal tubule cell proliferation. In contrast, 17α-estradiol, deoxoestrone, indenestrone, and 11β-methylestradiol, all weakly estrogenic and noncarcinogenic agents, had relatively little effect on tubule cell proliferation. The hydrolyzed Premarin exhibited a maximal 2.0-fold cell proliferative response at 10 nM.

The present results provide clear evidence that, in the hamster kidney, the degree of carcinogenicity of a given estrogen correlates with its ability to induce proximal tubule cell proliferation in vitro. Therefore, the ability of estrogens to enhance tubule cell proliferation is a more accurate indicator of its carcinogenicity in this system than either the estrogen-responsive end points used or the amount of catechol metabolites generated in this tissue as reported earlier.

INTRODUCTION

Numerous epidemiological studies have established causal relationships between estrogen use and increased risk for some of the predominant cancers in women, namely, breast and endometrium (1–3). Ingestion of estrogens has also been associated with some less prevalent cancers at hepatic, cervico-vaginal, and ovarian sites (4–6). It is estimated that at least 20 million women in the United States take estrogenic hormones, largely but not solely for contraception, for the relief of menopausal symptoms, and to reduce the risk of osteoporosis and cardiovascular disease that afflicts aging women. On a worldwide basis, this number is most certainly multiplied many times. Menopausal estrogens have become one of the most widely prescribed drugs, with a current usage rate of over 32% among women ages 50–70 years (7). Despite increasing study, there is still little understanding of the cellular and molecular mechanisms whereby these estrogen-related neoplastic events occur.

The estrogen-induced renal tumor has emerged as one of the primary experimental models to evaluate the carcinogenic properties of estrogens, whether they possess steroidal or nonsteroidal structures (8–10). Recent advances in estrogen carcinogenesis indicate that cell proliferation may play a critical early role in estrogen-induced tumorigenic processes (11, 12). Interestingly, this concept has received considerable support from earlier studies using numerous experimental models in hormonal carcinogenesis employing estrogens as well as other sex steroids (13–17).

The current report presents data correlating in vivo carcinogenicity data of estrogens and in vitro cell proliferation of primary renal epithelial hamster cells in culture, grown in serum-free chemically defined conditions, as well as other parameters of estrogenicity.

MATERIALS AND METHODS

Chemicals and Reagents. [2,4,6,7-3H]-17β-estradiol (115 Ci/mmol), [1,2,6,7-3H]-progesterone (103 Ci/mmol), and [17α-methyl-3H]-R5020 (86 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Radiinert chromatographic grade estradiol, estrone, and progesterone were purchased from Calbiochem (Behring, CA), and all other nonlabeled steroids were obtained either from Sigma Chemical Co. (St. Louis, MO) or Steraloids (Wilton, NH). Deoxoestrone was prepared by Dr. Luke K. T. Lam (LKT Laboratories, Minneapolis, MN). Indenestrone B and indenestrone were generously provided by Dr. Manfred Metzler (University of Kaiserslautern, Kaiserslautern, Germany); MOX3 (11β-methoxyethylstradiol) by Dr. J. P. Raynaud (Roussel UCLAF, Paris, France); and 16α-hydroxyestrone by Dr. Jack Fishman (IVAX Corp., Miami, FL). All estrogens investigated exhibited purities of at least 95% as shown by HPLC analyses by using a Waters model 840 liquid Chromatograph equipped with a Waters 490 programmable multi-wavelength detector. Estrogen samples (10 µg), dissolved in tetrahydrofuran, were injected and eluted on two tandem octadecyl (C18) columns, 0.46 x 25 cm (IBM, Wallingford, CT), by using 29% acetonitrile in water isocratically at a column temperature of 35°C and at a flow rate of 3 ml/min. Conjugated estrogens were hydrolyzed from Premarin (Wyeth-Ayerst, Philadelphia, PA)

Received 4/17/95; accepted 7/27/95.

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1 This investigation was supported by National Cancer Institute, NIH Grants CA58030 and CA22088 and a Kansas Masonic Oncology Research Center grant.

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3 The abbreviations used are: MOX, Moxestrol; PRL, prolactin; ER, estrogen receptor; DES, diethylstilbestrol; PR, progesterone receptor; EE, ethinylestradiol.
by using sulfatase, type H-1 (Sigma), for 5 h under N₂ in 0.2 N sodium acetate, pH 5.0. HPLC separation of the unconjugated estrogens in hydrolyzed Premarin is shown in Fig. 1. The composition of the hydrolyzed Premarin prepared was estrone (42%), equilin (17%), 17α-dihydroequilenin (3.4%), 17α-estradiol (2.4%), 8-dihydroestrone (18%), 17β-dihydroequilenin (0.7%), equilenin (4.3%), 17α-dihydroequilenin (10%), 17β-estradiol (1.5%), and 17β-dihydroequilenin (0.7%).

Animals and Treatments. Young adult castrated male Syrian golden hamsters, weighing 85–95 g, were purchased from Harlan Sprague-Dawley (Indianapolis, IN). All animals were acclimated 1 week before treatment or use. Hamsters were housed in compliance with the guidelines of the United States Department of Health and Human Resources (NIH). Animals were housed in a facility certified by the American Association for the Accreditation of Laboratory Animal Care. Hamsters were maintained on a 12-h light/12-h dark cycle and fed rodent certified chow 5012 Purina diet (Ralston Purina Co., Richmond, IN) and tap water ad libitum. Castrated animals in the treatment groups (9–12/group) were implanted s.c. with the following pellets (20 mg): 17β-estradiol, MOX, 11β-methoxyestradiol, 11β-methylestradiol, 16a-hydroxyestrone, 17α-estradiol, deoxoestrone, DES, indenestrol B, indanestrol, or hydrolyzed Premarin as described previously (18, 19). To maintain estrogenic levels of each compound constant, additional pellets were implanted every 2.5 months. The pellets were prepared by Hormone Pellet Press (Westwood, KS), and their release rates were adjusted so that mean daily absorptions were similar (111 ± 11 µg). However, 11β-methoxyestradiol, like EE, exhibited a mean daily absorption rate of 259 ± 12 µg. For PR induction and serum PRL levels, hamsters were treated for 3.0 and 9.0 months, respectively, with various estrogens as reported elsewhere (19–21).

Competitive Binding Studies. For ER, pure renal tumors were excised from hamsters treated with DES for 9–11 months as described previously (22), homogenized in 9.0 volumes of TED buffer (10 mM Tris-HCl, 1.5 mM EDTA, 1 mM dithiothreitol, pH 7.4), and centrifuged at 100,000 × g. Competitive binding studies of the 8S ER were carried out on filtered cytosols (22-µm Millipore filter; Millipore) as described earlier, by using 5 nM [3H]-17β-estradiol and various concentrations (1–100-fold excess) of either radioinert steroidal or nonsteroidal estrogens (22, 23). Prewashed dextran-charcoal was used to remove free hormone and some lower-affinity-binding components (1 h). For PR, renal cytosol fractions from 3.0-month-old DES-treated hamsters were incubated with 5 nM of either [3H]-progesterone or [3H]-R5020, alone or in combination with corresponding radioinert hormones (100-fold excess). Dextran-charcoal treatment was 10 min (20–22). Protein concentrations of the renal tumor and kidney cytosols were determined by the method of Lowry et al. (24).

Hamster PRL Bioassy. To determine PRL levels in hamster serum samples, the Nb₂ node lymphoma bioassay was used as described previously in detail (19, 25). Serum samples were obtained at 9.0 months from each of the estrogen-treated animals via the inferior vena cava. The Nb₂ node rat lymphoma cells were kindly provided by Dr. R. L. Noble and Dr. C. T. Beer, (University of British Columbia, Vancouver, British Columbia, Canada). Hamster serum samples were diluted 1:10 with filtered Fischer medium containing 10% horse serum and added to wells in aliquots of 50 and 100 µl. Each hamster serum sample was bioassayed in triplicate.

Detection of Renal Tumor Foci. The occurrence of renal tumor foci induced by the various steroidal and nonsteroidal estrogens was assessed by procedures described earlier (19, 26, 27). Briefly, serial frozen kidney sections (10-µm thick) were prepared immediately after excision and embedding whole kidneys in ornithine carbamyl transferase (Tissue TEK, Elk Hart, IN). By using a CTF Microtome cryostat (Damon, IEC, Needham Heights, MA), maintained at −18°C, 25–30 unfixed frozen sections were taken at regular intervals from each kidney. Each frozen section was stained for nonspecific esterase activity by using α-naphthyl butyrate as substrate (18, 28). Tumor foci were identified under microscopic examination by the markedly reduced esterase activity in the tumor compared to surrounding normal kidney tissue and by morphological criteria.

Isolation of Renal Tubules. Kidneys were removed from castrated male hamsters immediately after decapsulation. The kidneys were decapsulated, and the cortices were separated from the medullas. The procedure for isolating renal tubules has been described elsewhere (29, 30). Briefly, the kidneys were minced and homogenized in an all-glass homogenizer on ice (University of British Columbia, Vancouver, British Columbia, Canada). Homogenate was filtered through 10– and 3-mm nitrocellulose filters, and the filtrate (renal tubules) was washed with 0.1% EDTA and disaggregated to single cells with 0.25% trypsin and 0.1% EDTA. The cells were then counted in a hemocytometer by a procedure described elsewhere (30). The cell proliferation data are expressed as a stimulation index, the ratio of the number of cells grown in the presence of estrogen versus the number of cells grown without estrogen.

![Graph showing absorbance over time](image)
RESULTS

Estrogenicity Studies. To assess the relative estrogenicity of the various steroidal and nonsteroidal estrogens studied in the hamster, competitive binding to kidney tumor cytosolic ER, induction of PR, and serum PRL levels were studied. Untreated normal levels of PR and PRL in castrated male hamsters were 3.3 ± 0.2 fmol/mg protein and 34 ± 5 pg/ml, respectively. Generally, there was a good correlation among these parameters concerning the relative estrogenic potency of the steroidal and nonsteroidal compounds studied (Table 1).

Utilizing the hormonal end points described herein, it is evident that MOX, 17β-estradiol, and DES were the most potent estrogens, followed closely by indenestrol B, 16α-hydroxyestrone, and 11β-methylestradiol with moderate estrogenic potencies, whereas 11β-methyltestosterone, 17α-estradiol, indenestrol, and deoxoestrone exhibited distinctly lower estrogenic activities (Table 1). The expected strong estrogenicity of hydrolyzed Premarin (unconjugated estrogens) was confirmed by its competitive binding to renal tumor ER (1.0-fold excess) at 42 ± 3%, its induction of hamster kidney PR to 53 ± 2 fmol/mg protein, and its elevation of serum PRL levels to 339 ± 93 μg/ml.

Carcinogenic Activity of Various Estrogens. As reported previously (19, 26), 17β-estradiol and DES were equally carcinogenic in the hamster kidney. These potent carcinogenic estrogens served as positive references for the rest of the steroidal and nonsteroidal estrogens studied. MOX was found to be the most potent carcinogenic estrogen in the hamster kidney (Table 1), effecting a 100% tumor induction of PR in both kidneys and elevating serum PRL levels to 539 ± 76 ng/ml.

Steroidal

<table>
<thead>
<tr>
<th>Estrogen</th>
<th>Renal tumor % of ER competitive binding&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Hamster kidney induction of PR&lt;sup&gt;b&lt;/sup&gt; (fmol/mg protein)</th>
<th>Serum prolactin (ng/ml)</th>
<th>No. of animals with tumors (%)</th>
<th>Combined no. of tumor foci in both kidneys</th>
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<tr>
<td>17β-Estradiol</td>
<td>55 ± 2</td>
<td>48 ± 6</td>
<td>390 ± 76</td>
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<tr>
<td>11β-Methoxy EE (MOX)</td>
<td>52 ± 4</td>
<td>60 ± 2</td>
<td>330 ± 65</td>
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<td>22 ± 3</td>
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<tr>
<td>16α-Hydroxyestrone</td>
<td>48 ± 3</td>
<td>45 ± 7</td>
<td>387 ± 35</td>
<td>38</td>
<td>3 ± 1</td>
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<tr>
<td>11β-Methoxyestradiol</td>
<td>30 ± 6</td>
<td>35 ± 6</td>
<td>389 ± 17</td>
<td>25</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>11β-Methylestradiol</td>
<td>14 ± 1</td>
<td>18 ± 2</td>
<td>149 ± 69</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>17α-Estradiol</td>
<td>34 ± 5</td>
<td>6 ± 1</td>
<td>129 ± 17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Deoxoestrone</td>
<td>14 ± 1</td>
<td>8 ± 1</td>
<td>94 ± 20</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Diethylstilbestrol</td>
<td>46 ± 4</td>
<td>50 ± 4</td>
<td>449 ± 89</td>
<td>100</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>Indenestrol B</td>
<td>46 ± 2</td>
<td>49 ± 5</td>
<td>281 ± 90</td>
<td>100</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Indenestrol</td>
<td>10 ± 1</td>
<td>29 ± 5</td>
<td>103 ± 40</td>
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Steroidal estrogens at varying concentrations (0.01–100 nM) under serum-free chemically defined conditions. Estrone attained a maximal 2.4-fold increase in renal cell proliferation between 0.1 and 10 nM; 4-hydroxyestradiol exhibited a similar maximum proliferative response at 10 nM. Additionally, 4-hydroxyestrone exposure resulted in a maximal 2.1-fold rise in tubule cell proliferation at 1.0 nM. It is noted that 2-hydroxylated metabolites of 17β-estradiol and estrone exhibited lower renal cell proliferative activity compared to their corresponding 4-hydroxylated counterparts (data not shown). Interestingly, renal proximal tubule cell proliferation increased only a maximal of 1.7-fold after exposure to EE. At 100 nM, all of the above indicated estrogens exhibited a decline in tubular cell proliferation.

Exposure of the proximal tubule cell cultures to MOX resulted in a consistently high cell proliferation response over the concentration range of 0.1–10 nm (Fig. 3A). Moreover, 17β-estradiol resulted in a similar 2.4-fold rise in tubule cell proliferation at 1.0 nM. At 1 nM concentration, however, exposure of the tubule cells to either 16α-hydroxyestrone or 11β-methoxyestradiol elicited a lower 1.4- and 1.3-fold increase in cell proliferative response, respectively. The hydrolyzed Premarin exhibited a 2.0-fold increase in tubule cell proliferation with a maximum occurring at 10 nM. On the other hand, 11β-methylestradiol exhibited little ability to effect renal tubule cell proliferation (Fig. 3A). In contrast, DES exhibited a strong cell proliferative response, between 1.0 and 10 nm, when exposed similarly to proximal tubule cells in culture (Fig. 3B). Indenestrol B exposure also showed a strong, 2.2-fold, cell proliferative response at 1.0 nm concentration. However, exposure of hamster renal tubule cells to weak estrogens, such as 17α-estradiol, indenestrol (Fig. 3A and B), and deoxoestrone (data not shown), did not result in significant renal tubule cell proliferative responses in these cultures.

DISCUSSION

Previous studies from our laboratory (19, 26, 27) have concluded that a good correlation exists between hormonal activity and carcinogenicity...
CARCINOGENIC ACTIVITY OF ESTROGENS AND CELL PROLIFERATION

Fig. 2. Effect of various steroidal estrogens (0.1–100 nM) on proliferative outgrowth of kidney tubule cells grown on PF-HR9 basement membrane in serum-free chemically defined medium. Hamster cortical explants (~500/flask) were plated in separate 25 cm² flasks. Twelve days after initiation of the culture after ± estrogen exposure, all tubules were coated and treated with 0.1% EDTA. Both parent explants and monolayer cells were removed and disaggregated into single cells. The cells were then counted in a hemocytometer. Points, mean of six counts from three individual flasks; bars, mean ± SEM. The data are expressed as a stimulation index, the ratio of the number of cells grown in the presence of estrogen versus the number of cells grown in the absence of estrogen. The steroid estrogens shown are estrone (V), 4-hydroxyestradiol (Ø), 4-hydroxyestrone (Y), ethinylestradiol (C), and 17a-estradiol (B). In regard to PR induction in the hamster kidney, 2-hydroxyestradiol was shown to be a poor inducer when compared to 4-hydroxyestradiol (data not shown). This observation is consistent with the relatively stronger estrogenic activity reported for 4-hydroxyestradiol in other target tissues when compared to 2-hydroxyestradiol (39, 40).

The relative abilities of estrone, 4-hydroxyestradiol, 4-hydroxyestrone, and EE to elicit tubule cell proliferation essentially coincides with the abilities of these estrogens to induce renal tumors in the hamster (27). Similarly, the ability of DES and indenestrol B to effect tubule cell proliferative responses also correlates well with the ability of these nonsteroidal estrogens to induce kidney tumors. It should be noted that the relative estrogenicity of these stilbene estrogens reported herein is similar to those reported elsewhere using uterotrophic activity studies in the mouse (41).

Although determination of hormonal activity described in these studies is a useful marker of estrogenicity, and the relative amounts of catechol estrogen metabolites generated by various estrogens on the hamster kidney have been assessed (42, 43), neither of these parameters has adequately predicted the carcinogenic potential of a given estrogen. A more accurate indicator of carcinogenicity for an estrogen in the hamster kidney is the ability of the hormonal agent to enhance cell proliferation in the proximal tubule cells. For example, neither 16α-hydroxyestrone nor 11β-methoxyestradiol (data not shown) elicited strong proliferative responses in these cells, consistent with their observed lower carcinogenic activity. Although exposure of these renal tubule cells to highly potent carcinogenic estrogens (MOX, estrone, 17β-estradiol, DES, and indenestrol B) in the hamster kidney resulted in significant renal tubule cell proliferative responses, estrogens that were either poorly carcinogenic or exhibited no tumor development showed markedly less renal cell proliferation activity (11β-methyleneestrol, indenestrol, deoxoestrol, and 17α-estradiol). In contrast to our finding that 2-methylenestradiol is devoid of carcinogenic activity in the hamster kidney, it has been reported by others that this estrogen induced renal tumors in 2 of 10 hamsters after 7.0 months of treatment, but no renal neoplasms in 0 of 10 hamsters after 8 months of treatment (42). Because estrone comprised >40% of hydrolyzed Premarin, it was not surprising that this preparation was highly carcinogenic in the hamster renal tumor model and also significantly affected renal tubule cell proliferation. It should be noted among estrogens tested in the hamster kidney. However, EE was a notable exception because it exerted poor carcinogenic activity at this organ site (10% tumor incidence), and yet is considered to be at least five times more estrogenic than 17β-estradiol by standard uterus assay (32) and is also evidently a strong estrogen in the hamster by most of the estrogenic criteria used herein (19, 27). It is clear, however, from the present results and those reported elsewhere (33) that various estrogens can have profoundly different effects in the same target tissue. For example, compared to other potent estrogens, hamsters treated with EE exhibited a very different series of morphological changes in the kidney. After only brief treatment (≤1.0 month), consistent unusual hyperplasia was seen in a subset of proximal tubule cells located deep in the cortex adjacent to the renal pelvis (33). This unique hyperplasia observed in EE-treated hamsters was only uncommonly observed in either DES or 17β-estradiol treated animals. This finding may in part help explain the low carcinogenic activity of EE at this organ site. On the other hand, in the rat and human, MOX has an estrogenic potency at least five times greater than EE and about ten times that of 17β-estradiol and its metabolites (2- and 4-hydroxylation; Refs. 34, 35) by virtue of its lower affinity to plasma proteins, slower dissociation from ER, and markedly reduced metabolism, particularly to catechol intermediates, in both human liver and hamster kidney (35, 36). As a result of these properties, it was not surprising that MOX exhibited strong estrogenic activity in the hamster based on the hormonal end points studied, but is also the most potent carcinogenic estrogen tested thus far in the hamster kidney.

Although a good association exists between estrogenicity and carcinogenicity by using the hormonal end points described herein, the correlation is apparently limited in some respects. For example, 16α-hydroxyestrone exhibited appreciable estrogenicity by these end points and yet yielded only a moderate, 38% tumor incidence. It should be noted that 16α-hydroxyestrone, unlike in humans, is not formed from either 17β-estradiol or estrone in the hamster kidney or liver (12, 37, 38). Moreover, the significant binding of 17α-estradiol to renal ER in the hamster did not result in substantial elevation in PR or serum PRL levels in this animal. Previous studies in our laboratory have demonstrated that 2-hydroxyestrone (estradiol and estrone) metabolites were devoid of carcinogenic activity in the hamster kidney (27). In contrast, 4-hydroxyestrone metabolites, particularly estradiol, exhibited significant carcinogenic activity at this organ site. In regard to PR induction in the hamster kidney, 2-hydroxyestradiol was shown to be a poor inducer when compared to 4-hydroxyestradiol (data not shown). This observation is consistent with the relatively stronger estrogenic activity reported for 4-hydroxyestradiol in other target tissues when compared to 2-hydroxyestradiol (39, 40).

Fig. 3. Effect of different steroidal estrogens (A) and nonsteroidal stilbene estrogens (B) at varying concentrations (0.01–100 nM) on proliferative outgrowth of kidney tubule cells grown on PF-HR9 basement membrane in serum-free chemically defined medium. All culture conditions are the same as described in Fig. 2. A, stimulation by steroidal estrogens 17β-estradiol (Ø), MOX (V), hydrolyzed Premarin (Y), 16α-hydroxyestrone (C), and 11β-methyleneestrol (B). B, stimulation by nonsteroidal stilbene estrogens DES (C), indenestrol B (Ø), and indenestrol (V).
that in human and animal studies, it is the unconjugated or “free” form of Premarin that is the hormonally active form because the conjugated form has been found to be hydrolyzed by target-tissue sulfatases (44, 45).

Therefore, it is possible that the disparity observed among some estrogens in the hamster kidney, as it concerns hormonal activity and the response to estrogen-dependent cell proliferation, may be due to the fact that elevation of serum PRL and renal PR (46) requires the expression of only a few genes, which may be more readily expressed by virtually all estrogens to varying degrees. However, cell proliferation elicited by most potent estrogens likely requires the expression of about 24 genes in a highly coordinated and concerted process (47, 48) which may not necessarily be elicited by all estrogens in a given tissue. The data presented in this study are consistent with recent evidence implicating estrogen-mediated cell proliferation as an early critical event in a multistage process of estrogen carcinogenesis in the hamster kidney (11, 12).

ACKNOWLEDGMENTS

We are grateful for the useful editorial comments of Valerie Hahn and for her assistance in the preparation of the manuscript.

REFERENCES


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