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ARTICLES

Properties and Regulation of 17β-Hydroxysteroid Oxidoreductase of OVCAR-3, CAOV-3, and A431 Cells: Effects of Epidermal Growth Factor, Estradiol, and Progesterone

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Abstract Although there is a growing body of evidence that 17β-hydroxysteroid oxidoreductase plays a role in the regulation of steroid levels in epithelial tumors of the endometrium and breast, our knowledge of its role in other gynecologic tumors is limited. In this investigation, the 17β-hydroxysteroid oxidoreductase activity of cell lines derived from two ovarian tumors (OVCAR-3, CAOV-3) and an epidermoid tumor of the vulva (A431) was assayed under conditions which differentiate between 17β-hydroxysteroid oxidoreductase type 1, a cytosolic isoform highly specific for estradiol, and type 2, a membrane bound isoform reactive with both estradiol and testosterone. On the basis of estradiol/testosterone activity ratios, all three cell lines appear to have type 2-like activity, with the specific activity of A431 markedly greater than that of the other cell lines. Estradiol, progesterone, or EGF, alone or in combination, were without effect on the enzymatic activity of OVCAR-3 cells. EGF decreased the activity of CAOV-3 cells slightly. In contrast, EGF stimulated A431 17β-hydroxysteroid oxidoreductase activity 7–8-fold over a 5-day exposure. Estradiol or progesterone, singly or in combination, also did not effect the enzymatic activity of A431 cells. However, progesterone inhibited the increase in activity seen in the presence of EGF. With EGF, estradiol, and progesterone together, the increase in enzymatic activity was comparable to that with EGF alone. The effects of estradiol and progesterone appear to result from steroid actions following binding of EGF to low-affinity receptors on A431 cells.

Key words: gynecologic tumor cells, cancer cells, steroids, steroid metabolism, hydroxysteroid dehydrogenase

17β-Hydroxysteroid oxidoreductase (17-HOR, E.C. 1.1.1.62) catalyzes pyridine nucleotide-dependent oxidation and reduction at the C-17 position of various C_{18} and C_{19} steroids, and by so doing can influence intracellular levels of the biologically active 17β-hydroxysteroids, estradiol (E2) and testosterone (T), and their relatively inactive 17-keto forms, estrone (E1) and androstenedione (A). There is now a large body of evidence not only that 17-HOR plays an important role in steroid-producing organs such as ovary, testis, and placenta, but that one or more isoforms may regulate steroid levels in steroid-responsive peripheral tissues and tumors as well [Reed, 1991].

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There is also evidence of an end-effect coupling between 17-HOR levels and the regulation of cell proliferation by E2 and progesterone (P) in some tissues. Early studies by Tseng and Gurpide [1974, 1975] showed that the level of 17-HOR activity in uterine endometrium was subject to regulation by P. Their findings led to the concept of an antiestrogenic action of P-induced 17-HOR whereby the conversion of E2 to E1, catalyzed by 17-HOR, terminates the proliferation-inducing action of E2 on cells of the glandular epithelium [Tseng et al., 1977]. Thus in endometrial cells, there is a coupling between the two E2-end effects, stimulation of cell proliferation and synthesis of P receptor. Evidence of a similar coupling of E2 and P end-effects on cell proliferation and 17-HOR activity in normal and neoplastic mammary tissue has been presented [Lübbert and Pollow,

1978; Fournier et al., 1985; Mauvais-Jarvis et al., 1987].

Recently, however, results from a number of laboratories have demonstrated the complexity of the 17-HOR enzymology of human breast glandular and adipose tissue, as well as breast tumors [Tait et al., 1989; Mann et al., 1991]. It has also been shown that 17-HOR of a number of cell lines derived from breast tumors can be modulated in vitro by various steroids and nonsteroidal growth factors [Adams et al., 1988; Malet et al., 1991; Couture et al., 1993]. These findings have raised questions about the general applicability of the uterine model to other normal tissues or tumors [Horwitz, 1993].

In particular, although E2 and P receptors and 17-HOR activity have been detected in various gynecologic tumors, a strong correlation between receptor content and 17-HOR activity is not readily apparent [Vierikko et al., 1983]. Additionally, information about interactions between E2, P, and growth factors, such as epidermal growth factor (EGF), in regulating the growth and differentiation of gynecologic tumors, aside from being very limited, is suggestive, in some cases, of a discordance among steroid end-effects.

Growth of the gynecologic tumor cell lines OVCAR-3 and CAOV-3 of ovarian origin and the A431 cell line derived from an epidermoid carcinoma of the vulva can be regulated by EGF and steroids [Gill and Lazar, 1981; Kawamoto et al., 1984; Kamata et al., 1986; Nash et al., 1989; Zhou and Leung, 1992], but there are also observations consistent with an uncoupling of steroid end-effects in these cell lines. For example, Nash et al. [1989] reported that although E2 stimulated the formation of P receptors in OVCAR-3 cells, it had no effect on cell growth.

This investigation was undertaken (1) to characterize the 17-HOR activity of OVCAR-3 cells, in which E2 stimulates the formation of P receptor but does not stimulate growth [Nash et al., 1989], CAOV-3 cells, which also contain receptors for E2, P, and EGF [Zhou and Leung, 1992], (Leung, unpublished data) and A431 cells, which over-express EGF receptor [Fabricant et al., 1977]; and (2) to test the applicability of the uterine model for 17-HOR function by examining the effects of E2, P, and EGF on 17-HOR activity with E2 and T.

MATERIALS AND METHODS

Materials

Reagents and supplies were purchased from the following sources: [6,7-^{3}H]E2 (1.5 TBq/mmol, 40 Ci/mmol), Amersham Corp., Arlington Heights, IL; [1,2-^{3}H]T (1.9 TBq/mmol, 52.5 Ci/mmol), Dupont NEN Products, Boston, MA; unlabeled steroids, Steraloids, Inc., Wilton, NH; Bicine (N,N-bis[2-hydroxyethyl]glycine), Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), bovine serum albumin, calf thymus DNA, *bis*-benzimide trihydrochloride (Hoechst 33258), insulin, mouse EGF, Sigma Chemical Co., St. Louis, MO; fetal bovine serum, Biologos, Naperville, IL; NAD, Boehringer Mannheim, Indianapolis, IN; silica gel HL plates, Analtech, Inc., Newark, DE; Ecolume, ICN Schwarz Mann, Cleveland, OH, NIH:OVCAR-3, CAOV-3, A431 cells, American Type Culture Collection, Rockville, MD.

Methods

Cell culture. Monolayer cultures of tumor cell lines were maintained in a humified incubator at 37°C with an atmosphere of 95% air and 5% carbon dioxide. Growth media were as follows: CAOV-3 and A431 (Dulbecco's modified Eagle's medium); OVCAR-3 (RPMI 1640). Fetal bovine serum was added to 10% for CAOV-3 and A431 cells and to 20% for OVCAR-3 cells. Penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), and Hepes (15 mM) were included in all media. Insulin was added (10 μg/ml) to the OVCAR-3 cultures as well.

Preparation of cell homogenates and sonicates. Cells were harvested from monolayers by gentle scraping and washed twice with Dulbecco's phosphate buffered saline. The resulting pellets were suspended in ice-cold buffer containing 20% (v/v) glycerol, 10 mM 2-mercaptoethanol and 40 mM potassium phosphate, pH 7.0, and homogenized by hand in an all-glass Dounce homogenizer. Alternatively, the suspensions were sonicated for 5 min at room temperature in a water-bath type sonicator (Heat Systems Ultrasonics, Farmingdale, NY). All preparations were stored at 4°C.

17-HOR activity. 17-HOR specific activity was measured as described previously [Blomquist et al., 1993]. Briefly, a 10 μl aliquot of sample was combined with 10 μl of reaction mixture containing 1.0 mM NAD and 2.0 μM

[^{3}H]E2 or [^{3}H]T in 0.08 mM Bicine, pH 9.0. Assays were run at 37°C for time intervals giving linear rates of product formation. At the end of the incubation, reaction mixtures were transferred in total to the preadsorbant layer of silica gel plates and, after the addition of unlabeled carriers, fractionated by thin layer chromatography with benzene:acetone (4:1) as the solvent. Steroids were located by a light spraying with water. The plates were air dried and steroid-containing spots scraped into 10 ml of Ecolume for liquid scintillation counting. Product formation was quantitated in terms of cpm in product as percent of total cpm in substrate and product and specific activity expressed as nmol product/mg protein or DNA · 30 min [Blomquist et al., 1984]. Modifications of this assay in particular experiments are indicated in the figure legends.

Protein and DNA quantitation. Protein was quantitated by the method of Markwell et al. [1981] with bovine serum albumin as the protein standard. DNA was quantitated fluorometrically by the method of Labarca and Paigen [1980]. Calf thymus DNA was used as the standard.

Data analysis. Multiple means were compared by analysis of variance in combination with the Newman-Keuls multiple comparisons test. When Bartlett's test indicated a non-Gaussian distribution of values, the Kruskal-Wallis non-parametric test was used. Paired means were compared by *t*-test. Apparent Michaelis constants and maximum velocities were estimated graphically by the method of Eisenthal and Cornish-Bowden [1974], as described previously [Blomquist et al., 1984].

RESULTS

17-HOR Specific Activity of OVCAR-3, CAOV-3, and A431 Cells

Human ovary contains two forms of 17-HOR, which are also present at high levels in placenta [Blomquist et al., 1994]. One, 17-HOR type 1, is a cytosolic activity highly specific for E2 and E1, with a low affinity for T [Tremblay et al., 1989]. A second activity, associated with microsomes, is reactive with both E2 and T [Blomquist et al., 1984; Pittaway et al., 1983; Barbieri, 1992]. This latter form of the enzyme is referred to as 17-HOR type 2 [Wu et al., 1993]. Aside from differences in subcellular localization, the two enzyme forms differ markedly in their E2/T activity ratios. When assayed at pH 9.0 with E2 or T at 1.0 μM, the E2/T activity ratio for purified 17-HOR type 1 is approximately 200, that for 17-HOR type 2 of washed microsomes is approximately 2 [Blomquist et al., 1984, 1985]. The assay used in this investigation specifically differentiates between the two forms on the basis of this difference. As shown in Table I, activity with both E2 and T was detected in all three cell lines. Activity with E2 or T of A431 cells was greater than that of either CAOV-3 or OVCAR-3 cells. However, the E2/T activity ratios for the three cell lines did not differ significantly and are comparable to the ratios for other 17-HOR type 2-containing tissues [Blomquist et al., 1985, 1994].

Effects of E2, P, and EGF on 17-HOR Activity

Exposure of uterine, proliferative-phase endometrium to P in vitro results in an approximately 10-fold increase in 17-HOR type 2-like activity within 48–72 h [Tseng, 1978]. A stimulatory effect of P on 17-HOR activity of normal breast epithelial cells in culture has been reported [Prudhomme et al., 1984]. In contrast with those observations, when CAOV-3, OVCAR-3, and A431 cells were exposed to E2 (10 nM) or P (5.0 μM), alone or in combination, at steroid levels shown previously to stimulate ovarian cancer cell proliferation and P receptor formation [Nash et al., 1989; Wimalasena et al., 1992] or the 17-HOR activity of endometrial tissue in organ culture [Tseng, 1978], we were unable to detect any statistically significant changes in 17-HOR activity over 5-day incubations.

In a previous investigation in this laboratory, it was observed that EGF stimulated cell growth and DNA synthesis in OVCAR-3 cells but inhibited both growth and DNA synthesis in CAOV-3

TABLE I. 17-HOR Activities of CAOV-3, OVCAR-3, and A431 Cells

	17-HOR activity[a]		
Cell line	E2	T	E2/T
CAOV-3	0.13 ± 0.01	0.11 ± 0.04	1.24 ± 0.27
OVCAR-3	0.05 ± 0.03	0.03 ± 0.01	1.79 ± 0.09
A431	4.04 ± 0.53	2.50 ± 0.51	1.64 ± 0.15

[a]The values for specific activity (nmol/mg protein · 30 min) and the E2/T ratio are the mean ± SE of 2–3 experiments in which triplicate cultures were combined and assayed in duplicate.

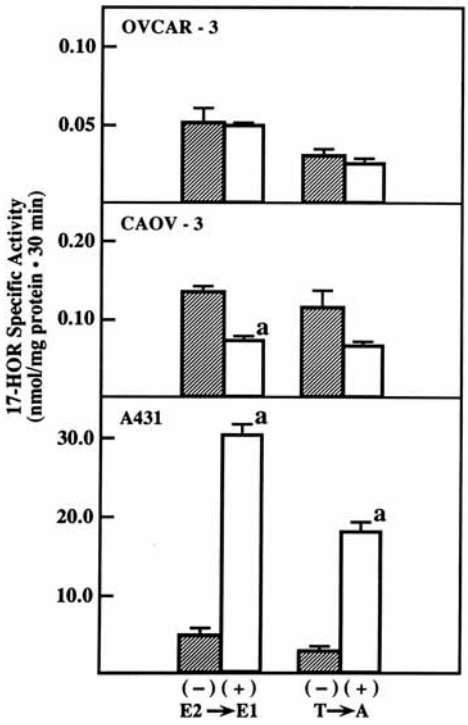

Fig. 1. Effect of EGF exposure on 17-HOR specific activity. Log-phase cultures were exposed to EGF (10 ng/ml) for 120 h. Triplicate cultures were combined and assayed in duplicate. The values are the mean ± SE of 2–3 separate experiments. $^{a}P < 0.001$, different from cultures not exposed to EGF.

cells [Zhou and Leung, 1992]. It has also been shown that EGF at low concentrations stimulates the proliferation of A431 cells but inhibits cell growth at higher concentrations [Gill and Lazar, 1981; Kawamoto et al., 1984; Kamata et al., 1986]. On the basis of this variation in EGF effects, as well as the steroid receptor content of these cell lines, it was felt to be of interest to examine the effect of EGF alone and in combination with steroids on 17-HOR activity.

Exposure of OVCAR-3 cells to EGF for up to 5 days had no effect on 17-HOR activity. In similar experiments with CAOV-3 cells, small decreases in activity with E2 and T were noted. In marked contrast, exposure of A431 cells to EGF at 10 ng/ml stimulated 17-HOR activity 7- to 8-fold during a 5-day period (Fig. 1). Stimulation required levels of EGF (5–10 ng/ml) shown previously [Gill and Lazar, 1981; Kamata et al., 1986] to inhibit proliferation of A431 cells (Fig. 2).

In time-course experiments, little change in 17-HOR activity was detected during the initial 16 h of exposure. However activity began to increase at approximately 16 h, and increased 3- to 4-fold by 48 h (Fig. 3).

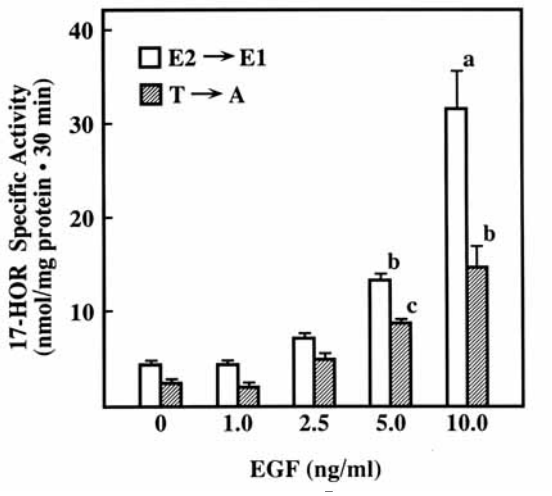

Fig. 2. Dependence of A431 17-HOR on EGF levels. Triplicate cultures were exposed to the indicated levels of EGF for 120 h, combined, and the homogenates assayed for activity with E2 and T as indicated under Materials and Methods. The values are the mean ± SE of duplicate assays; $^{a}P < 0.001$, $^{b}P < 0.01$, $^{c}P < 0.05$, different from cultures not exposed to EGF.

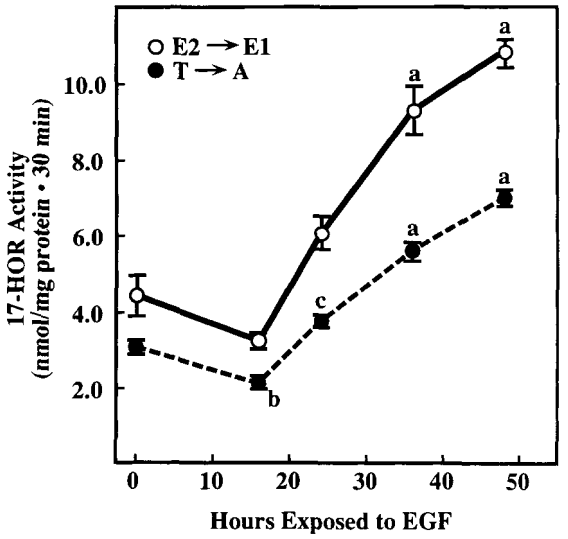

Fig. 3. 17-HOR activity of A431 cells exposed to EGF for different periods of time. Cells were cultured for 48 h. Over this time period, EGF (10 ng/ml) was added to the medium at intervals such that cells were exposed to the growth factor for 0 (EGF not added), 16, 24, 36, or 48 h. After the 48-h culture period, triplicate cultures at each exposure time were combined and assayed for 17-HOR activity with E2 and T. The values are the mean ± SE of duplicate assays with each substrate. $^{a}P < 0.001$, $^{b}P < 0.01$, $^{c}P < 0.05$, different from cells not exposed to EGF.

The delay in the increase in 17-HOR activity suggested enzyme biosynthesis was being affected rather than enzyme modulation by a mechanism such as a change in phosphorylation state. To examine this question further, cells

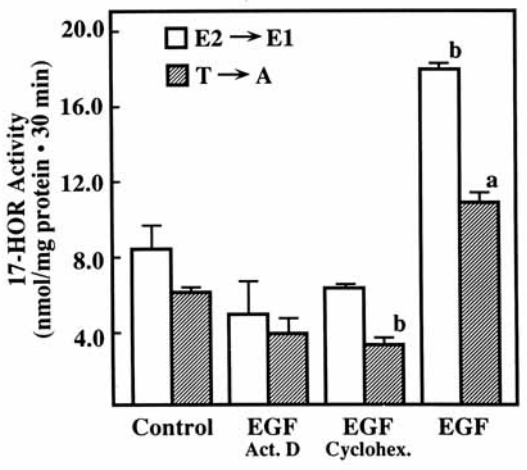

Fig. 4. Effects of actinomycin D and cycloheximide on 17-HOR activity of EGF-treated cells. Cells were cultured in triplicate for 48 h with or without EGF (10 ng/ml). When added, actinomycin D was at 1.0 μM and cycloheximide at 2.0 μM. The values are the mean ± SE of duplicate assays with each substrate. $^{a}P < 0.01$, $^{b}P < 0.05$, different from controls.

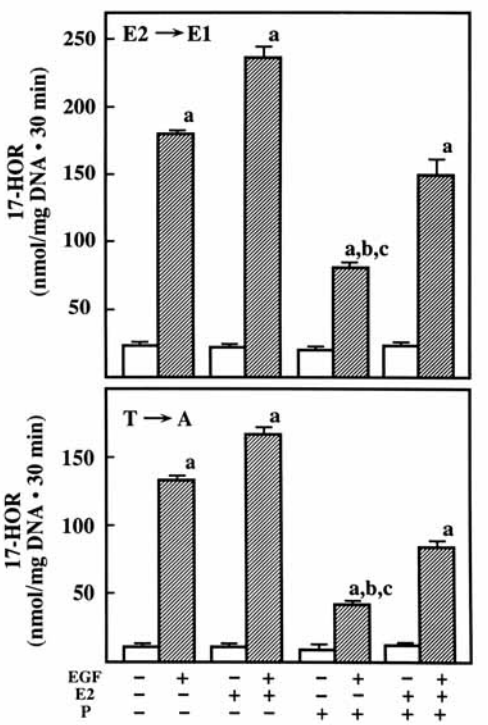

Fig. 5. Effects of EGF, E2 and P on 17-HOR activity of A431 cells. Cells were cultured in duplicate for 120 h in the presence or absence of EGF (10 ng/ml), E2 (10 nM), and P (5.0 μM), singly or in combination, as indicated. The values are the mean ± SE of duplicate assays with E2 and T. [a]Different from EGF(−), $P < 0.001$; [b]different from EGF(+), E2(−) and EGF(+), E2(+), $P < 0.001$; [c]different from EGF(+), E2(+), P(+), $P < 0.001$.

were exposed to EGF in the presence of actinomycin D or cycloheximide. As shown in Figure 4, the EGF-stimulated increase in 17-HOR specific activity was inhibited by both agents.

The possible roles of E2 and P as modulators of EGF action is a subject of much current interest. This led us to examine the possibility that E2 or P, singly or in combination, could either affect the susceptibility of OVCAR-3 or CAOV-3 cells to stimulation by EGF or modulate the magnitude of the change in 17-HOR activity in A431 cells. The lack of an effect of EGF on 17-HOR activity of OVCAR-3 cells and the decrease in activity in CAOV-3 cells were not influenced by E2 or P (data not shown), and as indicated above, the steroids had no effect on the 17-HOR activity of A431 cells. However, steroid effects were observed in cultures of A431 cells exposed to EGF (Fig. 5). E2 alone had a significant enhancing effect on the increase in 17-HOR activity seen in the presence of EGF. In contrast, P alone inhibited the increase by 50–70%. When both E2 and P were present, along with EGF, the increase in 17-HOR activity approximated that seen with EGF alone.

Properties of 17-HOR

An E2/T activity ratio of approximately 2 is characteristic of 17-HOR type 2 of ovary, endometrium, and placenta [Blomquist et al., 1984, 1985; Tseng et al., 1981]. The constancy of the ratio among the cell types suggested that all three lines contain a 17-HOR type 2-like activity. Because of the particularly high level in A431 cells, the 17-HOR activity of these cells was characterized further.

When A431 cells were fractionated by centrifugation into cytosol and a particulate fraction enriched in microsomes, over 90% of the total activity with either E2 or T was microsomal, characteristic of the type 2 enzyme (data not shown).

To examine the possibility that EGF might be stimulating the formation of an isoform of 17-HOR different from that in unstimulated cells, the Michaelis constants for E2 and T were determined for 17-HOR activity of cells cultured in the presence or absence of EGF. Sonicates were assayed in duplicate at pH 9.0 with 0.5 mM NAD and 0.5, 1.0, 1.5, and 2.0 μM E2 or T. As shown in Figure 6, maximum velocities for both substrates were increased with no change in Michaelis constants. Michaelis constants for E2 were 0.37 μM, minus EGF, 0.47 μM, plus EGF; for T the values were 0.75 μM, minus EGF, 0.68 μM, plus EGF. Maximum velocities (nmol/mg protein · 30 min) for E2 were 4.4, minus EGF, 31.8,

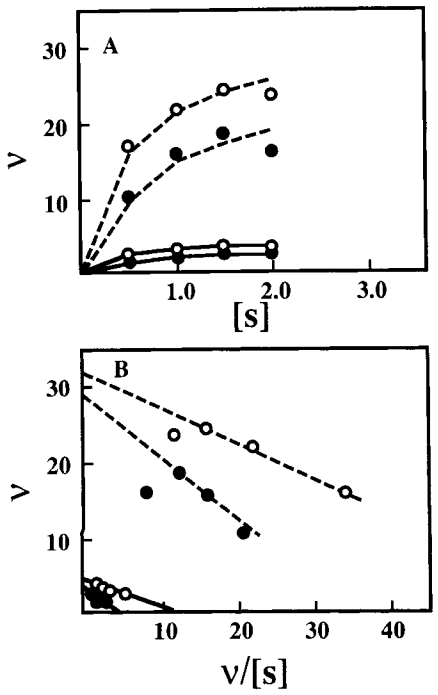

Fig. 6. Plots of (A) v vs. [S] and (B) v vs. v/[S] for A431 activity with E2 (*open circles*) and T (*filled circles*) of untreated cells (*solid line*) and cells exposed to EGF for 120 h (*dashed line*). The lines and intercepts were estimated by the linear graphical procedure of Eisenthal and Cornish-Bowden [1974] as indicated under Materials and Methods. The values are the mean of duplicate cultures combined and assayed in duplicate.

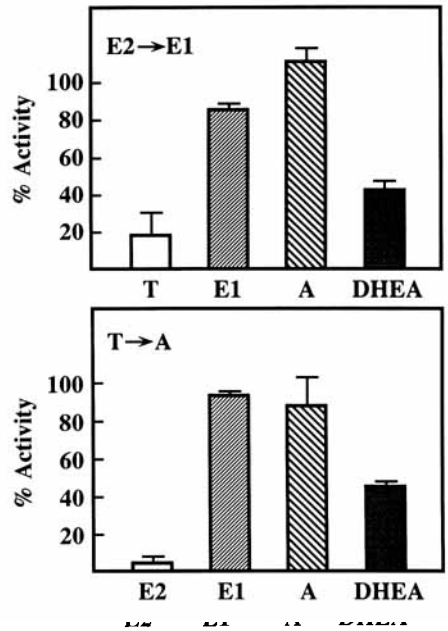

Fig. 7. Inhibition of A431 17-HOR of EGF-treated cells by various steroids. Reaction mixtures (20 μl) contained microsomes (3.2 mg protein/ml), 0.5 mM NAD, 1.0 μM ^{3}H-E2 or ^{3}H-T, and 100 μM unlabeled E2, T, E1, A, or DHEA. The values, shown as percent of controls without added steroid, are the mean ± SE of duplicate assays. Activities in the absence of putative inhibitors were 10.35 nmol/mg protein · 30 min with E2 as substrate and 5.07 nmol/mg protein · 30 min with T.

plus EGF; for T the values were 3.6, minus EGF, 27.0, plus EGF. There was no change in the E2/T activity ratio.

In contrast with 17-HOR type 1, which is reactive with both NAD and NADP, 17-HOR type 2 is highly specific for NAD [Wu et al., 1993]. When NAD and NADP were compared in assays of A431 cell sonicates, the Michaelis constant for NAD with 1.0 μM steroid substrate was 0.18 mM. Activity with NADP was at the limit of detection (data not shown).

Because of their differing substrate specificities, 17-HOR types 1 and 2 can also be differentiated on the basis of the ability of C_{19} 17β-hydroxysteroids such as T to inhibit reaction with E2 [Blomquist et al., 1984, 1985]. When A431 cell sonicates or microsomes were assayed with ^{3}H-E2 as substrate and the C_{19} steroids T, A, and dehydroepiandrosterone (DHEA) as putative inhibitors, activity was inhibited by T, but was insensitive to inhibition by the 17-ketosteroids, E1 and A. Similarly, activity with T was inhibited by E2 but not by E1 or A. A partial inhibition of activity with either substrate was observed with the 3β-hydroxy,17-ketosteroid (DHEA) (Fig. 7). This inhibitor specificity is characteristic of 17-HOR type 2 [Blomquist et al., 1984].

DISCUSSION

Ovarian cancer cell lines have been shown to respond to and produce a variety of growth factors [Berek and Martinez-Maza, 1994]. Information on their capacity for steroid production and metabolism is limited [Thompson et al., 1988; Wimalasena et al., 1991]. This investigation was designed to characterize the 17-HOR activity of OVCAR-3, CAOV-3, and A431 cells and to examine the applicability to these cell lines of gynecologic tumor origin of the uterine model in which P stimulates 17-HOR type 2 formation.

Three forms of 17-HOR have now been cloned and sequenced. 17-HOR type 1 is a soluble enzyme highly specific for E2 and E1 [Tremblay et al., 1989]. It occurs at high levels in E2-producing placental syncytiotrophoblast and granulosa cells of the ovary [Tremblay et al., 1989; Blomquist et al., 1994]. 17-HOR type 2 is a membrane-bound form reactive with E2, T, and 20α-

dihydroprogesterone [Wu et al., 1993]. It is particularly enriched in placental microsomes [Blomquist et al., 1994], and appears also to be the P-dependent form present in glandular epithelium of the endometrium [Tseng et al., 1981] and normal breast [Prudhomme et al., 1984]. 17-HOR type 3 is a reductase reactive with 17-ketosteroids and localized to the testis [Geissler et al., 1994].

The 17-HOR specific activity of OVCAR-3 and CAOV-3 cells (Table I) is comparable to that of primary ovarian tumors of epithelial origin [Vierikko et al., 1983] and to that of human ovarian stroma/theca [Blomquist et al., 1994], as is the E2/T activity ratio, suggesting that these cells contain a 17-HOR type 2-like activity. The lack of an effect of E2 or P alone or in combination suggests further that the uterine model is not applicable to these cell lines. Thus although 17-HOR may influence the relative levels of C_{18} and C_{19} 17β-hydroxysteroids and 17-ketosteroids within these cells, it appears unlikely that progestins or antiprogestins would be able to effect cell proliferation or differentiation by altering 17-HOR activity. However because the cell lines used in these studies are not primary cultures, the general applicability of this conclusion to primary ovarian or vulvar epithelial tumors will require further study.

In marked contrast with the ovarian tumor cell lines, A431 cells have a level of 17-HOR activity comparable to that of proliferative endometrium exposed to progestins. And after exposure to EGF, the level exceeds that reported previously for normal endometrium [Tseng, 1978]. The low E2/T activity ratio, comparable to that of CAOV-3 and OVCAR-3 cells, as well as the comparable Michaelis constants for E2 and T, the specificity for NAD, the low affinity for 17-ketosteroids, and the localization of activity to membrane fractions suggest A431 cells also contain 17-HOR type 2 or an isoform very similar to type 2 in its kinetic properties.

Although the 17-HOR type 2-like activity of A431 cells is similar in its properties to that in human endometrium [Tseng et al., 1981] and normal breast glandular epithelium [Prudhomme et al., 1984], both of which can be stimulated by progestins in vivo [Kokko et al., 1982] and in vitro [Tseng, 1978; Prudhomme et al., 1984], we were unable to detect an affect of either E2 or P, singly or in combination, on 17-HOR after exposure of cells to steroid for up to 5 days. It is of interest in this regard that endometriosis lesions, which are similar to endometrial glandular epithelium in many of their properties, and which have 17-HOR type 2-like activity at a level comparable to normal endometrium, do not show an increase in enzymatic activity in response to P or medroxyprogesterone acetate [Vierikko et al., 1985].

The basis for the marked stimulatory effect of EGF on the 17-HOR activity of A431 cells remains to be clarified. The A431 cell line was derived from an epidermoid carcinoma of the vulva. The cells are hypotetraploid with 78 chromosomes [Shimizu et al., 1984] and are particularly enriched in EGF receptors [Fabricant et al., 1977]. Cell proliferation is stimulated at concentrations of EGF of 0.1 ng/ml or less, while growth is inhibited by EGF at 1.0 to 10.0 ng/ml [Gill and Lazar, 1981; Kamata et al., 1986]. It has been proposed that this dual effect reflects the presence of two classes of receptors differing in affinity for EGF [Gill and Lazar, 1981; Kawamoto et al., 1984]. The findings of this investigation suggest the increase in 17-HOR results from occupation of the low affinity receptors.

The relatively slow time-course of the increase in 17-HOR, as well as its sensitivity to inhibition by actinomycin D and cycloheximide, are consistent with an indirect response not mediated by the rapid, tyrosine kinase-mediated events immediately following exposure to EGF. Our results suggest, instead, that exposure to EGF slowly induces a cellular state in which 17-HOR activity is subject to modulation by both P and E2. In these experiments the levels of E2 (10 nM) and P (5.0 μM) to which the cells were exposed, and the much lower levels which would be remaining in extracts of washed cells at the time of assay, are well below those generally observed to affect 17-HOR types 1 and 2 at the level of catalysis [Blomquist et al., 1978, 1984]. Our findings are more consistent with a mechanism in which the actions of E2 and P occur as part of a sequence of events affecting 17-HOR activity at the level of de novo mRNA and protein synthesis.

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REFERENCES

Adams EF, Coldham NG, James VHT (1988): Steroidal regulation of oestradiol-17β dehydrogenase activity of the human breast cancer cell line MCF-7. J Endocrinol 118: 149–154.

Barbieri RL (1992): Human ovarian 17-ketosteroid oxidoreductase: Unique characteristics of the granulosa-luteal cell and stromal enzyme. Am J Obstet Gynecol 166:1117–1126.

Berek JS, Martinez-Maza O (1994): Molecular and biologic factors in the pathogenesis of ovarian cancer. J Reprod Med 39:241–248.

Blomquist CH, Kotts CE, Hakanson EY (1978): Inhibition of human placental 17β-hydroxysteroid dehydrogenase by steroids and nonsteroidal alcohols: Aspects of inhibitor structure and binding specificity. Arch Biochem Biophys 186:35–41.

Blomquist CH, Lindemann NJ, Hakanson EY (1984): Inhibition of human placental 17β-hydroxysteroid dehydrogenase (17-HSD) activities of human placenta by steroids and non-steroidal hormone agonists and antagonists. Steroids 43:571–586.

Blomquist CH, Lindemann NJ, Hakanson EY (1985): 17β-hydroxysteroid and 20α-hydroxysteroid oxidoreductase activities of human placental microsomes: Kinetic evidence for two enzymes differing in substrate specificity. Arch Biochem Biophys 239:206–215.

Blomquist CH, Hensleigh HC, Block DL, Feeney LA (1993): Placental 17β-hydroxysteroid oxidoreductase, lactate dehydrogenase and malate dehydrogenase during the latter half of pregnancy in the mouse. J Steroid Biochem Mol Biol 46:61–67.

Blomquist CH, Bealka DG, Hensleigh HC, Tagatz GE (1994): A comparison of 17β-hydroxysteroid oxidoreductase type 1 and type 2 activity of cytosol and microsomes from human term placenta, ovarian stroma and granulosa-luteal cells. J Steroid Biochem Mol Biol 49:183–189.

Couture P, Theriault C, Simard J, Labrie F (1993): Androgen receptor-mediated stimulation of 17β-hydroxysteroid dehydrogenase activity by dihydrotestosterone and medroxyprogesterone acetate in ZR-75-1 human breast cancer cells. Endocrinology 132:179–185.

Eisenthal R, Cornish-Bowden A (1974): The direct linear plot: A new graphical procedure for estimating enzyme kinetic parameters. Biochem J 139:715–720.

Fabricant RN, De Larco JE, Todaro GJ (1977): Nerve growth factor receptors on human melanoma cells in culture. Proc Nat Acad Sci USA 74:565–569.

Fournier S, Brihmat F, Durand JC, Sterkers N, Martin PM, Kutenn F, Mauvais-Jarvis P (1985): Estradiol 17β-hydroxysteroid dehydrogenase, a marker of breast cancer hormone dependency. Cancer Res 45:2895–2899.

Geissler WM, Davis DL, Wu L, Bradshaw KD, Patel S, Mendonca BB, Elliston KO, Wilson JD, Russell DW, Andersson S (1994): Male pseudohermaphroditism caused by mutations of testicular 17β-hydroxysteroid dehydrogenase 3. Nature Genet 7:34–39.

Gill GN, Lazar CS (1981): Increased phosphotyrosine content and inhibition of proliferation in EGF-treated A431 cells. Nature 293:305–307.

Horwitz KB (1992): The molecular biology of RU486. Is there a role for antiprogestins in the treatment of breast cancer? Endocr Rev 13:146–163.

Kamata K, Chida K, Rikimaru K, Horikoshi M, Enomoto S, Kuroki T (1986): Growth-inhibitory effects of epidermal growth factor and overexpression of its receptors on human squamous cell carcinomas in culture. Cancer Res 64:1648–1653.

Kawamoto T, Mendelsohn J, Le A, Sato GH, Lazar CS, Gill GN (1984): Relation of epidermal growth factor receptor concentration to growth of human epidermoid carcinoma A431 cells. J Biol Chem 259:7761–7766.

Kokko E, Jänne O, Kauppila A, Vihko R (1982): Effects of tamoxifen, medroxyprogesterone acetate, and their combination on human endometrial estrogen and progestin receptor concentrations, 17β-hydroxysteroid dehydrogenase activity, and serum hormone concentrations. Am J Obstet Gynecol 143:382–388.

Labarca C, Paigen K (1980): A simple, rapid and sensitive DNA assay procedure. Anal Biochem 102:344–352.

Lübbert H, Pollow K (1978): Correlation between the 17β-hydroxysteroid dehydrogenase activity and the estradiol and progesterone receptor concentrations of normal and neoplastic mammary tissue. J Mol Med 3:175–183.

Malet C, Vacca A, Kuttenn F, Mauvais-Jarvis P (1991): 17β-Estradiol dehydrogenase (E2DH) activity in T47D cells. J Steroid Biochem Mol Biol 39:769–775.

Mann VZ, Newton CJ, Tait GH (1991): 17β-Hydroxysteroid dehydrogenase in human breast tissues: Purification and characterization of soluble enzymes and the distribution of particulate and soluble forms in adipose, non-adipose and tumour tissues. J Mol Endocr 7:45–55.

Markwell MK, Haas SM, Tolbert NE, Bieber LL (1981): Protein determination in membrane and lipoprotein samples: Manual and automated procedures. Methods Enzymol 72:296–303.

Mauvais-Jarvis P, Kuttenn F, Gompel A (1987): Antiestrogen action of progesterone in breast tissue. Hormone Res 28:212–218.

Nash JD, Ozols RF, Smyth JF, Hamilton TC (1989): Estrogen and anti-estrogen effects on the growth of human epithelial ovarian cancer in vitro. Obstet Gynecol 73:1009–1016.

Pittaway DE, Andersen RN, Coleman SA Jr, Giverns JR, Wiser WL (1983): Human ovarian 17β-hydroxysteroid oxidoreductase activity: A comparison of normal and polycystic ovarian tissues. J Clin Endocrinol Metab 56:715–719.

Prudhomme JF, Malet C, Gompel A, Lalardrie JP, Ochoa C, Boue A, Mauvais-Jarvis P, Kuttenn F (1984): 17β-Hydroxysteroid dehydrogenase activity in human breast epithelial cell and fibroblast cultures. Endocrinology 114: 1483–1489.

Reed MJ (1991): Oestradiol-17β hydroxysteroid dehydrogenase: Its family and function. J Endocrinol 129:163–165.

Shimizu N, Kondo I, Gamou S, Ali Behzadian M, Shimizu Y (1984): Genetic analysis of hyperproduction of epidermal growth factor receptors in human epidermoid carcinoma A431 cells. Somatic Cell Mol Genet 10:45–53.

Tait GH, Newton CJ, Reed MJ, James VHT (1989): Multiple forms of 17β-hydroxysteroid oxidoreductase in human breast tissue. J Mol Endocrinol 2:71–80.

Thompson MA, Adelson MD, Kaufman LM, Marshall LD, Coble DA (1988): Aromatization of testosterone by epithelial tumor cells cultured from patients with ovarian carcinoma. Cancer Res 48:6491–6497.

Tremblay Y, Ringler GE, Morel Y, Mohandas TK, Labrie F, Straus JF III, Miller WL (1989): Regulation of the gene for estrogenic 17-ketosteroid reductase lying on chromosome 17cen-q25. J Biol Chem 264:20458–20462.

Tseng L (1978): Steroid specificity in the stimulation of human endometrial estradiol dehydrogenase. Endocrinology 102:1398–1403.

Tseng L, Gurpide E (1974): Estradiol and 20α-dihydroprogesterone dehydrogenase activities in human endometrium during the menstrual cycle. Endocrinology 94:419–423.

Tseng L, Gurpide E (1975): Induction of human endometrial estradiol dehydrogenase by progestins. Endocrinology 97: 825–833.

Tseng L, Gusberg SB, Gurpide E (1977): Estradiol receptor and 17-beta dehydrogenase in normal and abnormal human endometrium. Ann NY Acad Sci 286:190–198.

Tseng L, Mazella J, Tseng L (1981): Kinetic studies of human endometrial hydroxysteroid dehydrogenase. J Steroid Biochem 14:437–442.

Vierikko P, Kauppila A, Vihko R (1983): Cytosol and nuclear estrogen and progestin receptors and 17beta-hydroxysteroid dehydrogenase activity in non-diseased tissue and in benign and malignant tumors of the human ovary. Int J Cancer 32:413–422.

Vierikko P, Kauppila A, Ronnberg L, Vihko R (1985): Steroidal regulation of endometriosis tissue: Lack of induction of 17β-hydroxysteroid dehydrogenase by progesterone, medroxyprogesterone acetate, or danazol. Fertil Steril 43:218–224.

Wimalasena J, Meehan D, Cavallo C (1991): Human epithelial ovarian cancer cell steroid secretion and its control by gonadotropins. Gynecol Oncol 41:56–63.

Wimalasena J, Dostal R, Meehan D (1992): Gonadotropins, estradiol, and growth factors regulate epithelial ovarian cancer cell growth. Gynecol Oncol 46:345–350.

Wu L, Einstein M, Geissler WM, Chan HK, Elliston KD, Andersson S (1993): Expression cloning and characterization of human 17β-hydroxysteroid dehydrogenase type 2, a microsomal enzyme possessing 20α-hydroxysteroid dehydrogenase activity. J Biol Chem 268:12964–12969.

Zhou L, Leung BS (1992): Growth regulation of ovarian cancer cells by epidermal growth factor and transforming growth factors alpha and $beta_1$. Biochim Biophys Acta 1180:130–136.