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ESTRADIOL BINDING AND METABOLISM IN HUMAN ENDOMETRIAL HYPERPLASIA AND ADENOCARCINOMA

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SUMMARY

The levels of estradiol receptors (E_2R), estimated from the amounts of E_2 tightly bound to nuclei after incubation of tissue with excess [^{3}H]-E_2, were high in normal proliferative endometrium and postmenopausal hyperplasia. Postmenopausal adenocarcinoma, well- or poorly-differentiated, showed lower levels in untreated patients and higher in patients who had taken Premarin. Significantly lower values were found in normal secretory endometrium and premenopausal adenocarcinoma, likely due to progestational effects. A decline in E_2R levels was seen after administration of Provera to postmenopausal patients with endometrial cancer.

The $E_2 17\beta$ dehydrogenase (E_2DH) activities, estimated from the rate of conversion of [^{3}H]-E_2 to [^{3}H]-estrone under substrate and NAD saturating conditions, were low in all specimens of hyperplastic and neoplastic endometrium from postmenopausal patients. These levels correspond to those of normal proliferative or postmenopausal tissue. Higher levels, typical of normal secretory endometrium, were found in the premenopausal, well-differentiated adenocarcinoma analyzed. Provera treatment of patients with endometrial carcinoma increased the levels of E_2DH.

The E_2R and E_2DH levels observed in patients with endometrial cancer can be explained on the basis of the hormonal environment of the tissue at the time of sampling (e.g., relative proportion of E_2 and progesterone) without postulating drastically altered binding and metabolism of E_2 in abnormal endometrium.

INTRODUCTION

A typical approach in cancer research is to compare biochemical parameters in neoplasms and in the normal tissue from which they originate. Human endometrium provides a particularly interesting target for this type of study since normal, hyperplastic and neoplastic tissue of various degrees of differentiation can be examined at different physiologic hormonal environments.

Estrogens influence not only the proliferation of normal endometrium but, under conditions of chronic stimulation in the absence of progesterone, they can also cause the development of endometrial hyperplasia and cancer. For instance, a high incidence of hyperplasia and adenocarcinoma is found after the menopause, in patients with estrogen-producing ovarian tumors, and in subjects receiving exogenous estrogens [1–4]. The particularly high incidence of endometrical carcinoma in obese, postmenopausal women has been related to an increased formation of estrogens derived from the adrenal precursor androstenedione [5, 6].

Previous *in vitro* work conducted in our laboratories on normal endometrium has provided us with background information on estradiol receptor (E_2R) levels [7] and estradiol 17β dehydrogenase (E_2DH) activities [8] against which we can compare similar measurements in abnormal endometrium. These parameters are relevant because they affect the intracellular concentrations of estradiol (E_2) and its biologic action. Furthermore, the studies on normal endometrium revealed an influence of progesterone on E_2R and E_2DH levels which needs also be investigated in hyperplastic and neoplastic tissue.

Aryl sulfatase is another endometrial enzyme which was considered worthy of measurement since changes in its activity might influence the intracellular levels of E_2 by regulating the conversion of circulating estrone sulfate, the estrogen in highest concentration in plasma [9], to estrone and estradiol.

The efficacy of progestin treatment in provoking remissions of endometrial adenocarcinoma in some patients [10, 11] makes the study of the effects of progesterone on estradiol receptors, estradiol dehydrogenase and aryl sulfatase, relevant to the problem of endometrial cancer.

Experimental

Estradiol receptor levels were measured by the method illustrated in Fig. 1. and reported elsewhere [7]. Slices of proliferative endometrium were incubated in glass vials with 2 ml of Earl's balanced salt solution (GIBCO) containing glucose (1 mg/ml) and 6,7-[^{3}H]-E_2 for 2 h at 37°C, with shaking. The tracer (48 Ci/mmol) was purchased from New England Nuclear Corp.; its purity was verified by mixing an aliquot with authentic E_2 and measuring specific activities after t.l.c. or crystallization. The initial concentrations of E_2 in the incubation medium (approximately 10^{-6} M) were reduced by no more than 50%

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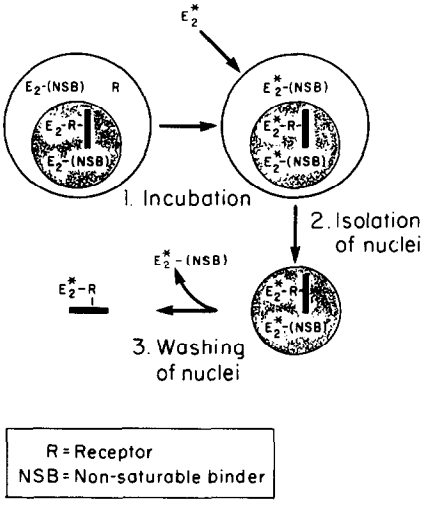

Fig. 1. Tissue slices were incubated with 10^{-6} M [^{3}H]-E_2 (E_2^*). Non-specifically bound [^{3}H]-E_2 (E_2^*-NSB) was removed from the nuclear pellet isolated after incubation, by washing with 0.1% Triton, in Tris–EDTA buffer once, and plain buffer, seven times. The [^{3}H]-E_2 bound to the chromatin was measured after chromatographic purification of the steroid. Results were expressed as pmol E_2/mg DNA.

during the incubations. The intracellular concentrations of [^{3}H]-E_2 at the end of the incubation were always higher than 20 pmol/mg of DNA. It has been shown that at these levels of E_2, available cytoplasmic receptors are translocated to the nucleus and the endogenous hormone is replaced by the exogenous [^{3}H]-E_2. After incubation, the tissue was rapidly washed with cold isotonic saline and homogenized at 0–4°C in Tris (10 mM) and EDTA (1.5 mM) buffer, pH 7.4, in a glass tissue grinder. Aliquots of the homogenate were taken and the rest was centrifuged at 800 *g* at 0–4°C. The nuclear pellet was washed several times by resuspension in fresh buffer, and transferred to another tube containing [^{14}C]-E_2 indicator and carriers of estrone and estradiol in methanolic solution. Labeled steroids were measured in each wash and in the final nuclear pellet, as well as in the samples of whole homogenate and incubation medium. The amounts of precipitated protein and DNA were determined by the method of Lowry[12] and Burton[13], respectively. The extracts were chromatographed on Silica Gel GF (Analtech) thin layer plates, using the system chloroform–ethyl acetate, 4:1 v/v. Radioactivity was measured with a liquid scintillation spectrometer (Isocap 300, Nuclear Chicago). Losses of E_2 were estimated from the ^{3}H/^{14}C ratio in the isolated E_2. Concentrations of labeled estradiol were calculated on the basis of the concentration of radioactivity and the S.A. of the incubated E_2. Results were expressed as pmol/mg prot or pmol/mg DNA (homogenate and washed nuclear pellet). The results on concentrations of estradiol receptors obtained by these methods were not affected by increases in the concentration of the incubated [^{3}H]-E_2 or by prolonging the time of incubation. When another portion of the same specimen was incubated with [^{3}H]-E_2 and excess diethylstilbestrol, a negligible amount of [^{3}H]-E_2 remained in the washed pellet, an indication that all non-specifically bound E_2 was removed during the washing procedures. When tritiated estriol [14] or tritiated ethylestradiol [15] were used instead of [^{3}H]-E_2, similar receptor levels were obtained. However, only 65% of the value obtained with estradiol was achieved during incubations with tritiated estetrol [15]. This finding was interpreted to indicate heterogeneity in the binding sites of E_2 in the nucleus.

The method used to measure E_2DH activity has been described in detail elsewhere [8]. Briefly, the 800 *g* supernatant from an endometrial homogenate (50 mM Tris buffer, pH 8, approx. 0.08 mg protein/ml) was mixed with NAD (1.4 mM) and ^{3}H-E_2 (3.5×10^6 c.p.m./ml, 37 μM) and kept at 37°C. Aliquots of the reaction mixture were taken at various intervals, between 0.5 and 8 min. The reaction was stopped with methanol containing [^{14}C]-E_1 and unlabeled E_1 and E_2 carriers. Duplicate aliquots of the assay mixture were taken to determine protein concentrations. Estrone and estradiol were separated using t.l.c. as described above. Losses were estimated from the recovery of the [^{14}C]-E_1 indicator added to the sample. The amount of E_1 formed from E_2 was calculated by dividing the amount of [^{3}H]-E_1 by the specific activity of the [^{3}H]-E_2 used as substrate. The rate of conversion of E_2 to E_1 was estimated from 5 values obtained at different incubation times and was expressed as nmol of E_1 formed/(mg protein × h).

In order to measure the aryl sulfatase activity, an aliquot of the 800 *g* supernatant of the endometrial homogenate was incubated at 37°C with 6×10^6 c.p.m./ml (12 μM) tritiated estrone sulfate (potassium salt) obtained from New England Nuclear. At different intervals between 0–15 min, 0.1 ml aliquots were taken, diluted to 1 ml with water containing [^{14}C]-E_1 and mixed with 10 ml of toluene-based counting solution (Econofluor, New England Nuclear). After vortexing, the mixture was frozen, the solvent transferred to another vial and washed with 1 ml of water. This operation was repeated until a constant ^{3}H/^{14}C ratio was achieved. From this ratio, the amount of tritiated estrone in the sample was calculated. The adequacy of this procedure to estimate the content of [^{3}H]-E_1 in the sample was verified by the more conventional method involving isolation of estrone by t.l.c.

RESULTS

Table 1 shows the results obtained by measuring estradiol levels in different types of endometrial tissue.

It can be seen that the levels of receptors are high in proliferative endometrium and decline during the secretory phase, as previously reported [7]. That the decline in the endometrial receptor levels in the luteal phase is due to the influence of progesterone was

Table 1. Estradiol receptor (E_2R) levels

Type of endometrium	n	E_2R Levels pmol/mg DNA $\bar{X}$	SD
Normal			
Proliferative	39	3.1	1.3
—, after progestin administration	9	1.5	0.7
Secretory			
Early (days 17–19)	6	1.6	0.5
Mid (days 20–23)	7	0.6	0.3
Late (days 24–28)	2	0.5	—
Hyperplastic (postmenopausal)	7	2.8	0.6
Adenocarcinoma (postmenopausal)			
Well-differentiated	17	2.3	1.1
—, exogenous estrogen users	4	3.8	0.8
Poorly-differentiated	3	2.0	1.5

shown by administering Provera to patients in the follicular phase of their menstrual cycle. The levels of estradiol receptors in endometrium taken before ovulation were lower than control values corresponding to proliferative endometrium [16].

Hyperplastic endometrium obtained from perimenopausal and postmenopausal patients showed estradiol receptor levels comparable to those of proliferative endometrium. Some of these patients were taking exogenous estrogens.

The specimens of endometrial adenocarcinoma from the postmenopausal patients studied showed, in average, estradiol receptor levels lower than those measured in proliferative endometrium but higher than those present in early secretory endometrium. No distinction could be made between the E_2R levels in well-differentiated and poorly-differentiated endometrium. A significant difference, however, was seen in the E_2R levels of well-differentiated endometrial adenocarcinoma from pateints receiving exogenous estrogens and from patients which were not under hormonal treatment. These observations indicate that estrogens may stimulate the synthesis of E_2R. An approx. normal distribution of E_2R concentration values in well-differentiated adenocarcinoma was noted. Three out of the 17 tissues studied (corresponding to about 18%) had receptor levels as low as those seen in early secretory endometrium.

Some patients with endometrial adenocarcinoma were studied before and after treatment with oral Provera at a dose of about 60 mg per day during 2 to 10 days. As shown in Fig. 2, 3 out of the 8 patients showed a clear reduction in E_2R levels in samples of endometrium taken before and after the treatment. No attempts at estrogen-priming, which may influence the concentration of progesterone receptors and the response of the tissue to exogenous progestins, were made in these studies.

Table 2 shows activities of estradiol 17β dehydrogenase measured in different types of endometrium. It can be seen that enzymatic activity in proliferative endometrium is low; a marked increase (approx. 14-fold) occurs during the secretory phase [8]. This increase can be attributed to the secretion of progesterone by the corpus luteum, on the basis of both *in vivo* and *in vitro* results. *In vivo*, oral administration of Provera during the follicular phase resulted in a clear increase in the estradiol dehydrogenase activities [17], as shown in Table 2. *In vitro*, induction of the enzyme was observed during incubations of proliferative endometrium for 2 to 3 days in medium containing progesterone, in concentrations as low as

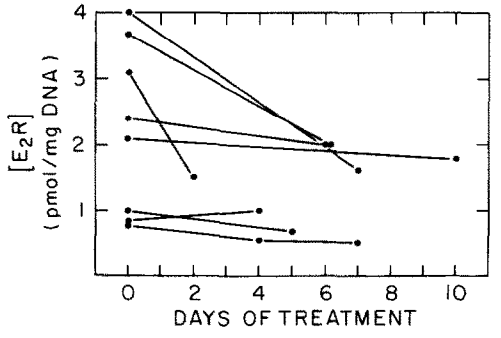

Fig. 2. Each patient was treated with Provera (60 mg/day) for the period of time indicated in the graph. Estradiol receptor levels were estimated in tissue specimens obtained before and after treatment.

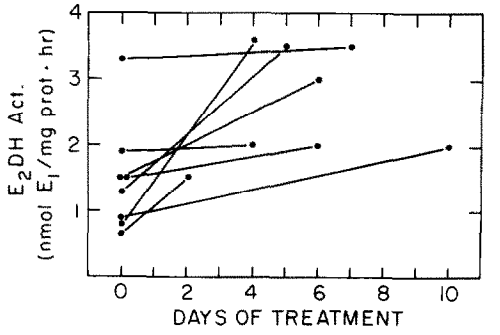

Fig. 3. Each patient was treated with Provera (60 mg/day) for the period of time indicated in the graph. Estradiol dehydrogenase was assayed in tissue specimens obtained before and after treatment.

Table 2. Estradiol 17β dehydrogenase (E_2DH) activities

Type of endometrium	n	E_2DH Activities nmol E_1 formed from E_2 mg prot × h $\bar{X}$	SD
Normal			
Proliferative	20	1.4	0.5
—, after progestin administration	5	4.7	2.0
Secretory			
Early (days 17–19)	6	17	5
Mid (days 20–23)	7	18	5
Late (days 24–28)	7	11	4
Hyperlastic (postmenopausal)	5	1.2	0.6
Adenocarcinoma (postmenopausal)			
Well-differentiated			
—, exogenous users	3	1.3	0.5
Poorly differentiated	3	1.2	0.4

20 ng/ml. This induction could be inhibited by addition to the medium of actinomycin D, cordecypin, puromycin or cycloheximide. Other progestational agents such as norgestrel, medroxyprogesterone, medroxyprogesterone acetate, R5020 (Roussel Uclaf), and norethynodrel were also effective inducers of the enzymatic activity, whereas estradiol, testosterone and cortisol were not [18].

The E_2DH activity in postmenopausal hyperplastic endometrium or adenocarcinoma was found to be as low as in proliferative endometrium, a result to be expected if progesterone were a necessary inducer of this enzymatic activity.

In vitro studies on the dynamics of estrone and estradiol in normal endometrium, previously reported [19], have shown that E_2 is almost exclusively converted to estrone, and that estrone leaves the tissue. Although estrone can be converted to estradiol, the interconversion between these two estrogens favors the oxidative direction. Under these conditions, an increase in estradiol dehydrogenase lowers the ratio of concentrations of estradiol in tissue and medium, and presumably, in tissue and plasma, under *in vivo* conditions. Therefore, one mechanism by which progestins may act as an antiestrogen is through an induction of estradiol dehydrogenase. Estrone, formed as a product of estradiol metabolism in tissue, may not have a direct estrogenic effect, as no chromatin-bound estrone–receptor complexes were found in human endometrium, even after incubations of tissue slices with labeled estrone [20].

The pattern of uptake and metabolism of estrone and estradiol in postmenopausal endometrial adenocarcinoma, corresponded to that of normal proliferative endometrium, e.g., E_2 was preferentially converted to estrone, and estrone, rather than estradiol, left the tissue. It can then be concluded that an increase in estradiol dehydrogenase in endometrial carcinoma will also result in a decline of estradiol intracellular concentrations.

Table 3 presents the results obtained by measuring aryl sulfatase activities in normal, hyperplastic, and neoplastic endometrium. These data show that the activity of the enzyme is approx. equal in all types of tissue studied. No changes in activity during the menstrual cycle were noted.

Treatment of patients with adenocarcinoma of the endometrium for 2 to 10 days with Provera (60 mg/day) altered the levels of endometrial E_2DH, as can be seen in Fig. 3. In 5 out of the 8 patients studied, a definite increase was noted.

DISCUSSION

The 3 parameters studied, i.e. E_2R levels, E_2DH and aryl sulfatase activities, had essentially the same values in endometrial adenocarcinoma, hyperplastic endometrium from postmenopausal subjects, and nor-

Table 3. Aryl sulfatase activity

Type of Endometrium	n	Aryl sulfatase activity nmol E_1 formed from E_1S mg prot × h $\bar{X}$	SD
Normal			
Proliferative	6	1.1	0.21
Secretory	5	1.0	0.26
Hyperplastic (postmenopausal)	1	1.2	—
Adenocarcinoma (postmenopausal)	3	1.1	0.75

mal proliferative endometrium. These tissues are exposed to an hormonal environment characterized by the absence of progesterone. Some patients with endometrial adenocarcinoma, however, had low estradiol receptor levels, as low as those found in secretory endometrium. Furthermore, some of these patients failed to respond to progestational treatment with an increase in estradiol dehydrogenase or a decrease in estradiol receptor levels, whereas all of the normal subjects in the follicular phase responded. This difference in responsiveness among specimens of endometrial carcinoma, which can also be inferred by the failure of progestin therapy in some patients, may be related to several factors, one of them being the tissue levels of progesterone receptors.

The levels of estradiol receptors in endometrial carcinoma are in general agreement with those reported by other workers [21–25] although we did not find higher E_2R levels in poorly-differentiated than in well-differentiated adenocarcinoma, as reported by others [24].

The effect of progesterone on estradiol dehydrogenase appeared to be specific for endometrium. Myometrium has low levels of these enzymatic activities which does not increase during the luteal phase or during pregnancy [18]. Histochemical studies conducted in our laboratories by Scublinsky and Marin[26] showed that the enzyme is localized in the glands of secretory endometrium. No enzymatic activity was detected by this method in the stroma of secretory endometrium or in either glands or stroma of proliferative endometrium and endometrial adenocarcinoma. The specificity of the regulation of the intracellular levels of estradiol by progesterone through the activity of estradiol dehydrogenase may explain the lack of mitotic activity in glands during the secretory phase, even at the luteal peak of estradiol plasma levels, whereas the second mitotic wave is observed in the stroma [27].

The results of elegant studies by Hagenfeldt and Landgren[28] showed a decrease in the tissue/plasma ratio of estradiol concentrations upon intrauterine delivery of progesterone. These results are in agreement with the effects of progesterone on endometrial E_2R levels and E_2DH activities proposed here.

Further development of clinical studies correlating efficacy of hormonal therapy for endometrial carcinoma with levels of estradiol and progesterone receptors, as well as responsiveness to progestins evaluated *in vivo* or *in vitro* by morphologic and biochemical criteria, are urgently needed.

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DISCUSSION

Munck. Have you checked to see whether the induction of a dehydrogenase is an activation of enzyme or new protein synthesis?

Gurpide. We do not see activation when adding progesterone to the enzyme assay system or during 2 h incubations with progestins; longer incubation periods are necessary to enhance the enzymatic activity. Actinomycin D, puromycin, cycloheximide and cordecypin are effective inhibitors of the inductive effect of progesterone on the estradiol dehydrogenase. We have not been able to obtain a more direct evidence for increased synthesis of the enzyme since no antibodies are available. Dr. Engel was kind enough to give us an antiserum against purified human placental estradiol dehydrogenase; we found that this antiserum does not cross-react with the solubilized endometrial enzyme under conditions that lead to complete neutraliz-

ation of equal amounts of placental estradiol dehydrogenase activity.

Pasqualini. In your system, is it possible to detect the action of the estradiol dehydrogenase on the estradiol complex itself. Is there the possibility of some transformation of the steroid while bound to the receptor.

Gurpide. I have no answer to your question, but I should mention that extensive conversion of estradiol to estrone can be observed even when more than 60% of the intracellular estradiol is bound to receptors in the nucleus, during superfusions of endometrium with very low concentrations of estradiol.