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SPECIFIC BINDING OF OESTRADIOL IN HUMAN UTERINE TISSUE

By

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ABSTRACT

Oestradiol receptors in human uteri were measured by the uptake-competition technique with tissue slices and by agar gel electrophoresis of soluble cytoplasmic oestradiol receptor complexes. The concentration of spare receptor was found to be markedly higher during the proliferative phase as compared to the secretory phase of the cycle and was inversely correlated to the concentrations of free oestrogen in the blood.

It is generally accepted that oestradiol requires binding to specific receptors in the target organ in order to exert its full biological activities. At present, a two-step mechanism of oestradiol fixation is proposed involving two different proteins (Gorski et al. 1968; Jensen et al. 1968, 1971; Jungblut et al. 1967, 1970). From recent experiments it is evident that the degree of oestrogen binding is dependent on the hormonal state of the animal (Eisenfeld & Axelrod 1966; McGuire & Lisk 1968; Whalen & Maurer 1969; Feherty et al. 1970; Lee & Jacobson 1971; de Hertogh et al. 1971). After the presence of specific oestrogen receptors had been demonstrated in human uterine tissue by Wyss et al. (1968), Meister et al. (1970), Wagner (1970, personal communication), Hähnel (1971), it was of interest to study whether the oestradiol binding capacity of the uterus varies at different stages of the menstrual cycle and during the postmenopausal period in relation to the plasma concentrations of...
endogenous oestrogen or of administered oestrogenic substances. It was hoped that the results of this study would provide guide lines for the characterization of target organ tumours by the assay of spare receptors.

MATERIAL AND METHODS

Chemicals

\([6,7-3H]17\beta\text{-oestradiol (spec. act. 48 Ci/mm)}\) and \([2,4,6,7-3H]17\beta\text{-oestradiol (spec. act. 100 Ci/mm)}\) were purchased from New England Nuclear Corp., Boston, Mass. The purity (> 94.0 %) was checked by thin layer chromatography. The anti-oestrogenic compound U. 11.100 (Nafoxidine) was provided by the Upjohn Company, Kalamazoo, Mich. All other chemicals were obtained from E. Merck AG, Darmstadt or Boehringer, Mannheim and were of analytical grade. Agar purum, human serum albumin and rabbit antiserum against human serum albumin were purchased from Behringwerke, Marburg (Lahn).

Processing of tissue

Human uterine tissue was placed on ice immediately after hysterectomy of Caesarian section. Tissue slices of 0.5 mm thickness were cut with a Stadie-Riggs-microtome within two hours after removal of the organ. Tissue specimens used for the preparation of extracts were immediately dissected and stored at ~20°C until processing.

Receptor assay by the uptake-competition technique (Jensen et al. 1967)

Uptake control: 14 tissue slices (5–10 mm in diameter) were incubated and stirred magnetically in 200 ml of a 10⁻¹⁰ M solution of \([6,7-3H]17\beta\text{-oestradiol in Krebs-Ringer NaHCO}_3 – 0.1 % \text{glucose buffer at 37°C. Uptake-competition: same conditions as in uptake control but with the addition of 10}^{-5} \text{ M Nafoxidine. (Substance dissolved in 0.5 ml glycerol, the same amount of glycerol was added to the uptake control.)}

Two tissue slices were removed from the incubation flasks at 15 min intervals (as indicated in Fig. 1), washed in cold buffer for 3 min, dried on filter paper, lyophilized and 2–3 mg aliquots were ashed in an oxygen atmosphere according to the procedure of Maurer (1968). After cooling the counting vials in a methanol-dry ice bath, the condensed tritiated water was dissolved in 10 ml of the scintillation fluid according to Hayes (1963) (7.0 g PPO, 0.3 g dimethyl-POPOP, 100 g naphthalene in 1000 ml dioxane). The actual radioactivity of the individual samples was measured in a Packard Tricarb scintillation counter, model 3880, and ranged from 800 to 3000 cpm. All counts were corrected to 100 % efficiency by external standardization.

Evaluation

The radioactivity of the samples (dpm/mg dry weight) from the incubations with and without Nafoxidine were plotted against time of incubation. (Scale: abscissa-time: 2 mm/min; ordinate - radioactivity: 10 mm = 100 dpm/mg dry weight). The area within the two curves was then measured with a planimeter (Ott, Kempten, Germany) and was expressed as dpm/mg dry weight. A typical example of a “positive” and a “negative” tissue specimen is given in Fig. 1. Only those cases were taken as “positive” for oestrogen receptors in which the area was greater than 25 cm². This borderline value was derived from the average plus one standard deviation of the dpm/mg dry weight values for “negative” tissue specimens.

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[6,7-3H]17β-oestradiol binding in slices of human uterine tissue (myometrium). Determination by the uptake-competition technique according to Jensen et al. (1967). With regard to oestrogen receptors, left: negative (area within the two curves 9.9 cm²); right: positive (area within the two curves 131.8 cm²).

Assay of spare cytoplasmic receptor

Preparation and incubation of uterine tissue extracts

The frozen tissue specimen (0.2–2.0 g) was pulverised with four volumes/weight of Tris-EDTA-buffer (0.01 M, pH 7.5, 0.001 M NaN₃) in a porcelain mortar, which was immersed in liquid nitrogen. After thawing, the sample was centrifuged in a SW 56 rotor (L 2 – 65 B, Beckman Instr.) at 2°C and 40 000 r. p. m. (157 000 × g) for 90 min. The supernatant was removed by pipetting and used immediately. One part of the extract was heated at 48°C for 60 min, the other part was kept at 2°C for the same time. [6,7-3H]17β-oestradiol was added to both samples to a final concentration of 10⁻⁹ M, and incubated for 120 min at 2°C. At the end of the incubation period, aliquots of both samples were subjected to gel electrophoresis.

Agar gel electrophoresis

The gel electrophoresis was performed according to the technique of Wagner (1970). Gel layers were prepared on a 85 × 100 mm glass slide with a 1% agar solution (0.05 M Michaelis buffer, pH 8.2). In the centre line of the gel plate, wells of 3.5 mm diameter were punched out and 20 µl aliquots of the labelled extracts were applied. The distance between wells was 3.5 mm and three wells were charged with material from one specimen. The prepared gel plates were then placed on a teflon coated brass plate within an airtight electrophoresis chamber (constructed at the Max-Planck-Institut für Zellbiologie, Wilhelmshaven, Germany). The plate was cooled to 1°C with methanol using a cryostat (Colora Messtechnik, Lorch, Germany). Electrophoresis
was carried out for 90 min at 110 mA/300 V. After the run, the gel was divided lengthwise (3 wells per strip) and then cut into 3 mm wide sections. Radioactivity was eluted from the strips with scintillation fluid according to Hayes (1963) for at least 20 hours before counting.

**Determination of tissue protein**

The total protein content of the extracts was determined according to Lowry et al. (1951). The amount of serum proteins was estimated by measuring the albumin content by means of a radial immunodiffusion test according to Ouchterlony (1949) as modified by Mancini et al. (1965) and Augustin & Hayward (1957). Total tissue protein content was then calculated by the formula used by Wagner (1971): tissue protein = total protein - serumalbumin × 100/60.

**Determination of plasma oestrogens**

Oestrogens were measured as total oestrogens without prior chromatographic separation. The method of Abraham (1969) and Mikhail et al. (1970) was used as modified by Lehmann et al. (1972).

a) *Antiserum.* – The antiserum was a gift of Dr. Vande Wiele, Columbia University, N.Y. The antiserum was diluted 1:30,000 and 0.1 ml of this dilution used per test tube. The specificity of the antiserum was investigated by adding various steroids to a solution containing labelled oestradiol and antiserum. The relative binding affinity was (oestradiol = 100 %) 40 % for oestrone, 0.1 % for oestradiol. Other steroids did not displace any labelled oestradiol up to 100 ng/test tube.

b) *Sample preparation.* – 1 ml of plasma was extracted twice with 10 ml of diethyl ether. The ether phases were transferred into another glass tube and the extract was dried under an air jet in a water bath at 38°C. The residue was re-dissolved in 0.6 ml phosphate buffered saline (9 g NaCl and 1 g NaN₃ were dissolved in 1000 ml 0.1 M phosphate buffer, pH 6.9). A 0.5 ml aliquot was taken for the assay.

c) *Radioimmunoassay.* – Each assay was performed in duplicate. Samples and oestradiol standards (20 to 1000 pg) were extracted and re-dissolved in phosphate buffered saline. 0.5 ml was added to assay tubes, followed by 0.2 ml 0.1 % gelatine, 0.1 ml (15 pg) [2,4,6,7-3H]oestradiol and 0.1 ml antiserum. All dilutions were made with phosphate buffered saline. After 2 h incubation at 4°C, bound and free tracer were separated by addition of 0.5 ml of gelatine-dextran-charcoal suspension (0.1 % - 0.025 % - 0.25 %) to each tube, followed by a brief agitation on a vortex mixer and standing for 45 min at 4°C. Tubes were then centrifuged for 45 min at 3500 r.p.m. (1200 × g) and 4°C (Christ Zeta 20) and the total supernatants (1.4 ml) containing the bound tracer were decanted into counting vials with 18 ml scintillation fluid. (Insta-Gel, Packard Instrument Comp.).

d) *Precision.* – The within assay precision (coefficient of variation) was ± 10.6 %. 10 replicate determinations of samples of 250 pg/ml were carried out. Between-assay precision was determined by carrying a sample from a low level pool (80 pg/ml) and from a high level pool (620 pg/ml) through 5 assays. The coefficient of variation for the first sample was ± 18.6 % and for the second ± 9.8 %.

e) *Recovery.* – The recovery of [2,4,6,7-3H]oestradiol from 10 samples was 82.4 ± 4.9 % (sp). The recovery of unlabelled oestradiol added to buffer or steroid-free plasma was estimated for the range of 50 to 700 pg per assay tube and was found to be 96.6 ± 11.3 %.
RESULTS

In a first series of experiments, we determined the binding of oestradiol by 190 specimens of either the myometrium only or of the myometrium and endometrium by the in vitro technique of Jensen et al. (1967). The results are listed in Table 1. In most cases the myometrium was used. Specific binding of oestradiol was observed in 75% of the myometrium specimens obtained from pre-menopausal patients and in 90% of the post-menopausal myometrium specimens. Oestradiol fixation by endometrium was found in all the post-menopausal uteri (6) and in 25 out of 28 pre-menopausal uteri. The myometrium of pregnant uteri at term completely lacked the ability to bind oestradiol. Out of 8 endometrial carcinomas (all patients were in the post-menopause), 6 gave evidence of the presence of oestrogen receptors.

Since a higher percentage of menstruating than of post-menopausal patients displayed no oestrogen binding, it was suggested that the day of the cycle on which the specimen was removed might influence the oestrogen binding capacity. Hence the oestradiol binding capacity was plotted against the day of the cycle on which the specimen was taken. As demonstrated in Fig. 2, the uptake of oestradiol is significantly reduced during the mid-cycle and the luteal phase as compared to the follicular phase. This difference is highly significant.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Oestradiol binding</th>
<th>No binding*</th>
</tr>
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<tbody>
<tr>
<td>Pre-menopause</td>
<td>M 102</td>
<td>76 = 75%</td>
<td>26 = 25%</td>
</tr>
<tr>
<td></td>
<td>E 28</td>
<td>25 = 89%</td>
<td>3 = 11%</td>
</tr>
<tr>
<td>Post-menopause</td>
<td>M 39</td>
<td>83 = 90%</td>
<td>4 = 10%</td>
</tr>
<tr>
<td></td>
<td>E 6</td>
<td>6 = 100%</td>
<td>0</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>M 7</td>
<td>0</td>
<td>7 = 100%</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>8</td>
<td>6 = 75%</td>
<td>2 = 25%</td>
</tr>
</tbody>
</table>

M – Myometrium.
E – Endometrium.
* – A DPM/mg dry weight < 25.
(P < 0.001) for the endometrium but myometrium specimens also displayed the same tendency (P < 0.5).

In a further series of 347 cases, we determined the oestradiol binding using the gel electrophoresis technique, a more specific and sensitive method (Wagner 1970). By this technique the 9 S and 4 S cytoplasmic transport factors can be determined. Both factors are extractable from the tissue of the target organs. They bind 3H-oestradiol specifically in vitro and are easily destroyed by heat. The unspecific oestradiol-albumin-complex is dissociated during electrophoresis and the free steroid is shifted towards the cathode. The oestradiol receptor complex migrates towards the anode. The radioactivity found at the anodical

![Graph](image)

[6,7-3H]17β-oestradiol binding in slices of human uterine tissue in relation to the day of the menstrual cycle on which hysterectomy was performed (determination of oestradiol binding by the technique of Jensen et al. 1967). The difference of oestradiol binding between the follicular and the luteal phase is significant for the myometrium (P < 0.5) and for the endometrium (P < 0.001). △ Endometrium. ● Myometrium.
peak represents the amount of bound oestradiol and is calculated as mol per mg of tissue protein of the extract. The mean values of these determinations obtained from patients at various stages of the menstrual cycle are shown in Fig. 3. The difference in oestradiol binding between the follicular and luteal phase becomes clearly evident. The values at mid-cycle and in the luteal phase are significantly lower than those obtained in the follicular phase. In view of these data, it was of interest to correlate the oestradiol binding capacity with the plasma levels of free oestrogens. Fig. 4 illustrates the values of oestradiol binding capacity in correlation to the plasma levels of free oestrogens as obtained at the time of hysterectomy. The receptor capacity in uterine tissue is lower in cases with a high plasma oestrogen level ($P < 0.5$). This tendency is especially pronounced when plasma oestradiol values exceed 300 pg/ml. These cases are either pregnant patients at term or post-menopausal patients who had been treated pre-operatively with ethinyloestradiol in doses of 3 to 9 mg per patient.
The data presented confirm the specific binding of oestradiol by the human uteri. The results obtained with tissue slices as well as those obtained with tissue extracts clearly indicate changes in the oestrogen binding capacity during the course of the menstrual cycle. A decrease in oestrogen binding is noted at mid-cycle and during the luteal phase.

These findings could be explained by "saturated" receptor sites, since the decrease in oestrogen binding capacity is closely correlated to the increase of endogenous oestrogen production. A correlation is clearly evident when rather high oestrogen concentrations are present in the blood, but disappears with oestrogen concentrations in the 100 pg/ml range. This might be due to methodological problems, e.g. submaximal saturation of available sites by the incubation with only $1 \times 10^{-9}$ M oestradiol. (At present, no one-step method is available to determine the total content of specific receptor proteins in target organs. Usually, only the amount of spare "unoccupied" receptors is determined). All
uteri of pregnant patients at term and of oestrogen pre-treated patients with plasma oestrogen levels ranging from 350 to 2000 pg/ml contain minimal concentrations of spare receptor. In these cases, newly synthesized receptor is apparently quickly “used up” by the abundance of circulating hormone (Hughes et al. 1969; Jensen et al. 1971; Scarff & Gorski 1971).

Cyclic fluctuations in the spare receptor content of human uteri were also observed by Evans & Hähnel (1971) and by Robertson et al. (1971). While the results of the former are in good agreement with our own, the latter concluded from their data a maximal concentration of spare receptor in the late proliferative and early luteal phase of the cycle, an interpretation not supported by the earlier in vivo studies of Brush et al. (1967). In rats, both Feherty et al. (1970) and Lee & Jacobson (1971) found the lowest concentrations of spare receptor during pro-oestrus and the highest during oestrus.

From a clinical point of view, the oestrogen-dependent concentration of spare receptor in target organs has to be taken into consideration, when the receptor content of tumour biopsies is used to evaluate their hormone-dependence. This has been demonstrated for breast cancer (Maass et al. 1972) and also holds true for carcinoma of the uterus as has been shown in this study.

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REFERENCES


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