21354265

Request # 21354265 **NOV 28, 2006**

Mail To:

Fordham Health Sciences Library (OhioLINK#547)
Interlibrary Loan
3640 Colonel Glenn Highway
Dayton, OH 45435-0001

DOCLINE: Journal Copy EFTS Participant

Title:	Journal of steroid biochemistry
Title Abbrev:	J Steroid Biochem
Citation:	1984 Apr;20(4B):1057-60
Article:	Oestrogen binding proteins in the female genital t
Author:	Bergink EW;Kloosterboer HJ;van der Vies J
NLM Unique ID:	0260125 Verify: PubMed
PubMed UI:	6727351
ISSN:	0022-4731 (Print)
Publisher:	Pergamon Press, Oxford
Copyright:	Copyright Compliance Guidelines
Authorization:	barb
Need By:	N/A
Maximum Cost:	**$15.00**
Patron Name:	Glaser, Rebecca - TN: 102003
Referral Reason:	Lacking
Library Groups:	GMRRG
Phone:	1.937.775-4110
Fax:	1.937.775-2232
Email:	fill@www.libraries.wright.edu
Ariel:	130.108.121.58
Alternate Delivery:	Ariel,Email(PDF)
Comments:	**GMR-RL PLEASE ARIEL, ODYSSEY OR EMAIL IF POSSIBLE.**
Routing Reason:	Routed to OHUCIN in Serial Routing - cell 2
Received:	Nov 29, 2006 (08:36 AM EST)
Lender:	University of Cincinnati/ Cincinnati/ OH USA (OHUCIN)

Bill to: OHUDAC

Fordham Health Sciences Library (OhioLINK#547)
Interlibrary Loan
3640 Colonel Glenn Highway

J. steroid Biochem. Vol. 20, No. 4B, pp. 1057–1060, 1984
Printed in Great Britain.

0022-4731/84 $3.00 + 0.00

IV. Estriol and Cancer

OESTROGEN BINDING PROTEINS IN THE FEMALE GENITAL TRACT

E. W. BERGINK, H. J. KLOOSTERBOER and J. VAN DER VIES
Scientific Development Group, Organon International B.V., Oss, The Netherlands

Summary—We have estimated the binding characteristics of an oestrogen binding protein in the human vagina and in the human myometrium. The specificities of these binding proteins were analyzed in binding studies using competitors and ligands with different structural features representing the various elements considered to be of importance for binding. The specificity of an oestrogen binding protein in human myometrium was found to be similar to those of oestrogen binding proteins in human breast tumour tissue and in human MCF-7 cells. This specificity was also similar to those of oestrogen receptors in myometrium, endometrium and vagina of the rat. However, the specificity of an oestrogen binding protein in the vagina of postmenopausal patients was different. The vaginal oestrogen-binding protein displayed similar high affinities for 17β-oestradiol, 17α-oestradiol and oestriol but low affinity for diethylstilbestrol. The equilibrium dissociation constants for [^{3}H]oestriol and [^{3}H]17β-oestradiol were 4.3×10^{-10} M and 4.0×10^{-10} M respectively and the concentration of this protein varied between 30 and 170 fmol/mg vaginal cytosol protein. We conclude that the human vagina contains an oestrogen binding protein with characteristics different from those of oestrogen receptors present in myometrium and breast tumour tissue.

INTRODUCTION

Previous studies have shown similar specificities of various oestrogens for oestrogen receptors in human myometrium, human endometrium and human breast cancer tissues [1–6].

The ranking of competitive potencies of 17β-oestradiol, 17α-oestradiol, oestriol and oestrone was similar in these studies. The strongest competitive effect was always displayed by 17β-oestradiol, which is in agreement with its stronger uterotrophic potency and its stronger effect on the induction of progesterone receptors in human cancer cells than other oestrogenic compounds [7, 8].

However, 17β-oestradiol is not always the most powerful oestrogenic competitor. Oestrone can bind to an endometrial nuclear receptor from Rhesus monkeys with a higher affinity than that of 17β-oestradiol [9]. Results of competitive binding studies with the oestrogen receptor in human gingiva show that when [^{3}H]moxestrol was used as a ligand, oestriol was a stronger competitor than 17β-oestradiol [10]. Moreover, it has also been reported that the oestrogen receptors of eosinophils have a higher affinity for oestriol than for 17β-oestradiol [11].

Embryological evidence and the results of clinical studies and more recently, receptor binding studies show that like the human uterus, the human vagina, the trigone area of the human urinary bladder and the human urethra are target tissues for oestrogens [12, 13]. The short-acting oestrogens like oestriol are more effective in stimulating the vagina than the uterus; the dose-dependent stimulatory effect of oestriol on the endometrium and on liver protein synthesis is low compared to its effects on vaginal atrophy and urinary incontinence [14, 15]. In the light of the observed preferential vaginotrophic effect of oestriol, we have examined the relative binding affinity of various oestrogens for oestrogen binding sites in human myometrium, and human vagina with either [^{3}H]17β-oestradiol or [^{3}H]oestriol as ligand (set as 100%).

MATERIALS AND METHODS

The details of the procedure for receptor binding studies (saturation analysis and competitive binding studies) have been previously described [1].

Human myometrium and vaginal tissue were obtained from postmenopausal patients (age 60–82 years) who underwent surgery for nononcological disorders. All patients were treated with oestriol dihemisuccinate (Synapause®) (2 mg daily for at least 3 months; last administration 24 h prior to surgery).

RESULTS AND DISCUSSION

Determination of dissociation constants and concentration of cytoplasmic binding sites

Aliquots of the cytosol fraction of human myometrium and human vagina were incubated for 24 h at 4°C with increasing concentrations of [^{3}H]17β-oestradiol or the synthetic progestagen [^{3}H]Org 2058. We found high-affinity binding sites for 17β-oestradiol ($K_d = 1 \times 10^{-10}$ M) and Org 2058 ($K_d = 7 \times 10^{-10}$ M) in myometrial cytosol. There were 144 ± 37 (SEM) fmol/mg cytosol protein of oestrogen receptors and 470 ± 172 fmol/mg of progesterone receptors. Cytoplasmic binding of [^{3}H]17β-oestradiol

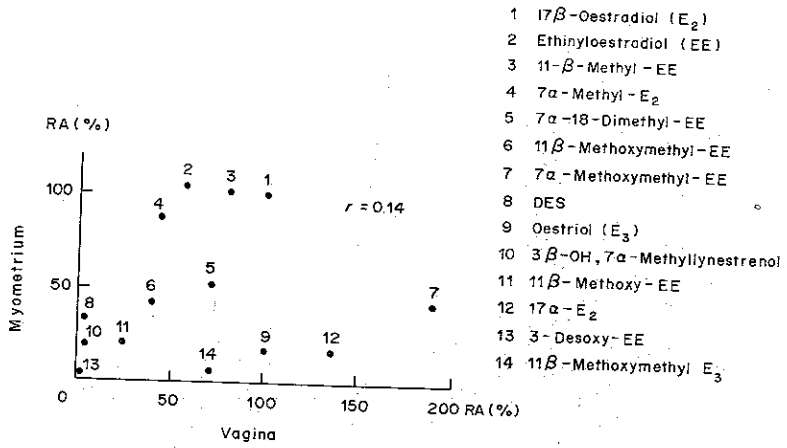

Fig. 1. Comparison of relative affinities for oestrogen binding proteins in human myometrium and vagina.

and [^{3}H]Org 2058 in cytosol from human vagina resulted also in the detection of high affinity binding sites for 17β-oestradiol ($K_d = 4.0 \times 10^{-10}$ M) and Org 2058 ($K_d = 9 \times 10^{-10}$ M). The concentration of binding sites for 17β-oestradiol and Org 2058 were 75 ± 26 fmol/mg cytosol protein and 63 ± 20 fmol/mg respectively. We also demonstrated in the cytosol fraction of human vagina a high affinity binding site for [^{3}H]oestriol with an equilibrium dissociation constant similar to that of 17β-oestradiol ($K_d = 4.3 \times 10^{-10}$ M).

The level of progesterone receptors in vaginal tissue of postmenopausal women pretreated with Synapause® (2 mg daily for at least 3 months) as found in the present study (63 ± 20 fmol/mg) was much higher than the values reported previously for progesterone receptors in the cytosol fractions from human vagina (< 5 fmol/mg) [12]. These investigators used tissues from premenopausal patients and from post-menopausal patients, who were either untreated or treated with a relatively low dose of 3×0.25 mg oestriol daily for 3 weeks. Our present results showing both oestrogen and progestagen binding sites in vaginal cytosol from women treated with Synapause® (2 mg daily) confirm that the human vagina is a target tissue for oestriol; priming with oestrogens may be a prerequisite for progesterone action as was found in other oestrogen target tissues.

COMPARATIVE *IN VITRO* BINDING AFFINITIES

The steroid specificities of the oestrogen binding sites in human myometrium and human vagina were examined by competitive binding studies between [^{3}H]17β-oestradiol and 14 reference compounds (Fig. 1). The reference compounds were selected on the basis of difference in structural features considered to be of importance for binding to the oestrogen receptor [6]. In a previous study we have compared the specificity of these compounds for the oestrogen receptors in human myometrium and human breast tumour and found an excellent correlation [6]. The coefficient of correlation (r) was 0.96 (Fig. 2) showing the similarity of these receptors. However, the results

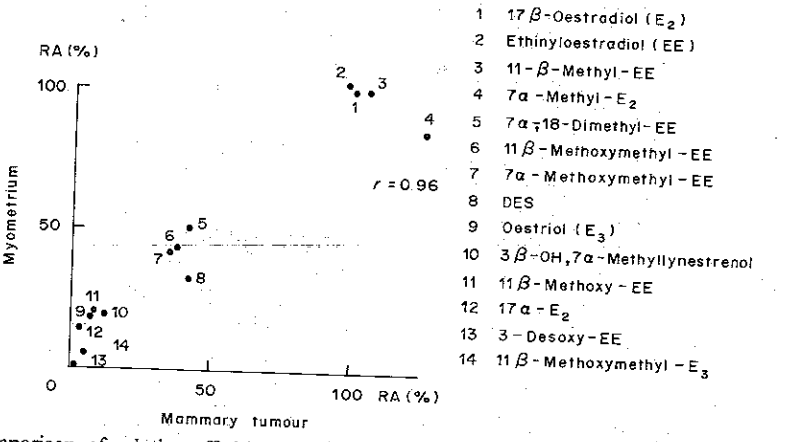

Fig. 2. Comparison of relative affinities for oestrogen binding proteins in human myometrium and mammary tumour.

in Fig. 1 show that there was no correlation between the specificities for the oestrogen receptor in myometrium and an oestrogen binding protein in human vagina.

This result is different from the observed similarity in the specificity of the same set of reference compounds for the oestrogen binding sites in vagina and myometrium from rat and rabbit [6]. When measured against binding by [^{3}H]oestriol (100%) the binding protein in the cytosol fraction of the human vagina was competed strongly for by 17β-oestradiol (80%), ethinyloestradiol (EE) (130%), 11β-methoxymethyl-oestriol (47%), oestrone (135%), and weakly by 7α-hydromethyl-EE (9%) and diethylstilbestrol (DES) (10%). The relative affinity of 7α-hydroxymethyl-EE (Org 3607) for the oestrogen receptor in human myometrium is less than 0.2% compared to 17β-oestradiol = 100% and diethylstilbestrol (DES) = 84%. To determine if the specificity of an oestrogen binding site in the human vagina for 7α-hydroxymethyl-EE and diethylstilbestrol was different from that of myometrium competitive inhibition was done on [^{3}H]17β-oestradiol binding. A double reciprocal plot revealed that [^{3}H]17β-oestradiol binding was inhibited by 7α-hydroxymethyl-EE (Org 3607; K_i = 7.1 nM) whereas inhibition of binding was much less with diethylstilbestrol (DES) (Fig. 3).

The present data demonstrate that an oestrogen binding protein in vaginal tissue from menopausal women on oestrogen medication (2 mg oestriol-succinate daily for at least 3 months) was different from the oestrogen receptor in human uterus. The origin of the oestrogen binding protein in vaginal tissue is uncertain. Several explanations for the observed differences in specificity are possible. Firstly, the binding protein is a receptor and oestrogen receptors found in different target tissues of the same species are of different origin. Secondly the binding protein is not a receptor but a tissue specific oestrogen binding protein resembling in this respect oestrogen binding proteins with high affinity for natural oestrogens and low affinity for stilbene derivatives as found in male rat liver [16], rat prostate [17] and human prostate [18]. Thirdly the binding protein is the classical oestrogen receptor as found in other oestrogen target tissues but the specificity of this receptor depends on its functional status. Several observations may also support this alternative. For example, the values reported in the literature for the relative affinities of 17β-oestradiol and oestriol and for the uterine receptor vary considerably [8]. It has also been found that a nuclear receptor in the endometrium of the Rhesus monkey interacted more strongly with oestrone than with 17β-oestradiol [9]. Moreover the processing of the oestrogen receptor in human MCF-7 cells has been shown to involve the formation of a receptor complex with a higher affinity for oestrone than for 17β-oestradiol [19].

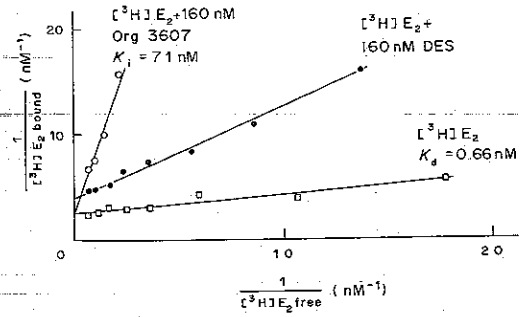

Fig. 3. Saturation analysis of oestrogen binding sites in human vaginal cytosol.

REFERENCES

1. Bergink E. W.: Oestriol receptor interactions: their biological importance and therapeutic implications. *Acta endocr., Copenh.* (Suppl.) **233** (1980) 9–16.
2. Makler A. and Eisenfeld A. J.: *In vitro* binding of ^{3}H-estradiol to macromolecules from the human endometrium. *J. clin. Endocr. Metab.* **38** (1974) 628–635.
3. Jänne O., Kontula K. and Vihko R.: Steroid binding properties of oestrogen receptors in human breast cancer. *Med. Biol.* **53** (1975) 214–223.
4. Hähnel R., Twaddle E. and Ratajzak T.: The specificity of the estrogen receptor of human uterus. *J. steroid Biochem.* **4** (1973) 21–31.
5. Katzenellenbogen J. A.: Comparative binding affinity of estrogen derivatives. *Cancer Treat. Rep.* **62** (1978) 1243–1249.
6. Bergink E. W., Kloosterboer H. J., van der Velden W. H. M., van der Vies J. and de Winter M. S.: Specificity of an estrogen binding protein in the human vagina compared with that of estrogen receptors in different tissues from different species, in Steroids and endometrial cancer (Edited by V. M. Jasonni, I. Nenci and C. Flamigni). Raven Press, New York (1983) pp. 77–84.
7. Jozan S., Kreitmann B. and Bayard F.: Different effect of oestradiol, oestriol, oestetrol and of oestrone on human breast cancer cells (MCF-7) in long term tissue culture. *Acta endocr., Copenh.* **98** (1981) 73–80.
8. Edwards D. P. and McGuire W. L.: 17β-Estradiol is a biologically active estrogen in human breast cancer cells in tissue culture. *Endocrinology* **107** (1980) 884–891.
9. Kreitmann-Gimbal B., Bayard F. and Hodgen G. D.: Changing ratios of nuclear estrone to estradiol binding in endometrium at implantation: Regulation by chorionic gonadotrophin and progesterone during rescue of the primate corpus luteum. *J. clin. Endocr. Metab.* **52** (1981) 133–137.
10. Vittek J., Hernandez M. R., Wenk E. J., Rappaport S. C. and Southren A. L.: Specific oestrogen receptors in human gingiva. *J. clin. Endocr. Metab.* **54** (1982) 608–612.
11. Tchernitichin A.: The role of eosinophil receptors in the non-genomic response to oestrogens in the uterus. *J. steroid Biochem.* **11** (1979) 417–424.
12. Van Haaften M., Wiegerinck M. A. H. M., Poortman J., Haspels A. A. and Thijsen J. H. H.: Progesterone receptors in human oestrogen target tissues. *Maturitas* **4** (1982) 57–66.
13. Saez S. and Martin P. M.: Evidence of estrogen receptors in the trigone area of human urinary bladder. *J. steroid Biochem.* **15** (1981) 317–320.
14. Bergink E. W., Crona N., Dahlgren E. and Samsioe G.: Effect of oestriol, oestradiol valerate and ethinyloestradiol on serum proteins in oestrogen-deficient women. *Maturitas* **3** (1981) 241–247.

15. Tzingounis V. A., Aksu F. and Greenblatt R. B.: Estriol in the management of the menopause. *J. Am. Med. Assoc.* **239** (1978) 1638–1641.
16. Miroshnichenko M. L., Smirnova O. V., Smirnov A. N. and Rozen V. B.: The unusual estrogen-binding protein (OEBP) of male rat liver: structural determinants of ligands. *J. steroid Biochem.* **18** (1983) 403–409.
17. Dubois R., Dubé J. Y. and Tremblay R. R.: Presence of three different estrogen binding proteins in rat prostate cytosol. *J. steroid Biochem.* **13** (1980) 1467–1471.
18. Hawkins E. F., Nijs M. and Brassine C.: Steroid receptors in the human prostate. 2. Some properties of the estrophilic molecule of benign prostatic hypertrophy. *Biochem. biophys. Res. Commun.* **70** (1976) 854–861.
19. Hansen E. R. and Brooks S. C.: Estrone receptor formation during the processing of estradiol–receptor complex in MCF-7 cells. *Cancer Res.* **42** (1982) 1967–1974.