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THE CYTOPLASMIC PROGESTERONE RECEPTOR OF HUMAN ENDOMETRIUM DURING THE MENSTRUAL CYCLE

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SUMMARY

The progesterone binding capacity of human endometrial 20,000 *g* supernatant has been determined during the menstrual cycle. Hyperplastic endometrium had a significantly higher progesterone binding capacity than proliferative or secretory phase endometrium ($P < 0.01$). The concentration of high-affinity binding sites was 2.3 ± 0.3 pmol/mg supernatant protein (mean ± S.E.) in the eight secretory phase endometrial supernatants studied and 4.3 ± 0.8 pmol/mg protein in five supernatants from hyperplastic endometrium. The concentration of endogenous progesterone in six secretory phase endometrial supernatants was 0.62 ± 0.15 pmol/mg supernatant protein (mean ± S.E.). In the five proliferative phase samples the concentration of endogenous progesterone was below the limit of detectability of the radioimmunoassay method used. The association constant of the endometrial progesterone receptor was $\sim 0.9 \times 10^9\ M^{-1}$ at +6°C when determined by equilibrium dialysis.

The relative affinity of seven synthetic progestins for the endometrial receptor was also determined. From this study it was concluded that an intact 3-keto-4-ene structure is essential for steroid binding to the receptor. Whereas the nature of the substituents at C-17 may be varied without considerable effect.

INTRODUCTION

Progesterone receptors are known to exist in the uteri of several mammals [1–3]. It has been shown that these receptors are subject to endocrine control as the progesterone binding capacity of myometrium varies during the estrous cycle [2, 4] and can be increased by estrogen priming [2, 5–7]. Estrogen receptors in human myometrium and endometrium have been investigated by several authors [8–13] whereas the nature of the progesterone receptor known to exist in the human endometrium [14, 15] is less well established.

Endometrium is a target organ whose functional state is highly dependant on estrogen and progesterone. The concentration of the oestradiol receptor in human endometrium varies during the menstrual cycle [9, 10, 13]. However, the concentration of the cytoplasmic progesterone receptor in human endometrium has not been measured during the different phases of the menstrual cycle.

The aim of the present study was to determine the amount of high-affinity progesterone binding in human endometrium during the menstrual cycle. The relative affinities of several synthetic progestins for this endometrial progesterone receptor was also studied.

MATERIALS AND METHODS

Chemicals

The chemicals used were the same as described previously [17]. Analytical grade petroleum ether, b.p. 30–60°C (Mallinckrodt Chemical Works, St. Louis, Mo., U.S.A.) was used without distillation. Analytical grade acetone, diethylether and ethyl acetate (Merck, Germany) were distilled before use. The preparation of tissue samples and the binding experiments were done in a medium containing 25 mM Tris–HCl, 1·25 mM EDTA and 250 mM sucrose, pH 7·4 (TES buffer).

Steroids

[1,2,6,7-^{3}H]-Progesterone (S.A. 81·1 Ci/mmol) was obtained from New England Nuclear Corporation, U.S.A. The purity of the radioactive progesterone was checked by t.l.c. (chloroform–acetone 9:1 v/v). The purity was 94–96% and did not change during use.

Progesterone (4-pregnene-3,20-dione), 17-hydroxyprogesterone (17-hydroxy-4-pregnene-3,20-dione) and oestradiol-17β (1,3,5(10)-oestratriene-3,17β-diol) were obtained from Ikapharm, Ramat-Gan, Israel. Testosterone (17β-hydroxy-4-androsten-3-one) was obtained from Steraloids, Inc., Pawling, N.Y., U.S.A. 20α-Hydroxy-4-pregnen-3-one was obtained from N.Y.

Organon, OSS, Holland. Cortisol was purchased from the Sigma Chemical Co., St. Louis, Missouri, U.S.A. Megestrol acetate (6-methyl-3,20-dioxo-4,6-pregnadien-17-yl-acetate) was obtained from BDH Chemicals Ltd., England and medroxyprogesterone acetate (6α-methyl-3,20-dioxo-4-pregnen-17-yl-acetate) from the Upjohn Co. Kalamazoo, Mich., U.S.A. Dr. Theodore Jackanicz (The Population Council, Biomedical Division, The Rockfeller University, N.Y., U.S.A.) kindly supplied the following synthetic steroids: norethisterone (17α-ethynyl-17β-hydroxy-4-estren-3-one), norethandrolone (17α-ethyl-17β-hydroxy-4-estren-3-one), norethynodrel 17α-ethynyl-17β-hydroxy-5(10)-estren-3-one), R-5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione), R-2323 (13-ethyl-17α-ethynyl-17β-hydroxy-4,9,11-gonatrien-3-one). We wish to thank Roussel-Uclaf (France) for the last two steroids mentioned. The purity of the non-radioactive standards was checked by g.l.c. on two different liquid phases. These standards were used without purification.

Tissue samples

The endometrium samples were obtained from the uteri of 31 patients undergoing hysterectomy, the indications for operation being endometriosis or uterine myomata. The patients were between 35 and 45-yr-old. The endometrium was scraped from the uterine cavity immediately after the removal of the uterus. The tissue was washed with cold 0·9% NaCl, a piece was taken for histological examination and the rest of the specimen was kept in an ice-box until processed, within 1 h.

Preparation of endometrium supernatants

The endometrial sample was weighed and then washed with ice-cold TES buffer pH 7·4 until all blood had been removed. The sample was homogenized in a glass homogenizer with a Teflon pestle using 1·0 ml of TES buffer per 100 mg of tissue wet weight. The homogenate was centrifuged at 20,000 *g* for 20 min at +2°C and the supernatant thus obtained was used in the progesterone binding studies. The supernatants were stored at −20°C. Binding studies were performed within 3 days of preparation of the samples.

Protein assay

Protein was determined using the method of Lowry *et al.* [16].

Equilibrium dialysis

The progesterone binding capacity of four endometrial supernatants was determined at +6°C by an equilibrium dialysis method the principles of which have been described in detail previously [15]. Albumin was added to the outer phase to reduce progesterone binding of low affinity and the dialyzing time was 19 h. The results obtained using the 19 h dialyzing period differed only minimally for those using a 40 h period and inactivation of the receptor at +6°C was avoided by employing the shorter 19 h dialysis. From the results obtained a Scatchard plot was constructed for the determination of the association constant.

Determination of progesterone binding capacity

The progesterone binding capacity of the remainder of the endometrial supernatant preparations was determined using a competitive protein binding assay (CPBA) method which has been described previously [17]. However, the following modifications were used: the incubation time at +6°C was 2 h and the reaction time with dextran-coated charcoal 10 min. The binding of [^{3}H]-progesterone was measured at a [^{3}H]-progesterone concentration of 0·2 nM in the absence and presence of 100 nM cortisol. Cortisol was added to eliminate binding by contaminating plasma corticosteroid binding globulin which was thought to be responsible for the progesterone binding abolished in part by the addition of cortisol. A cortisol concentration of 100 nM was used. This concentration was found to be optimal in reducing nonspecific binding without abolishing specific binding. The effect of cortisol on reducing the amount of 0·2 nM [^{3}H]-progesterone bound was not linear and only at a concentration of 200 nM (1000-fold excess relative to [^{3}H]-progesterone) did cortisol begin to compete with [^{3}H]-progesterone for the endometrial progesterone binding sites.

The binding at a [^{3}H]-progesterone concentration of 0·2 nM was measured at least in duplicate and for 19 of the 31 samples a CPBA curve was obtained by adding nonlabelled progesterone in increasing concentrations (from 1·7 nM to 1·7 μM) to the binding medium. Results are expressed as fmol of [^{3}H]-progesterone bound per mg of supernatant protein in the absence (total binding) and presence of 100 nM cortisol (specific binding). The binding site concentration of the supernatant was calculated by the reduced Scatchard plot method [8, 18].

The relative affinity of the progesterone receptor for various steroids was determined using the method of Korenman [19].

Radioimmunoassay (RIA)

The RIA method for progesterone determination was modified from a CPBA method [20]. Diethylether ethyl acetate (2:1, v/v) was used for extraction and progesterone was estimated by RIA instead of CPBA. The antibody (batch no. S.257 2) used was obtained from Dr. G. E. Abraham, University of California, Los Angeles, U.S.A. The concentration of progesterone in the endometrial 20,000 *g* supernatant was

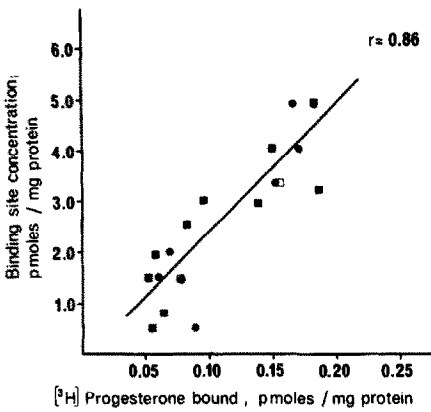

Fig. 1. Correlation between the two methods for measuring progesterone binding capacity of human endometrial 20,000 *g* supernatant. Ordinate: concentration of high-affinity binding sites determined from Scatchard plots as described in the text. Abscissa: binding of [^{3}H]-progesterone at a fixed steroid concentration of 0·2 nM. The protein concentration varied between 0·5–1·8 mg/ml. ●: [^{3}H]-progesterone 0·2 nM (total binding); ■: [^{3}H]-progesterone 0·2 nM and cortisol 100 nM (specific binding). The correlation coefficient has been calculated for the concentration of binding sites and the binding of [^{3}H]-progesterone in the presence of excess cortisol.

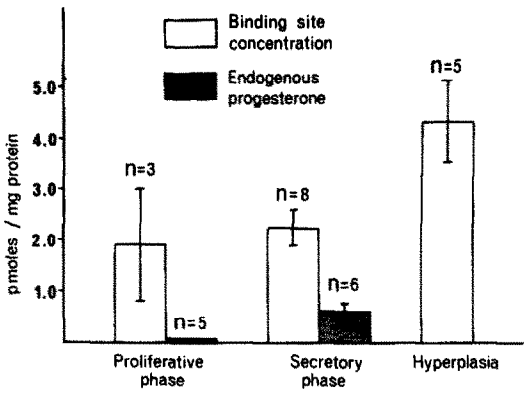

Fig. 2. The concentration of high-affinity binding sites for progesterone and concentration of endogenous progesterone in human endometrial 20,000 *g* supernatant during the menstrual cycle. The results are expressed as pmoles per mg of supernatant protein (mean ± S.E.). The progesterone concentration has been determined from different endometrium samples than the binding site concentration.

determined in different endometrium samples than the binding site concentration.

Measurement of radioactivity

Radioactivity was determined in a Wallac liquid scintillation counter 81,000 (LKB Wallac Inc., Turku, Finland) using Insta-Gel (Packard Instrument Co., Ill., U.S.A.) as the scintillation solution. The counting efficiency was 30%.

RESULTS

The capacity of individual samples of human endometrium 20,000 *g* supernatant to bind progesterone was measured using two different methods. The results are shown in Fig. 1. The correlation coefficient for the estimation of binding site concentration and the binding of 0·2 nM [^{3}H]-progesterone in the presence of excess cortisol was 0·86. Thus the correlation between the methods used was positive but not exact.

The amount of [^{3}H]-progesterone bound by endometrial supernatant is shown in Table 1. In the presence of 100 nM cortisol the amount of [^{3}H]-progesterone bound was reduced in half of the samples. The amount of high-affinity progesterone binding in the human endometrial supernatant could not be evaluated over the entire proliferative phase as only seven proliferative phase samples were obtained and of these three showed hyperplastic charactistics on histological examination. Hyperplastic endometrium showed a greater progesterone binding capacity per mg of supernatant protein than proliferative or secretory phase endometrium. The difference in binding capacity between secretory and hyperplastic endometrium was significant ($P < 0·01$) both in the absence and presence of excess cortisol.

In Fig. 2 progesterone binding in the different phases of the menstrual cycle is depicted as the high-affinity

Table 1. Binding of [^{3}H]-progesterone by human endometrium 20,000 *g* supernatant at a [^{3}H]-progesterone concentration of 0·2 nM in the absence (total binding) and presence of 100 nM cortisol (specific binding). The protein concentration varied between 0·5–1·8 mg/ml. The binding was determined with a CPBA method described in the text. The results are given as fmoles of [^{3}H]-progesterone bound per mg of supernatant protein; (mean ± S.D.)

	n	Total binding	Specific binding
Proliferative phase	3	86 ± 19	86 ± 19
Secretory phase	9	94 ± 32*	78 ± 42†
Hyperplasia	6	153 ± 26*	145 ± 23†

* $P < 0·01$.
† $P < 0·01$.

binding of [^{3}H]-progesterone at the 0·2 nM level The hyperplastic endometria contained the highest receptor concentration, 4·3 ± 0·8 pmol/mg protein (mean ± S.E.). The binding site concentrations in the proliferative and secretory phase supernatants were 1·9 ± 1·1 and 2·3 ± 0·3 pmol/mg protein, respectively. The difference in the binding site concentration between secretory and hyperplastic endometrium ($P < 0·05$) was not as significant as the difference in the binding of [^{3}H]-progesterone at the 0·2 nM level shown in Table 1.

The concentration of endogenous progesterone in the secretory phase supernatant preparations was always distinctly lower than the receptor concentration being 0·62 ± 0·15 pmol/mg protein (mean ± S.E.). The concentration of endogenous progesterone in all proliferative phase samples studied was below the limit of detectability of the RIA method used (0·16 pmol/mg protein).

The association constant of the progesterone receptor in human endometrium was 0·5 ± 0·3 × 10^9 M^{-1} (mean ± S.D.) when calculated from the results obtained using the CPBA and 0·9 ± 0·3 × 10^9 M^{-1} when calculated from the equilibrium dialysis measurements.

The endometrial supernatant prepared in TES buffer pH 7·4 was stable enough to stand freezing for at least 4 days. Under these conditions there was no loss of progesterone binding capacity. However, the receptor was not stable at +6°C and 35% of the binding capacity was lost during storage for 3 nights.

The steroid specificity of the receptor studied is outlined in Table 2. The low relative affinities of the physiological steroids tested emphasize the high specificity of the receptor for progesterone. Of the synthetic progestins studied, all, except norethynodrel had relative affinities greater than 70% (progesterone 100%). Norethynodrel has a nonconjugated double bond between carbon atoms 5 and 10 whereas all the other progestins studied have a 3-keto-4-ene structure in ring A. The side chains at C-17 may be varied to a much greater degree without affecting ligand binding to the endometrial receptor.

In the 19-nor steroids the progesterone side chain at C-17 could be replaced without effect by a 17α-ethynyl-17β-hydroxy group (norethisterone, R-2323) or by a 17α-ethyl-17β-hydroxy group (norethandrolone). The relative affinity of R-2323 was close to that of norethisterone. However, R-2323 has an additional methyl group at C-18 and two additional double bonds at C-9 and C-11. Pregnene derivatives methylated at C-6 with a 17α-acetate group were also bound by the receptor. The double bond at C-6 in megestrol acetate had no effect on binding. The 17,21-dimethyl derivative of 19-nor progesterone also had a high relative affinity for the receptor.

DISCUSSION

In the present study the capacity of human endometrial 20,000 *g* supernatant to bind progesterone was estimated as the high-affinity binding site concentration and as the ability of the supernatants to bind [^{3}H]-progesterone at a concentration of 0·2 nM. The correlation between the methods was not exact, the correlation coefficient being 0·86. However, this finding was to be expected as the human endometrium samples are usually very scanty, and the number of determinations possible with each sample is limited. Combined samples could have been used but then denaturation of the receptor during storage could not have been assessed reliably.

Table 2. The relative affinity of the human endometrial progesterone receptor for various steroids. The values were determined as described in the text and ref. no. 19

Trivial name	Systematic name	Relative affinity (%)
Progesterone	4-Pregnene-3,20-dione	100·0
Norethisterone	17α-Ethynyl-17β-hydroxy-4-estren-3-one	100·0
Norethandrolone	17α-Ethyl-17β-hydroxy-4-estren-3-one	100·0
Medroxyprogesterone acetate	6α-Methyl-3,20-dioxo-4-pregnen-17-yl-acetate	83·0
Megestrol acetate	6-Methyl-3,20-dioxo-4,6-pregnadien-17-yl-acetate	80·0
R-5020	17,21-Dimethyl-19-nor-4,9-pregnadiene-3,20-dione	77·0
R-2323	13-Ethyl-17α-ethynyl-17β-hydroxy-4,9,11-gonatrien-3-one	73·0
17-Hydroxyprogesterone	17-Hydroxy-4-pregnene-3,20-dione	5·8
Norethynodrel	17α-Ethynyl-17β-hydroxy-5(10)-estren-3-one	4·0
Testosterone	17β-Hydroxy-4-androsten-3-one	2·5
20α-Dihydroprogesterone	20α-Hydroxy-4-pregnen-3-one	0·9
Cortisol	11β,17α,21-Trihydroxy-4-pregnene-3,20-dione	0·1
Oestradiol	1,3,5(10)-Oestratriene-3,17β-diol	0·1

Progesterone receptors in myometrium are known to be subject to endocrine control. Estrogen priming increases the myometrial receptor concentration in animals [2, 5–7]. The estrogen receptors of human myometrium and endometrium have also been reported to vary in concentration during the menstrual cycle [9, 10, 13]. According to Robertson *et al.* [10] the oestradiol receptor concentration is highest during mid-cycle. Limpaphayom *et al.* [9] found a higher receptor concentration on day 21 than on day 8 of the oculatory cycle whereas Trams *et al.* [13] have reported the concentration of the oestradiol receptor in human uterus to be higher during the proliferative phase than during the secretory phase of the cycle. These somewhat conflicting results can probably be explained on the basis of part saturation of the high-affinity binding sites in the tissue samples studied by endogenous oestradiol. The concentration of spare oestradiol receptors in the human uterus has been reported to be inversely correlated to the concentration of free estrogen in the blood [13].

In our studies, hyperplastic endometrium, a consequence of estrogen excretion, had a higher binding capacity than secretory or simple proliferative phase endometrium indicating that the progesterone receptor of human endometrium is under estrogen control as are the progesterone receptors in the myometrium of several mammals [2, 4–7].

The progesterone binding capacity of individual endometrial samples with a similar histological pattern showed great variation (Fig. 2). However, as the measurements in early and late proliferative and secretory phase have not been grouped separately, the range of values in each characteristic phase is probably not as wide as shown in Fig. 2. Using a different technique the progesterone binding capacity of endometrium has been shown to increase from early to late proliferative phase [15].

The endogenous progesterone in human endometrial 20.000 *g* supernatant was determined in the samples prepared carefully in the cold for the measurement of receptor content. After preparation in this way the concentration of endogenous progesterone in the samples was lower than the receptor content indicating that the binding sites were not saturated in the secretory phase endometrial supernatants. However, the endogenous progesterone concentration was still high enough to interfere with the binding studies in a few secretory phase samples thus leading to erroneously low levels of binding at a [^{3}H]-progesterone concentration of 0·2 nM. The complex situation of receptor determination in the presence of endogenous steroid has been discussed in detail recently by Milgrom *et al.* [21].

The endogenous progesterone in four human menstrual cycle endometrium homogenates has been measured by Nilsson [22]. The present values are similar if expressed as pg/mg of protein.

The relative displacement potency of various endogenous steroids for the progesterone receptor in human endometrium has been studied by Wiest and Rao [14]. In agreement with their findings, the relative affinities of the physiological steroids tested in the present study were low reflecting the high specificity of the receptor for progesterone.

From the relative affinities of the synthetic progestins tested it could be concluded that the 3-keto-4-ene structure of these steroids was essential for steroid binding to the endometrial progesterone receptor. The side chain at C-17 of the progesterone molecule is not essential for ligand binding and can be replaced by serveral substituents. In addition to the other progestins, norethandrolone, which has strong anabolic and weak progestational activity [23], is bound by the receptor as well as progesterone.

In the present study human endometrial supernatant was used without purification. This endometrial preparation seems to be a suitable model for the study of the binding properties of synthetic steroids in an attempt to find steroids which bind to the receptor without exerting the biological activity of the physiological hormone. In endometrium such blockage of the receptor would probably lead to impairment of implantation or to premature menstruation. With such a mechanism of action the synthetic steroid would be behaving as an interceptive, a term proposed by Warren [24].

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REFERENCES

1. McGuire J. L. and DeDella C.: *Endrocinology* **88** (1971) 1099–1103.
2. Feil P. D., Glasser S. R., Toft D. O. and O'Malley B. W.: *Endocrinology* **91** (1972) 738–746.
3. Milgrom E., Perrot M., Atger M. and Baulieu E.-E.: *Endocrinology* **90** (1972) 1064–1070.
4. Milgrom E., Atger M., Perrot M. and Baulieu E.-E.: *Endocrinology* **90** (1972) 1071–1078.
5. Corvol P., Falk R., Freifeld M. and Bardin C. W.: *Endocrinology* **90** (1972) 1464–1469.
6. Saffran J., Loeser B. K., Haas B. M. and Stavely H. E.: *Biochem. biophys. Res. Commun.* **53** (1973) 202–209.
7. Faber L. E., Sandmann M. L. and Stavely H. E.: *J. biol. Chem.* **247** (1972) 8000–8004.
8. Méšter J., Robertson D. M., Feherty P. and Kellie A. E.: *Biochem. J.* **120** (1970) 831–836.

9. Limpaphayom K., Lee C., Jacobson H. I. and King T. M.: *Am. J. Obstet. Gynec.* **111** (1971) 1064–1068.
10. Robertson D. M., Měšter J., Beilby J., Steele S. J. and Kellie A. E.: *Acta endocr., Copenh.* **68** (1971) 534–542.
11. Henderson S. R. and Schalch D. S.: *Am. J. Obstet. Gynec.* **112** (1972) 762–771.
12. Rubin B. L., Gusberg S. B., Butterly J., Han T. C. and Maralit M.: *Am. J. Obstet. Gynec.* **114** (1972) 660–669.
13. Trams G., Engel B., Lehmann F. and Maass H.: *Acta endocr., Copenh.* **72** (1973) 351–360.
14. Wiest W. G. and Rao B. R.: In *Advances in the Biosciences, Schering Workshop on Steroid Hormone "Receptors"* (Edited by G. Raspé), Vol. 7. Pergamon Press, Vierveg (1970) pp. 251–264.
15. Haukkamaa M., Karjalainen O. and Luukkainen T.: *Am. J. Obstet. Gynec.* **111** (1971) 205–210.
16. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: *J. biol. Chem.* **193** (1951) 265–275.
17. Haukkamaa M.: *J. steroid Biochem.* **5** (1974) 73–79.
18. Sandberg A. A., Rosenthal H., Schneider S. L. and Slaunwhite W. R. In *Steroid Dynamics* (Edited by G. Pincus, T. Nakao and J. F. Tait). Academic Press, New York (1966) pp. 36–39.
19. Korenman S. G.: *Endocrinology* **87** (1970) 1119–1123.
20. Haukkamaa M., Luukkainen T. and Timonen H.: *Ann. clin. Res.* (1974) In press.
21. Milgrom M., Luu Thi M. and Baulieu E.-E.: In *Protein Synthesis in Reproductive Tissue* (Edited by E. Diczfalusy). Karolinska Symposia on Research Methods in Reproductive Endocrinology. 6th Symposium. Bogtrykkeriet Forum. Copenhagen (1973) pp. 380–397.
22. Nilsson L.: *Acta obstet. gynec. scand.* **51** (1972) 117–120.
23. Gibian H. and Unger R.: In *Die Gestagene* (Edited by K. Junkmann). Springer-Verlag, Berlin, Heidelberg, Vol. 1 (1968) p. 360.
24. Warren J. C.: *Biol. Reprod.* **8** (1973) 259–274.