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A SPECIFIC PROGESTERONE RECEPTOR OF MYOMETRIAL CYTOSOL FROM THE RHESUS MONKEY

DOREEN V. ILLINGWORTH*, CARLENE ELSNER†, KATE DE GROOT DE LA CRUZ‡, GEORGE L. FLICKINGER and GEORGE MIKHAIL
Endocrine Section, Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, PA, U.S.A.

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SUMMARY

A specific progesterone receptor of rhesus monkey myometrial cytosol is described. Characterization of the receptor by sucrose density gradient centrifugation showed two peaks at 4 *s* and 7.5 *s*. The 4 *s* peak seen in all groups (castrate; castrate + estrogen treated; castrate + estrogen and progesterone treated) contained little specific progesterone binding but the 7.5 *s* peak, seen only in the estrogen treated animal, was specific for progesterone. Competition studies showed the receptor affinities to be P > 5αP > melengestrol acetate norgestrel > DOC. There was little competition from corticosteroids and 5βP, 17αOHP. Receptor levels, measured from Scatchard plot analysis of equilibrium data, were 7.0 ± 4.2 fmol/mg cytosol protein (castrate), 45.2 ± 3.7 (E-treated), 10.5 ± 5.3 (E + P treated). The K_A was similar in all three groups and was approximately 5×10^{-9} M.

INTRODUCTION

The initiation of progesterone (P) action through interaction of the hormone with specific receptor proteins in target tissue cells has been described in several species [1–13]. This study characterizes the progesterone cytosol receptor of the myometrium of the rhesus monkey in different hormonal states. The methods were those routinely used in the laboratory for the characterization and identification of the progesterone receptor of human myometrial cytosol [14].

EXPERIMENTAL

Chemicals. [1,2-^{3}H]-progesterone (44 Ci/mmol) was purchased from New England Nuclear and was checked periodically for radiochemical purity. Non-radioactive steroids were obtained from Schwarz–Mann. The synthetic§ progestagens were generously provided, norgestrel by Wyeth Laboratories and melengestrol acetate by Upjohn Company. All other chemicals were of reagent grade.

Tissue. Myometrium was taken from three groups of monkeys: (a) castrate (C); (b) castrate treated with estrogen (E); (c) castrate treated with estrogen and progesterone (E + P). The details of the animal treatment and tissue preparation are described elsewhere [15]. Myometrium was the only tissue available in sufficient quantity to study progesterone receptor activity.

Preparation of cytosol. The preparation of the cytosol, using Tris glycerol (TG) buffer (50 mM Tris, 250 mM sucrose, 1 mM EDTA, 12 mM monothioglycerol, 30% glycerol, pH 7.4 at 4°C), progesterone cytosol receptor assay and sucrose density gradient centrifugation were carried out as previously described [14]. Endogenous steroids were routinely removed from the cytosol preparations by pre-treatment with dextran-coated charcoal [14]. For the progesterone cytosol radio-receptor assay, cortisol (approximately 10 nM) was included in the incubation in order to eliminate possible interference from CBG.

Competition study. Cytosol (0.2 ml) was incubated with 0.2 ml TG buffer containing 2000 d.p.m. [^{3}H]-progesterone (0.1 nM) and 10 nM competing steroids for 18 h at 4°C. Only one concentration of competing steroid could be used because of the small vol. of cytosol and the limited number of animals available.

RESULTS

Sucrose gradient analysis. Analysis was carried out on one castrate, one estrogen (E) treated, and one estrogen + progesterone (E + P) treated animal (Figs. 1–3). In the castrate and E + P treated animals a single 4 *s* peak of radioactivity was seen. In the castrate animal this peak was abolished by both cortisol

* Present Address: Department of Chemical Pathology, University of Leeds, Leeds, England.
† Present Address: Department of Obstetrics and Gynecology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC U.S.A.
‡ Present Address: Laboratory of Clinical and Experimental Endocrinology, Universitat-Eppendorf, Hamburg, West Germany.

§ The following trivial names are used: 5αP, 5α-pregnanedione = 5α-pregnane-3,20-dione; 5βP, 5β-pregnanedione = 5β-pregnane-3,20-dione; 17α OHP, 17α-hydroxyprogesterone = 17α- hydroxy-pregn-4-en-3,20-dione; DOC, desoxycorticosterone = 21-hydroxy-4-pregnene-3,20-dione; Norgestrel = DL-17α-ethynyl-13β-ethyl-17β-hydroxygon-4-en-3-one; Melengestrol acetate = 17α-acetoxy-6-methyl-16-methylene-pregn-4,6-diene-3,20-dione.

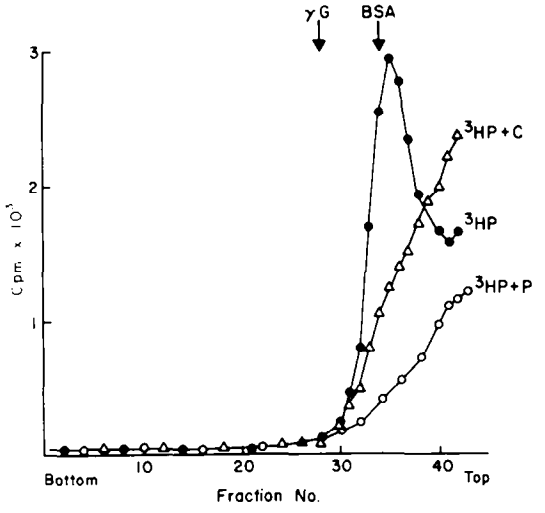

Fig. 1. Distribution of [^{3}H]-progesterone after sucrose gradient centrifugation of myometrial cytosol obtained from a castrated rhesus monkey. Cytosol was incubated in the presence of 10^{-9} M [^{3}H]-progesterone (^{3}HP) and either 10^{-7} M cortisol (^{3}HP + C) or progesterone (^{3}HP + P). Arrows indicate peaks of C^{14}-labelled rabbit gamma globulin (G) and bovine serum albumin (BSA).

and progesterone, while in the E + P treated animal the [^{3}H]-progesterone was displaced to a greater extent by progesterone than by cortisol. In the E-treated animal, not only was the amount of bound radioactivity much greater, but two peaks could be distinguished, at 4 and 7.5 *s*. Both peaks were abolished by the addition of excess progesterone, but when cortisol was used as the competitor, the 7.5 *s* peak was unaffected, only the 4 *s* peak was extensively reduced.

Competition studies. The combined results from three animals with significant specific high-affinity progesterone binding in the radio-receptor assay (two

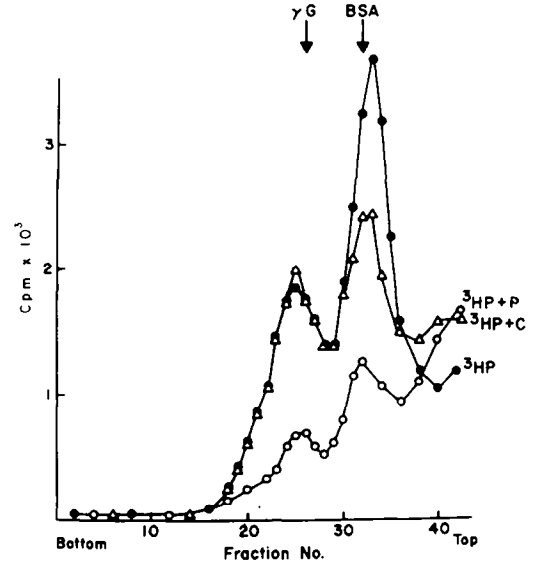

Fig. 2. Sucrose gradient analysis of progesterone binding by myometrial cytosol of a spayed monkey treated with oestrogen (see Fig. 1 for details).

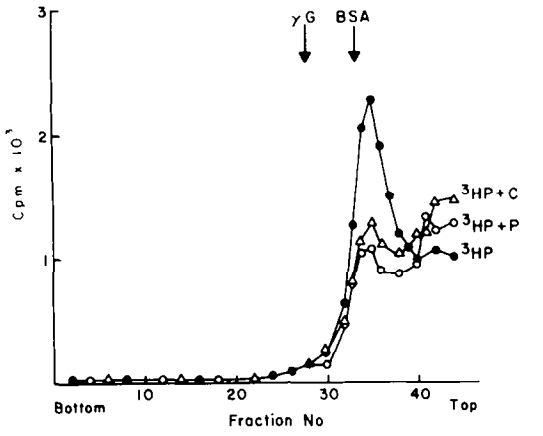

Fig. 3. Sucrose gradient analysis of progesterone binding by myometrial cytosol of a spayed monkey treated with estrogen and progesterone (see Fig. 1 for details).

E-treated, one E + P treated), are presented in Table 1. Because of the limited vol. of cytosol available, only five steroids could be studied in duplicate in one animal; some steroids (cortisol, corticosterone, progesterone, 5α-pregnanedione) were examined in more than one animal. The competing steroids were present at 100 × concentration of [^{3}H]-progesterone. The affinities agreed closely, and the mean value for each steroid is given. The corticosteroids, except deoxycorticosterone (26%) had virtually no competition at the steroid level examined. 5α-pregnanedione was an effective competitor, while the 5β isomer had no affinity for the receptor. The synthetic progestagens, norgestrel and melengestrol acetate had slightly lower affinities than 5α-pregnanedione.

Levels of progesterone receptor. The concentrations and affinity constant measurements of the progesterone receptor are shown in Table 2. Control experiments similar to those previously carried out [14] showed that equilibrium had been established after 18 h incubations and that the total number of receptor sites were measured. In 2/4 castrate and 1/3 E + P treated animals no high affinity binding could be distinguished. The amount of receptor present in both these groups was very low (7.0 and 10.5 fmol/mg cytosol protein). The E-treated animals, however, had considerably higher receptor concentrations (45.2 fmol/mg protein; $P = <0.01$), indicating the

Table 1. Relative steroid affinity for the cytosolic myometrial progesterone receptor in the rhesus monkey

Steroid	Relative Affinity
Progesterone	100
5α-dihydroprogesterone	81.9
Melengestrol Acetate	72.5
Norgestrel	53.0
Desoxycorticosterone	25.9
5β-dihydroprogesterone	1.2
17-hydroxyprogesterone	< 1
Corticosterone	< 1
Cortisol	< 1

Table 2. Progesterone receptor concentrations in myometrial cytosol

Treatment	No. of Animals	Plasma Concentration* Estradiol Pg/ml	Plasma Concentration* Progesterone Ng/ml	Binding Site Fmol/mg Prot.	$K_a \times 10^{-9}$ M
Castrate	4	11 ± 1	0.3 ± 0.05	7.0 ± 4.2	7.4 ± 0.6
E-Treated	4	62 ± 13	0.5 ± 0.02	45.2 ± 3.7	3.3 ± 0.5
E + P Treated	3	79 ± 26	1.8 ± 0.40	10.5 ± 5.3	6.1 ± 0.4

* Mean ± S.E.M.

stimulatory effect of estrogen. There was no correlation between the level of estradiol receptor and progesterone receptor in myometrium from the same animal (Fig. 4). The estradiol receptor levels were higher in all three groups (100–350 fmol/mg protein).

Measurement of nuclear receptor sites was also attempted using the exchange assay method described by Anderson *et al.*[16], but with the addition of 10% glycerol to the Tris–EDTA buffer. Three incubation conditions were tried, 37°C for one h, 20°C for two h and 4°C overnight. However, binding was extremely low and appeared both non-saturable and non-specific.

DISCUSSION

The myometrial progesterone receptor of the rhesus monkey is both qualitatively and quantitatively responsive to the hormonal status of the animal. Treatment of the ovariectomized monkeys with estrogen increased progesterone receptor levels six-fold. Estrogen stimulation of progesterone receptor concentrations has been described, for example, in the hamster [13], rat and mouse [10], guinea pig [3, 9], rabbit [8], and is also seen in the human [14]. Estrogen-treated postmenopausal women have much higher myometrial progesterone receptor levels than untreated postmenopausal patients.

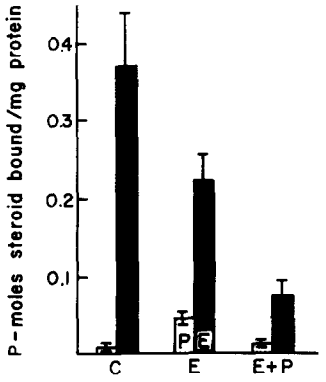

Fig. 4. Concentration of estrogen (E hatched bars) and progesterone (P plain bars) receptor in the myometrium of rhesus monkey. C = castrate; E = castrate treated with estrogen; E + P = castrate treated with estrogen and progesterone (see text).

Progesterone treatment, following estrogen stimulation, resulted in much lower levels of progesterone receptor, not significantly different from those in the castrate animals. Progesterone administration extensively reduces progesterone receptor levels in estrogen stimulated castrate guinea pigs [17], and in intact guinea pigs, lowest receptor levels are seen at times of high progesterone [7]. In women, progesterone receptor levels are lower during the secretory phase of the menstrual cycle than during the proliferative, and much reduced levels are seen in patients taking oral contraceptives [14]. Estrogen treatment also altered the sedimentation coefficient observed on sucrose density gradients. The heavier, 7.5 *s* form was only seen in the estrogen treated animal. This feature of progesterone receptors has been described in several animal species [3, 7, 8, 10, 12, 13].

[^{3}H]-progesterone was not displaced by cortisol from the 7.5 *s* receptor, but binding to the lighter component was considerably reduced by cortisol. In the castrate animal, binding on the sucrose gradient was totally abolished by cortisol competition, and in this animal no high affinity progesterone receptor was measurable from the Scatchard plot data. The animal treated with E + P also showed little specific binding on sucrose gradients that could not be displaced by cortisol and little high-affinity progesterone binding from the Scatchard plot data. These results indicate that most of the 4 *s* binding seen could be attributed to CBG and non-specific binding, but the 7.5 *s* form appears to be a specific progesterone receptor. A preliminary gradient analysis from an intact animal (stage of cycle unknown), showed both 7.5 and 4 *s* binding of [^{3}H]-progesterone, the former not displaced, the latter moderately reduced, by cortisol, but both components displaced by progesterone competition.

The competition study data shows the high affinity of the progesterone receptor for the synthetic steroids, which has been reported for other species [4, 6, 11, 12, 14, 18, 19]. Norgestrel had lower affinity than melengestrol acetate, both steroids lower than progesterone or 5α-pregnanedione. However, the norgestrel available for use in these experiments was the racemic dl-form. The l-isomer has been shown to be biologically inactive [20], and in the rat and rabbit only the d-isomer has an affinity for the uterine progesterone receptor [19, 21], so that true affinity of

the active isomer in the monkey should be much higher. Reports of the affinity of 5α-pregnanedione for uterine progesterone receptors are variable, from fairly high in the human [11, 14], guinea pig [19], mouse and rat [10] to low in the human [12] and hamster [13]. In the animals used in our present experiments, with the higher progesterone receptor levels, there was little competition with the corticosteroids, except desoxycorticosterone. This steroid has been shown to have significant affinity for the progesterone receptor [12, 13].

When compared to the levels of estrogen receptor, the progesterone receptor was present in distinctly lower concentrations. Taken individually, no significant correlation could be found between the levels of progesterone and estrogen receptor in the same animal.

The present experiments show that a specific progesterone receptor is present in the myometrial cytosol of the rhesus monkey and that the receptor has many properties in common with progesterone receptors described in other mammalian species.

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