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Progesterone Binding in Rabbit Oviduct and Uterus[1,2] (39191)

E. K. MUECHLER,[3] G. L. FLICKINGER, L. MASTROIANNI, JR., AND G. MIKHAIL

Endocrine Section, Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Progesterone binding to macromolecules of the rabbit uterus has been confirmed by several investigators (1-6). Both 4 S and 8 S progesterone receptorlike components have been reported in rabbit myometrium (1, 2). The association constant ranges from $7.8 \times 10^8\ M^{-1}$ in estrogen-primed castrated animals (2) to $1.6 \times 10^9\ M^{-1}$ in pregnant rabbits (5). In contrast to the uterus, the mammalian oviduct has received little attention. Only the chick oviduct has been studied extensively (7, 8). The present study was undertaken to examine progesterone binding in the rabbit oviduct and to compare it to the known characteristics of the rabbit uterus. Since progesterone may influence ovum transport, progesterone binding was studied during the first 6 days after mating.

Materials and Methods. *Animals.* New Zealand White rabbits, 3.5-5 kg body weight, were isolated for 25 days in individual cages. In one group of animals ovariectomy was performed at least 25 days prior to use. A second group was mated and sacrificed by cervical dislocation at intervals of 3, 12, 72, and 144 hr. Mating was considered successful when spermatozoa were found in the vagina at 3 hr or when corpora lutea were identified in both ovaries at other time intervals.

Steroids and buffers. [1,2-^{3}H]-Progesterone (SA 50.3 Ci/mmole), was purchased from New England Nuclear. Progesterone, 20α-hydroxypregn-4-en-3-one cortisol and estradiol were purchased from Schwarz/Mann. The buffer used for all procedures, except where specified otherwise, consisted of 0.01 *M* Tris-HCl, 0.001 *M* EDTA, 0.25 *M* sucrose, pH 8, at 4°. Dextran-coated charcoal suspension (250 mg Norit A and 50 mg T-70 dextran from Pharmacia per 100 ml of buffer) was prepared with 0.01 *M* Tris-HCl, 0.001 EDTA buffer, pH 8.

Cytosol preparation. Immediately after removal, the reproductive organs were placed in ice-cold buffer. After dissection from surrounding structures, the oviducts and uterus were rinsed three times in buffer, weighed, and minced. Tissues were homogenized in 10 vol (w/v) of buffer, except where otherwise indicated. Homogenization was performed with a Polytron instrument, using three pulses of 5 sec at a speed setting of 5, with 1-min intervals of cooling. The homogenate was centrifuged at 800*g* for 15 min. Nuclear pellets were used for measurement of DNA according to Burton (9). The supernatant was centrifuged at 105,000*g* for 60 min. The resulting cytosol was used immediately for the assay of progesterone-binding capacity. Aliquots of cytosol were used for determination of protein concentration by the method of Lowry (10).

Dextran-coated charcoal assay. Progesterone was pipetted into test tubes in amounts of 0, 50, 100, 200, 300, 500, 1000, and 10,000 pg. After evaporation to dryness, 1×10^{-13} mole of [^{3}H]progesterone in 0.2 ml of buffer containing 0.1% gelatin was added. Aliquots of cytosol (0.2 ml) were delivered to each tube and incubation was carried out at 4° for 3 hr. The tubes then received 1 ml of dextran-coated charcoal suspension. They were immediately vortexed and allowed to stand for 10 min in an ice bath before centrifugation at 3000 rpm for 5 min. The supernatant was decanted into scintillation vials, and 10 ml of scintilla-

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[2] Please address all correspondence to: Department of Obstetrics and Gynecology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104.

[3] Ford Foundation Fellow in Reproductive Biology. Present address: University of Rochester, Department of Obstetrics and Gynecology, Rochester, New York 14642.

tion fluid (4 g of Omniflour, 100 ml of BioSolv/liter of Toluene) was added. Tritium was counted with 46% efficiency using an intertechnique liquid-scintillation spectrophotometer.

All assays were carried out in duplicate. The protein-bound radioactivity was corrected for tritium not absorbed by charcoal. This blank value did not exceed 3% of unbound tritium in the presence (100 ng) or absence of unlabeled steroid hormone. Treatment of cytosol aliquots with charcoal prior to incubation with tracer and unlabeled steroid removed unbound progesterone. However, it was shown in preliminary experiments that preincubation of cytosol aliquots with physiological concentrations of unlabeled progesterone lowered the apparent equilibrium dissociation constant without affecting the receptor concentration. Therefore, endogenous progesterone levels were excluded from calculations of progesterone receptor concentrations. Progesterone-binding characteristics were determined from Scatchard plots (11). The concentration of high affinity binding sites were obtained by extrapolation to the X-intercept. Low affinity binding of the experimental curves was corrected for by the method of Rosenthal (12).

Sucrose density ultracentrifugation. Cytosol from estrous rabbits was obtained by homogenizing oviductal or uterine tissue in 3 vol of a buffer containing 0.01 *M* Tris-HCl, 0.001 *M* EDTA, 0.012 *M* thioglycerol, 10% glycerol, pH 8 at 4°. Following incubation of cytosol with [^{3}H]progesterone in the presence or absence of other steroids at 4° for 2 hr, 0.2 ml was layered onto 4.8 ml of 5-20% sucrose gradients containing 10% glycerol. After centrifugation at 40,000 rpm for 18 hr in a L3-50 Beckman centrifuge with a SW 50.1 rotor, the bottom of the tubes was pierced and equal fractions were collected for measurement of radioactivity. ^{14}C-labeled BSA and ^{14}C-labeled gamma globulin were used as markers.

Results. The temperature stability of the progesterone receptor was identical for uterine and oviductal cytosol. At 4°, maximum binding occurred after 3 hr of incubation and remained stable for 24 hr. Within 15-30 min of incubation at 25°, progesterone binding was 40% of the maximum binding at 4° and diminished steadily with time. At 30° there was minimal binding of progesterone (Fig. 1).

Competition studies were carried out using dextran-coated charcoal assay and sucrose gradients. Cortisol partially competes with progesterone binding by oviductal cytosol (Fig. 2a), but does not displace bound progesterone in uterine cytosol (Fig. 2b). Estradiol, testosterone, and 20α-hydroxy-

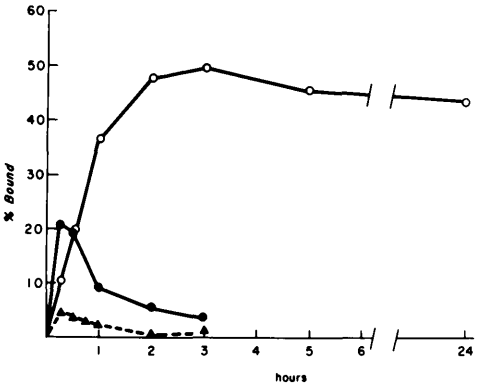

FIG. 1. Binding of [^{3}H]progesterone by uterine cytosol at 4° O- - -O, 25° ●- - -●, and 30° ▲- - -▲.

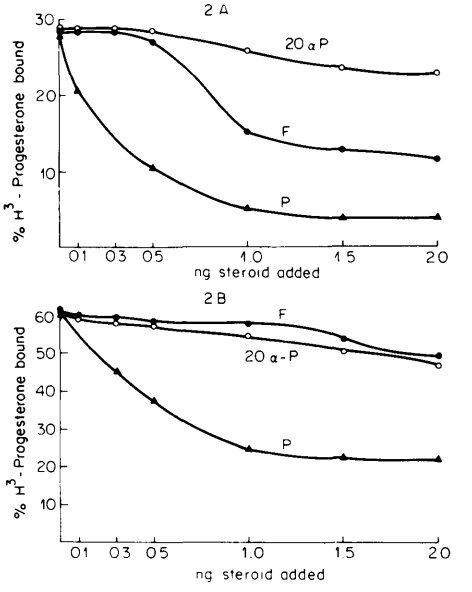

FIG. 2. Displacement of [^{3}H]progesterone from oviductal (2A) and uterine (2B) cytosol by unlabeled steroids. P, progesterone; F, cortisol; 20αP, 20α-hydroxy pregn-4-en-3-one.

pregn-4-en-3-one showed no significant competition in either tissue. Sucrose density ultracentrifugation confirms these results (Figs. 3a and b).

In estrous rabbits, macromolecules with sedimentation coefficients of 7–8 S and 4–5 S are identified in the oviduct. Unlabeled progesterone abolished the 7 S binding while cortisol exhibited modest competition. Neither cortisol nor progesterone affected the 4 S binder, suggesting that in the oviduct of estrous rabbits a 7 S specific progesterone receptor and a 4 S nonspecific binder of [^{3}H]progesterone are found. Similar results were obtained in castrated, estrogen-treated animals. In uteri obtained from the same animals, the 7 S specific progesterone receptor is dominant; however, a small 4 S peak (nonspecific) is also seen (Figs. 3a and b).

The calculated dissociation constant and progesterone receptor concentration (expressed as femtomole per microgram of DNA and per milligram of protein) in oviductal and uterine cytosol are shown in Tables 1 and 2. In the oviduct, the receptor levels in estrus and early pregnancy were not significantly different; however, lower values were obtained by the sixth day. No progesterone receptor was detected in castrate animals. In the uterus a decline in receptor concentration occurred after mating, reaching significantly lower levels (comparable to castrate) at 72 and 144 hr. The dissociation constant, ranged from $0.8 \pm 0.1 \times 10^{-9}$ M to $1.8 \pm 0.3 \times 10^{-9}$ M, was similar in both organs and in different animal groups.

Discussion. Biochemical studies have been unsuccessful in identifying a progesterone receptor in the mammalian oviduct (6); however, progesterone binding was demonstrated in the guinea pig oviduct by autoradiography (13). Our results provide evidence for the presence of a heat labile progesterone receptor in the cytosol of rabbit oviduct that is qualitatively similar in many respects to uterine progesterone receptor.

The K_d of progesterone receptor was similar in rabbit oviduct and uterus and showed no significant differences between estrus and early pregnancy. The values for K_d reported here are comparable to those described in the uterus of estrous rabbits (5). Under the experimental conditions used, the receptor in oviductal cytosol sedimented in glycerol containing sucrose gradients as a 7–8 S and 4–5 S component. Comparable results were found in uterine cytosol. The 7 S peak, more predominant in the uterine cytosol, appears to be the specific progesterone binder; however, in both the oviduct and uterus, competition studies strongly suggest that the 4 S component is nonspecific. Our data confirm reports demonstrating a 7 S progesterone receptor and is at variance with those reporting a 4 S peak for progesterone, particularly in the intact estrous rabbit (5).

A quantitative difference is seen in the sucrose gradients of the uterus and oviduct. Using equal dilution of cytosol from both organs, the 7 S peak in uterine cytosol is six

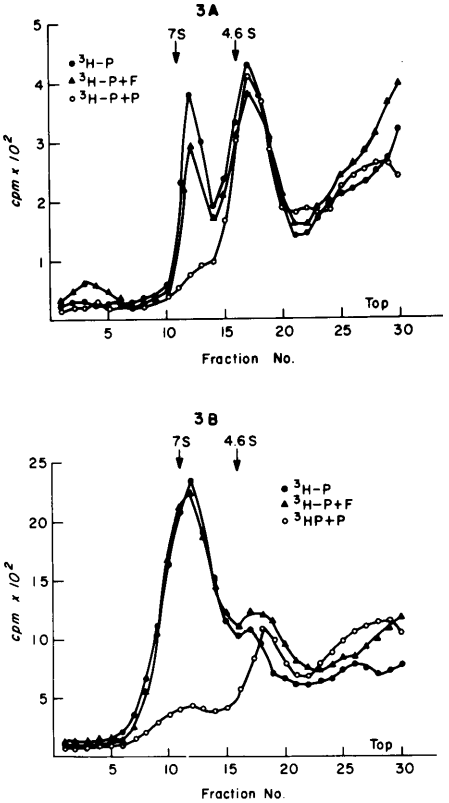

FIG. 3. Sucrose density gradients of oviductal (3A) and uterine (3B) progesterone receptors. Cytosol incubated with [^{3}H]progesterone (3×10^{-9} M) in the absence or presence of progesterone (P) or cortisol (F) (3×10^{-7} M).

TABLE I. PROGESTERONE-BINDING CAPACITY OF CYTOSOL FROM THE RABBIT OVIDUCT.[a]

Time of sacrifice[b]	Number of rabbits	Organ weight (g)	$K_d \times 10^{-9}$ M	fmole-bound progesterone per μg DNA	fmole-bound progesterone per mg protein
Estrus	6	0.75 ± 0.06	1.76 ± 0.30	10.52 ± 3.40	275 ± 81
3 hr pc	6	0.86 ± 0.07	1.30 ± 0.10	16.27 ± 1.73	327 ± 34
12 hr pc	6	0.85 ± 0.07	0.82 ± 0.12	10.12 ± 1.83	183 ± 26
72 hr pc	6	0.85 ± 0.05	1.78 ± 0.13	10.03 ± 2.03	323 ± 100
144 hr pc	4	0.81 ± 0.10	1.09 ± 0.01	4.13 ± 1.35	122 ± 13[c]
Castrate	7	0.41 ± 0.03[c]	0.82 ± 0.13	ND[d]	ND

[a] Values represent mean ± SE.
[b] pc, postcoitus.
[c] Significantly different from the estrous group ($P < 0.05$).
[d] ND, not detectable.

TABLE II. PROGESTERONE-BINDING CAPACITY OF CYTOSOL FROM THE RABBIT UTERUS.[a]

Time of sacrifice[b]	Number of rabbits	Organ weight (g)	$K_d \times 10^{-9}$ M	fmole-bound progesterone per μg DNA	fmole-bound progesterone per mg protein
Estrus	6	3.71 ± 0.48	1.33 ± 0.17	24.58 ± 3.46	1457 ± 194
3 hr pc	6	4.82 ± 0.50	1.12 ± 0.09	24.89 ± 4.15	1202 ± 146
12 hr pc	6	4.74 ± 0.45	0.88 ± 0.18	17.08 ± 2.99	1046 ± 173
72 hr pc	6	10.41 ± 0.54[c]	1.75 ± 0.19	16.85 ± 3.44	653 ± 143[c]
144 hr pc	4	10.57 ± 0.46[c]	1.41 ± 0.37	5.55 ± 1.46[c]	298 ± 109[c]
Castrate	7	1.82 ± 0.12[c]	0.86 ± 0.14	1.92 ± 0.28[c]	430 ± 68[c]

[a] Values represent mean ± SE.
[b] pc, postcoitus.
[c] Significantly different from the estrous group ($P < 0.05$).

times that of the oviduct. A similar relationship was found when values of the dextran-coated charcoal assay were compared in most animal groups where progesterone binding to uterine cytosol exceeded that of oviductal cytosol by two- to five-fold.

Steroid specificity appears to be a discriminating factor of progesterone binding in both organs. In oviductal cytosol dextran-coated charcoal adsorption and sucrose density ultracentrifugation both show significant competition for progesterone binding sites by cortisol. This is not seen in uterine cytosol. This suggests that factors are present in both tissues which exhibit different steroid specificity. Atger *et al.* found a variation in hormonal specificity in organs of guinea pigs. The competing efficiency of cortisol for progesterone binding varied from 2.1% in uterine horns to 13.6% in uterine cervix and 9.9% in vagina (14).

The displacement of [^{3}H]progesterone by cortisol in the cytosol of the oviduct (Fig. 2A) suggests more than one class of binding sites. Some of this heterogeneity may be due to CBG binding. However, displacement which occurs in the 7 S region of the sucrose gradient (Fig. 3A) can not be attributed to CBG since this protein is known to sediment in the 4 S region. Calculation of the levels of progesterone receptor took into consideration lower affinity binding proteins, and only the levels of the specific, high affinity receptor were measured.

If progesterone binding is compared in groups of animals at intervals after mating, important differences between oviduct and uterus are noted. In the oviduct a significant decrease in progesterone binding is observed from 3 hr to 12 hr after mating. This coincides with a sharp fall in ovarian estradiol and 20α-OH-progesterone secretion (15, 16). After intermittently higher levels at 72 hr, progesterone binding decreases to the lowest values observed at 144 hr after mating.

In the uterus, the progesterone binding pattern is that of steady decline from estrus

reaching significantly lower levels at 72 and 144 hr. Using a different assay procedure, the concentrations of uterine progesterone receptor reported here are lower than those reported by Davies (5); however, the pattern of the receptor concentration in early pregnancy is similar in the two studies.

As estradiol receptors were determined in the same group of animals (12), comparison can be made with progesterone receptor concentration. In the oviduct progesterone binding is higher than estrogen binding in all intact animal groups except 144 hr after mating. In uterine cytosol the ratio of progesterone binding to estrogen binding is higher than oviductal cytosol, reaching a miximum at 12 hr after mating.

During early pregnancy, functions of the reproductive tract are influenced by steroid hormones. Our findings suggest that temporal changes in the levels of steroid receptors in the oviduct and uterus may regulate hormone action between the time of ovulation and implantation.

Summary. Progesterone binding of high affinity with a dissociation constant of 10^{-9} *M* was identified in cytosol of rabbit oviduct and uterus. Macromolecules with sedimentation coefficients of 7–8 S and 4–5 S were present. Progesterone receptor concentration was two to fivefold lower in the oviduct when compared with the uterus. The receptor concentration declined steadily from 3 hr until 144 hr after mating in the uterus; however, the decline in oviductal receptor was not significant until the sixth day of pregnancy. Progesterone receptor concentration in rabbit oviduct and uterus in estrus and early pregnancy was greater than estradiol receptor levels.

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