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CYTOSOL AND NUCLEAR PROGESTERONE RECEPTOR IN CAT UTERUS AND OVIDUCT

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SUMMARY

Binding of progesterone (P) to a specific cytosol receptor and the *in vivo* translocation of the cytosol receptor complex to the nucleus was studied in the cat uterus and oviduct. Cytosol and nuclear fractions were prepared from the reproductive tracts of ovariectomized animals treated for 14 days with estradiol (E_2) and from those which were subsequently infused with a P solution for 15 min. *In vitro* cytosol incubation with [^{3}H]-P included cortisol to eliminate binding to CBG. The specific binding of [^{3}H]-P reached equilibrium within 90 min at 0°C and was stable for at least 4 h in both tissues. Competitive binding studies revealed the following binding affinities for the cytosol receptor; R5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione) = P $\gg$ corticosterone = E_2 = testosterone. Scatchard analysis of [^{3}H] P binding in cytosols from E_2-treated animals yielded a K_D of 3.2×10^{-8} M in the uterus and 2.5×10^{-8} M in the oviduct. The P binding protein had a sedimentation coefficient of 6.8 S. The content of nuclear receptor was assessed by an exchange assay using R5020. Equilibrium was reached within 4 h at 22°C and remained unchanged for another 2 h. The relative binding affinities for the nuclear receptor were; R5020 > P > corticosterone > testosterone > E_2. The K_D was 8.2×10^{-9} M in uterine nuclei and 6.0×10^{-9} M in oviduct nuclei from E_2 + P treated animals. Following P-infusion the decrease in the quantity of specifically bound [^{3}H]-P in the cytosol, and the increase in the quantity of specifically bound [^{3}H]-R5020 in the nuclei, were significant in both tissues.

INTRODUCTION

In ovariectomized cats exogenous E_2 induces hypertrophy and hyperplasia in the uterus and oviduct and increases cytoplasmic and nuclear E_2 receptor levels [1, 2]. Estradiol-induced morphological markers include the appearance of ciliated and secretory cells in the oviductal epithelium and the accumulation of basophilic secretory granules in the endometrial glandular epithelium. Administration of P to an E_2-primed ovariectomized cat causes a decrease in the cytoplasmic and nuclear E_2 receptor content in the uterus and oviduct [2]. However, the morphological response of these two organs to P differs in that the oviduct atrophies and dedifferentiates in spite of the continued presence of E_2, whereas the endometrial glands undergo further hypertrophy and synthesize glycogen. In addition, a delayed P antagonism is observed in the oviduct [3]. When P administration is begun prior to the E_2-treatment, E_2-induced hypertrophy and hyperplasia in the oviductal epithelium are not prevented, however, the antagonistic effects of P (atrophy and deciliation) do become evident on continuation of hormone treatment. A similar delayed P effect has not been observed in the uterus. These studies demonstrate the important regulatory role of P in the uterus and oviduct of the cat as well as the difference in the responses in the two tissues.

The regulatory mechanism of the ovarian steroid is, at least partially, mediated at the receptor level [4, 5]. In order to further clarify our understanding of the interaction of the ovarian steroids in the uterus and oviduct, it seems necessary to measure the cytoplasmic and nuclear P receptor levels in these organs. This report describes a method for measuring both the cytoplasmic and nuclear P receptor levels in cat uterus and oviduct. A direct assay with P was used to determine the binding sites in the cytosol fractions, whereas the synthetic progestin R5020 [6] was used to measure the content of nuclear receptor.

MATERIALS AND METHODS

Animals and experimental design

Domestic cats, bilaterally ovariectomized for at least four weeks, were treated with E_2 for 14 days by means of a tubular silastic implant (1.0 cm, Dow Corning No. 601–331) placed subcutaneously in the midscapular region [1]. The animals were then placed in one of three groups. The animals in Group I received no additional treatment. The entire reproductive tract was removed (E_2 treatment). The animals in Group II were infused with 5 μg/ml P at a rate of 6–8 ml/min for 15 min and then the entire reproductive tract was removed (E_2 + P treatment). One uterine horn and ipsilateral oviduct were removed in animals in Group III. These animals were then infused with P as described for Group II after which the contralateral uterine horn and oviduct were

removed. Peripheral blood samples were obtained by venipuncture or via indwelling catheter from the great saphenous vein contralateral to the one used for infusion. Blood was allowed to clot at room temperature for one hour, centrifuged, serum collected and stored frozen at −10°C until time of assay.

Preparation of cytosol and nuclear fractions

Excised oviducts and uteri were trimmed of extraneous membranes and fat, cut into small pieces (1–2 mm) and placed in ice-cold Buffer A (0.5 M sucrose, 2 mM $MgCl_2$, and 10 mM Tris–HCl, pH 7.4). All subsequent steps were carried out at 0°C. The tissue was homogenized in approximately 4 vol. Buffer A with two 5 s bursts of a Brinkman polytron (PT 10 generator) at a speed setting of five. The homogenate was centrifuged at 12,000 *g* for 10 min. The supernatant of the above centrifugation was then centrifuged at 105,000 *g* for 60 min. The clear supernates (cytosols) were retained and kept on ice until use. The crude nuclear pellets from the first centrifugation (12,000 *g*) were washed twice by gentle rehomogenization in a glass-Teflon homogenizer in Buffer B (Buffer A + 30% glycerol), and centrifuged at 12,000 *g* for 10 min after each wash. The washed nuclear pellets were resuspended in Buffer B and kept on ice until use.

Cytosol and nuclear assays

Aliquots of the cytosol or nuclear fractions (300 μl) were added to two parallel series of tubes; one series contained the [^{3}H]-steroid, and the other contained the same concentration of [^{3}H]-steroid as in the first plus a 250-fold excess of radioinert steroid. In addition, all cytosol tubes contained 10 μM cortisol to minimize the binding of P to corticosteroid-binding globulin (CBG) [7]. Cytosols were incubated at 0°C and nuclei at 22°C.

In cytosol, bound and free steroids were separated by adding 300 μl dextran coated charcoal (0.5% Norite A and 0.05% Dextran T-70 in 1.5 mM EDTA and 10 mM Tris–HCl, pH 7.4). After leaving for 10 min at 0°C the tubes were centrifuged at 2000 *g* for 10 min and radioactivity in the supernatants was determined. Nuclear incubations were terminated by centrifugation and the pellets were washed twice with 1 ml of Buffer C (0.2% Triton X-100 in Buffer A). The pellets were then solubilized in 300 μl 0.1 N NaOH at 90°C and counted.

Sucrose density gradients

Tissue was homogenized in Buffer D (10% glycerol, 12 mM thioglycerol, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4) and cytosol was prepared as described above. Following at least a 2 h incubation at 0°C, a 0.2 ml aliquot of cytosol or BSA (10 mg/ml) prepared in Buffer D was layered on top of linear 5 ml gradients of 5–20% sucrose prepared in Buffer D. The gradients were centrifuged for 16 h at 45,000 rev/min (189,000 *g*) at 0–4°C in an SW 50.1 rotor. Following centrifugation, the bottoms of the tubes were pierced and 10 drop fractions were collected and counted. The location of the BSA standard, 4.4 S [8], was obtained by measuring the absorbance at 280 nm.

Radioimmunoassay

Radioimmunoassay procedures described elsewhere [9, 10] were validated for measuring E_2 and P in cat serum before they were used in this study. Anti-estradiol-6-BSA serum GDN 224 and anti-progesterone-11-BSA serum GDN 337 were kindly supplied by Dr. G. D. Niswender of Colorado State University, Fort Collins, Colorado. Estradiol extracts assayed at different dose levels were parallel with the standard. Recovery of E_2 added to spay cat sera was quantitative with a correlation coefficient of 0.87 between expected versus observed values. The coefficient of variation for within assay variation was 7.8% and a coefficient of 13.5% was observed for between assay variation. Estradiol levels measured in sera obtained from spay cats treated with silastic implants were almost identical to the ones in another study (27 pg/ml vs. 28 pg/ml) where the same implants were used [11].

The inhibition curves obtained with extracts of the sera from P-infused cats (~58 ng/ml) had the same shape and slope obtained with the standard. When varying amounts of P were added to the serum from spay cats, the recovery of added P was complete over the useful range of the curve ($r = 0.95$). Within assay error was 4.6% and the between assay variation provided a coefficient of variation of 9.1%.

Other analytical methods

Protein was measured by the method of Lowry *et al.*[12] using BSA as standard and DNA was assayed by the method of Burton[13]. Radioactivity was counted in scintillation fluid composed of toluene, Triton X-100, and PPO-POPOP (RPI Scintillator) in a ratio of 2000:1000:126. The counting efficiency was approximately 28%. The dissociation constants and number of binding sites were determined from Scatchard plots [14]. Results are expressed as mean ± standard deviation. The significance of differences among mean values were determined using Student's *t*-test.

Steroids

[1,2-^{3}H]-Progesterone (55.7 Ci/mmol), [17α-methyl-^{3}H]-R5020 (86.0 Ci/mmol), [2,4,6,7-^{3}H]-Estradiol (91.8 Ci/mmol) and radioinert R5020 were obtained from the New England Nuclear Corporation. Radioinert E_2, P, cortisol, corticosterone, and testosterone of highest purity were obtained from Sigma Chemical Company.

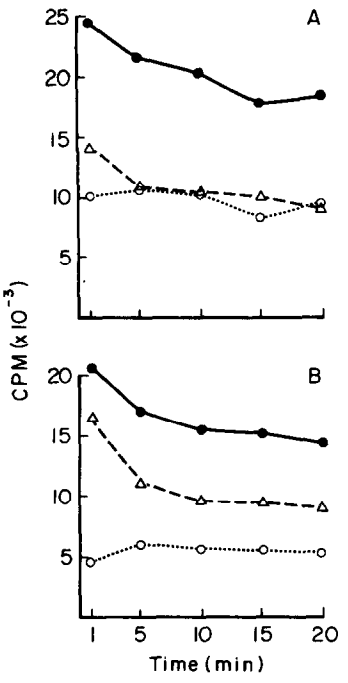

Fig. 1. Effect of charcoal treatment time on [^{3}H]-P binding in uterine (A) and oviduct (B) cytosols. Cytosols, prepared from animals in Group I, were incubated at 0°C for 90 min with 20 nM [^{3}H]-P and 10 μM cortisol in the presence or absence of a 250-fold excess of radioinert P prior to the addition of charcoal. Each point represents the mean of two determinations. ●——●, total binding; △---△, non-specific binding; ○---○, specific binding.

RESULTS

Hormone levels

The systemic level of E_2 on the day of tissue collection averaged 27 pg/ml in the 26 animals used in this study. Sixteen of these animals were infused with P and peripheral blood samples were obtained at 5 and 15 min. P averaged 58 ng/ml (N = 32) over the last 10 min of infusion. The levels of E_2 and P compared favorably with those found in estrus and in pregnancy [11].

Cytoplasmic progesterone receptor

Cytosols, prepared from the uteri and oviducts of E_2-treated animals (Group I), were used in experiments designed to characterize the cytosol receptor.

The effect of charcoal on [^{3}H]-P bound to the cytosol receptor was determined by exposing cytosols to charcoal for various lengths of time (Fig. 1). Cytosols were incubated at 0°C for 90 min with 20 nM [^{3}H]-P and 10 μM cortisol in the presence or absence of a 250-fold excess of radioinert P prior to the addition of charcoal. As shown in Fig. 1, approximately 6–7% of the specifically bound [^{3}H]-P is lost after a 10 min exposure, and 12–14% after a 20 min exposure. A 10 min exposure was used in all subsequent studies measuring [^{3}H]-P bound to cytosol receptor.

The rate of association was determined by incubating cytosols at 0°C with 20 nM [^{3}H]-P and 10 μM cortisol in the presence or absence of a 250-fold excess of radioinert P. The difference between the amount of [^{3}H]-P bound was used to represent the specific binding. After various periods of time the amount of specially bound hormone was measured. In both tissues, association was rapid, reaching equilibrium within 90 min and remaining stable for up to 4 h (Fig. 2). In all additional studies cytosols were incubated at 0°C for 90 min.

The specificity of the cytosol receptor was determined by incubating cytosols with 2 nM [^{3}H]-P, 10 μM cortisol, and various concentrations of competing steroids (4×10^{-10} to 4×10^{-6} mol/l). The

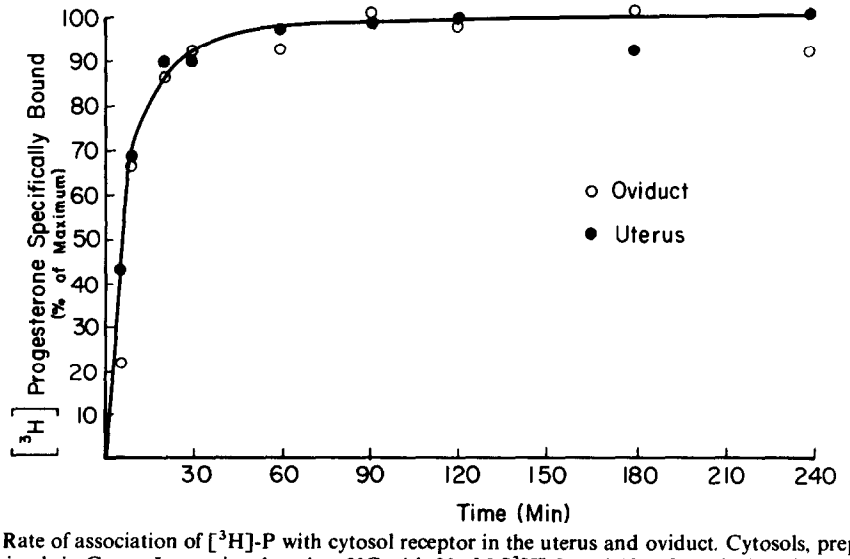

Fig. 2. Rate of association of [^{3}H]-P with cytosol receptor in the uterus and oviduct. Cytosols, prepared from animals in Group I, were incubated at 0°C with 20 nM [^{3}H]-P and 10 μM cortisol in the presence or absence of a 250-fold excess of radioinert P. Each point represents the mean of two determinations.

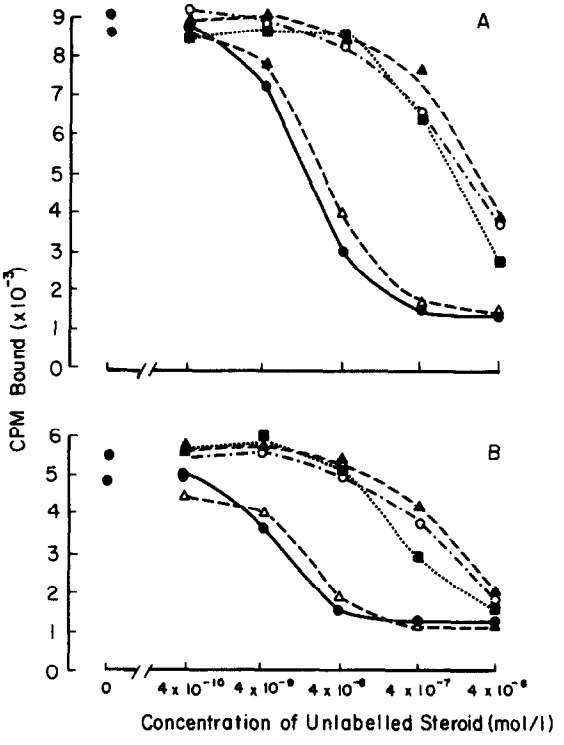

Fig. 3. Steroid competition for the cytosol receptor. Cytosols, prepared from animals in Group I, were incubated at 0°C for 90 min with 2 nM [^{3}H]-P, 10 μM cortisol, and various concentrations of competing steroids. Each point represents the mean of two determinations. A, uterus; B, oviduct; ●——●, R5020; △---△, P; ■-----■, corticosterone; ○—·—○, E_2; ▲---▲, testosterone.

synthetic progestin R5020 and P had almost identical competition curves in both tissues (Fig. 3). The relative binding affinities, calculated as the ratio of steroid concentration which inhibits 50% of the [^{3}H]-P binding [15], were <2% for corticosterone, and <1% for E_2 and testosterone of the binding affinity of P in both uterine and oviduct cytosols.

The saturation characteristics of the cytosol receptor were studied by incubating cytosols with various concentrations of [^{3}H]-P and 10 μM cortisol in the presence or absence of a 250-fold excess of radioinert P. The difference between total and non-specific binding was used to represent the quantity of [^{3}H]-P that was specifically bound (Fig. 4). The saturable binding appeared to be due to a single component since the data corresponded to a straight line on Scatchard analysis (Fig. 5). The equilibrium dissociation constants (K_D) were $3.2 \pm 1.0 \times 10^{-8}$ M in uterine and $2.5 \pm 1.3 \times 10^{-8}$ M in oviduct cytosols ($N = 4$). The difference was not statistically significant.

Our results indicate that [^{3}H]-P was bound to macromolecules that sedimented in the 6.8 S region in cytosols from both uteri and oviducts when centrifuged on 5–20% sucrose gradients containing 10% glycerol (Fig. 6). The radioactivity in the 6.8 S peak in uterine cytosols and the shoulder observed in oviduct cytosols was completely displaced by a 250-fold excess of radioinert P but not by 10 μM cortisol. The radioactivity detected in the 4.4 S region may be due to [^{3}H]-P bound to CBG as suggested previously in studies with decidualized hamster uterus [16] since the binding was displaceable by 10 μM cortisol.

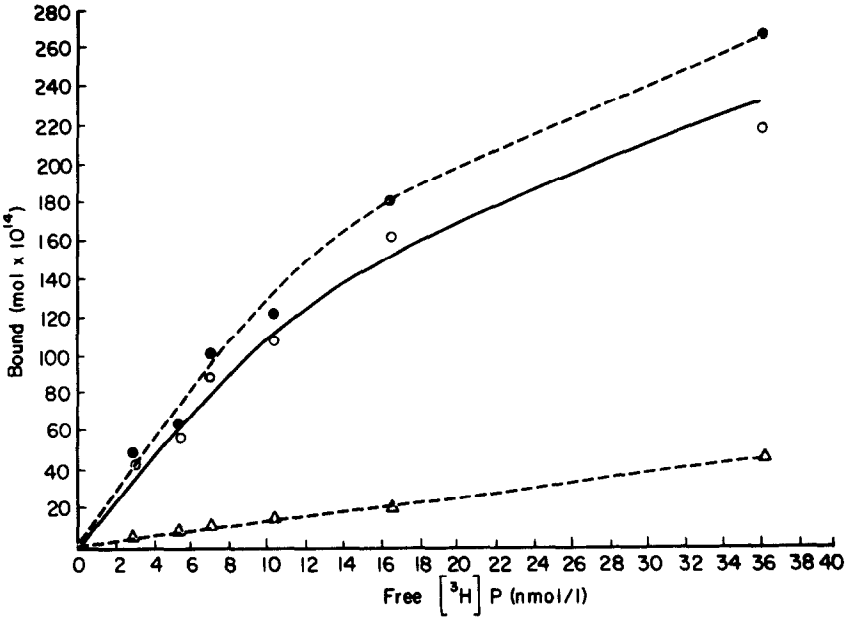

Fig. 4. Saturation analysis of [^{3}H]-P binding to uterine cytosol receptor. Cytosols, prepared from animals in Group I, were incubated at 0°C for 90 min with various concentrations of [^{3}H]-P (cortisol added to prevent binding to CBG) in the presence or absence of a 250-fold excess of radioinert P. ●---●, total binding; △---△, nonspecific binding; ○——○, specific binding.

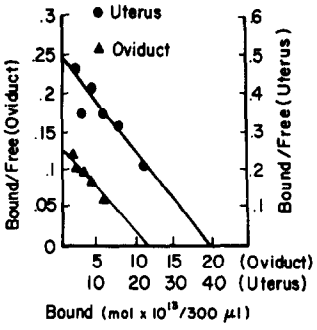

Fig. 5. Scatchard plot of cytosol receptor saturation data. Lines were fitted by linear regression. ●——●, uterus, $K_D = 2.8 \times 10^{-8}$ M, n = 14.4×10^{-13} mol [^{3}H]-P bound/mg protein; ▲——▲, oviduct, $K_D = 3.5 \times 10^{-8}$ M, n = 12.2×10^{-13} mol [^{3}H]-P bound/mg protein.

Nuclear progesterone receptor

Oviduct and uterine nuclear preparations from P-infused animals (Group II) were incubated at 0°C and 22°C for various periods of time with 10 nM [^{3}H]-R5020 in the presence or absence of a 250-fold excess of radioinert R5020. At 22°C, the quantity of specifically bound [^{3}H]-R5020 increased up to 4 h and remained relatively constant for two additional hours (Fig. 7). Degradation of the receptor occurred after 6 h of incubation. In the 0°C incubates the quantity of specifically bound [^{3}H]-R5020 increased for at least 24 h (Fig. 7). However, the maximum binding measured at 0°C was only 50% of that measured at 22°C in nuclear preparations from the same animals. In all additional studies nuclei were incubated with [^{3}H]-R5020 at 22°C for 4 h.

The specificity of nuclear binding was determined by incubating nuclei with 2 nM [^{3}H]-R5020 and various concentration of competing steroids (4×10^{-10} to 4×10^{-6} mol/l). As can be seen in Fig. 8, R5020 was more effective in displacing [^{3}H]-R5020 than P. The relative binding affinities were 1% for corticosterone, and <0.1% for testosterone and estradiol of that of R5020 in both the uterus and oviduct.

Nuclei, prepared from P-infused animals (Group II), were incubated with various concentrations of [^{3}H]-R5020 in the presence or absence of a 250-fold excess of radioinert R5020. Figures 9 and 10 show the saturation characteristics of the P nuclear receptor in the uterus and oviduct. As observed in the cytosols, Scatchard analysis yielded a straight line indicating that only one binding component was present (Fig. 10). The K_Ds were $8.2 \pm 3.1 \times 10^{-9}$ M in uterine and $6.0 \pm 2.5 \times 10^{-9}$ M in oviduct nuclei ($N = 4$). The values were not significantly different.

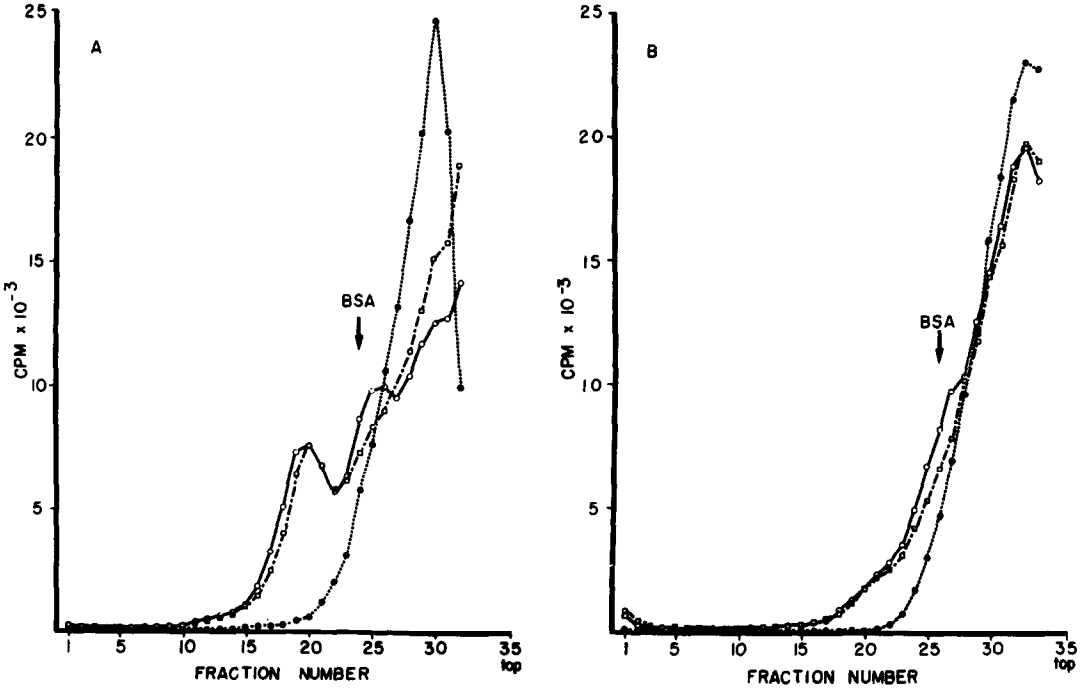

Fig. 6. Gradient profiles of [^{3}H]-P binding in the uterine and oviduct cytosols. Aliquots (0.2 ml) of incubates were layered on 5–20% sucrose gradients in Buffer D. Centrifugation was carried out for 16 h at 189,000 *g* at 0–4°C in a SW 50.1 rotor. BSA (4.4 S) was used as the sedimentation standard. The peak of specific [^{3}H]-P binding is at 6.8 S. ○——○, cytosol incubated with 20 nM [^{3}H]-P; □—·—□, cytosol incubated with 20 nM [^{3}H]-P and 10 µM radioinert cortisol; ●——●, cytosol incubated with 20 nM [^{3}H]-P, 10 µM radioinert cortisol, and 250-fold excess of radioinert P; A, uterus; B, oviduct.

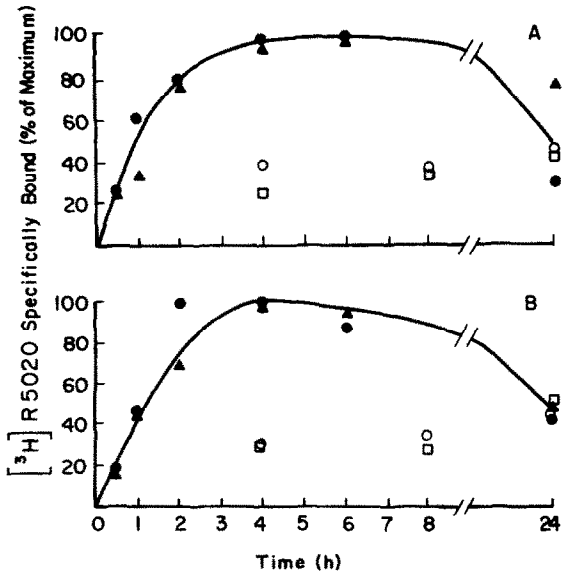

Fig. 7. Specific binding of [^{3}H] R5020 to the nuclear receptor as a function of time and temperature. Nuclei, prepared from animals in Group II, were incubated at 0°C (open symbols) and 22°C (closed symbols) for various periods of time with [^{3}H] R5020 (20 nM) in the presence or absence of a 250-fold excess of radioinert R5020. Each set of symbols are the determinations from the same animal. A, uterus; B, oviduct.

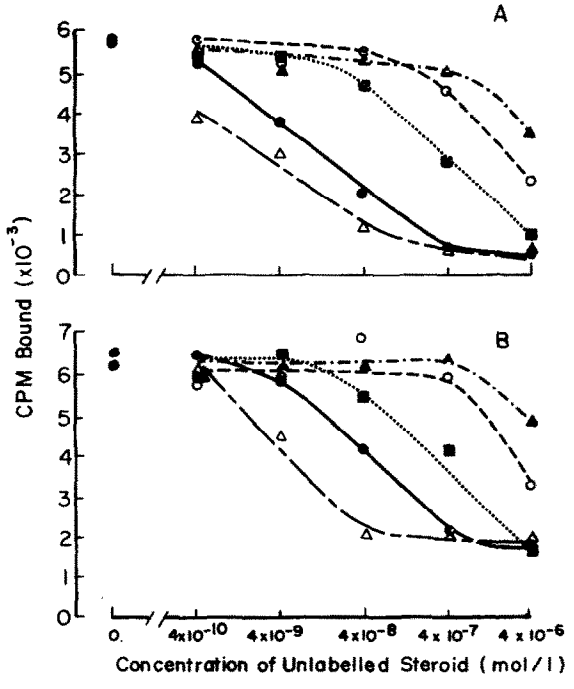

Fig. 8. Steroid competition for the uterine (A) and oviduct (B) nuclear receptor. Nuclei, prepared from animals in Group II, were incubated at 22°C for 4 h with 2 nM [^{3}H] R5020 and various concentrations of competing steroids. Each point represents the mean of two determinations. △---△, R5020; ●——●, P; ■-----■, corticosterone; ○---○, testosterone; ▲—·—▲, E_2.

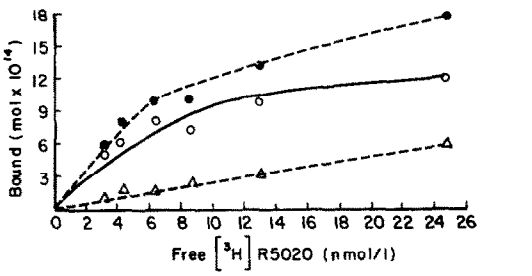

Fig. 9. Saturation characteristics of [^{3}H]-R5020 binding to the nuclear receptor. Uterine nuclei, prepared from animals in Group II, were incubated at 22°C for 4 h with various concentrations of [^{3}H]-R5020 in the presence or absence of a 250-fold excess of radioinert R5020. ●——●, total binding; △---△, nonspecific binding; ○——○, specific binding.

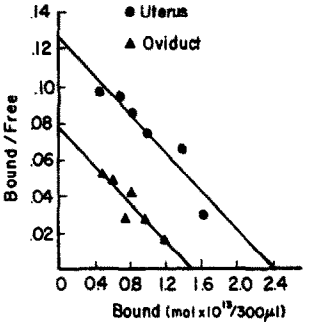

Fig. 10. Scatchard plot of nuclear receptor saturation data. Lines were fitted by linear regression. ●——●, uterus, $K_D = 6.5 \times 10^{-9}$ M, $n = 5.3 \times 10^{-15}$ mol [^{3}H]-R5020 bound/μg DNA; ▲——▲, oviduct, $K_D = 6.5 \times 10^{-9}$ M, $n = 8.4 \times 10^{-15}$ mol [^{3}H]-R5020 bound/μg DNA.

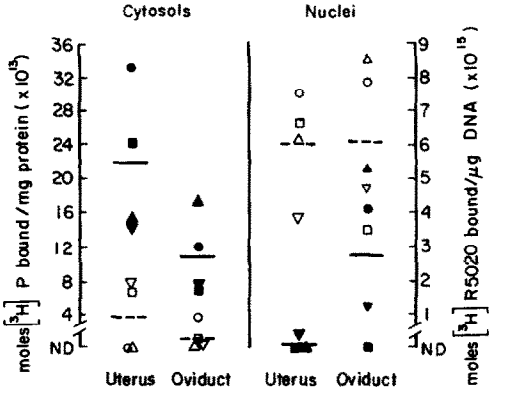

Fig. 11. *In vivo* translocation of the P receptor. Two cats in each of four experiments were treated simultaneously, their tissue pooled, and cytosol and nuclear fractions prepared from tissue obtained both before (closed symbols) and after (open symbols) P-infusion (Group III). The number of binding sites were determined by Scatchard analysis. Each set of symbols represents data from the same two animals. Solid lines are the arithmetic mean of tissue from animals treated with E_2 and broken lines are the arithmetic mean of tissue from animals treated with E_2 and P. ND = non-detectable specific binding.

Effect of P on the number of cytosol and nuclear binding sites

Uterine and oviduct cytosol and nuclear preparations obtained from the same animals both before and after P-infusion (Group III) were incubated as previously described and the number of binding sites determined by Scatchard analysis. The number of cytosol receptors in both the uterus ($21.8 \pm 8.9 \times 10^{-13}$ mol [^{3}H]-P bound/mg protein) and oviduct ($11.0 \pm 4.8 \times 10^{-13}$ mol [^{3}H]-P bound/mg protein) decreased to almost nondetectable levels in the contralateral uterine horn and oviduct of the same animals following infusion of P (Fig. 11). The number of nuclear binding sites in the E_2-treated uteri were nondetectable and increased to $6.0 \pm 1.6 \times 10^{-15}$ mol [^{3}H]-R5020 bound/μg DNA after P-infusion whereas the nuclear binding sites in the oviducts increased from 2.7 ± 2.5 to $6.1 \pm 2.4 \times 10^{-15}$ mol [^{3}H]-R5020 bound/μg DNA. The decrease in [^{3}H]-P binding in cytosols, and increase in [^{3}H]-R5020 binding in nuclei following P-infusion were statistically significant ($P < 0.05$) in both tissues.

DISCUSSION

This report demonstrates that the methodology used to characterize the cytosol P receptor in the uterus of the rabbit [15] and the cytosol and nuclear P receptor in the uterus of the rat [17–21], can be used, with slight modification, to measure the cytosol and nuclear P receptor in the uterus and oviduct of the cat.

The cytosol receptor of the uterus and oviduct of the cat had essentially identical hormonal specificities which are similar to those described for the uterine cytosol receptor of other species [15, 17, 18]. The cytosol receptor was found to have a high specificity for P and the synthetic progestin R5020. These two ligands demonstrated superimposable inhibition curves. The concentration of the non-progestin ligands E_2, testosterone, and corticosterone, required to obtain the same magnitude of inhibition had to be approximately 100-fold greater. The fact that P and R5020 cause an equivalent maximal displacement of [^{3}H]-P (Fig. 3) and also [^{3}H]-R5020 (data not shown), indicates that they both are bound to the same molecule. This observation provides further evidence that cortisol prevents the binding of P to CBG [7] since R5020 does not bind CBG [15, 22].

The saturability characteristics of the [^{3}H]P binding observed in this study compare favorably to those reported in previous studies [15, 17, 18]. The addition of 10% glycerol to our buffer system enabled us to determine a sedimentation coefficient of 6.8 S for cytosol receptor in the uterus. Oviduct cytosols, prepared from E_2-treated animals, also displayed [^{3}H]-P binding in the 6.8 S region which was displaced by P but not by cortisol. Previous investigators have reported a sedimentation coefficient of 6-7 S for the cytosol receptor in the uterus of the rat, mouse and rabbit using either [^{3}H]-P or [^{3}H]-R5020 [15, 18, 23, 25]. The uterine and oviduct K_Ds for the cytosol receptor were 3.2×10^{-8} M and 2.5×10^{-8} M respectively. These are somewhat higher than the K_Ds reported for the uterus of the rat [17], guinea pig [26], and hamster [27]. However they are very similar to those reported recently in the rat [18].

Evidence is accumulating to substantiate the hypothesis that the action of P in mammalian systems involves the translocation of the cytosol receptor to the nucleus, similar to what was first described for the E_2 receptor system [28, 29]. In parallel groups of ovariectomized, E_2-primed rats [20, 21] good evidence was presented for *in vivo* translocation of the cytosol receptor. The subcutaneous administration of P caused a significant depletion of cytosol receptor and a parallel increase in nuclear receptor within an hour [21]. This was even more clearly demonstrated in this study where the compartmental modulation of cytosol and nuclear receptor were measured in the same animals both before and after P-infusion (Fig. 11). The low level of specific cytosol binding observed after 15 min of P-infusion correlated with a marked increase in nuclear binding. Our results suggest that under the hormonal conditions used in this study, which were designed to mimic those of pregnancy in the intact cat [11], most of the cytosol receptor may be translocated to the nucleus.

The inhibition of [^{3}H]-R5020 binding to nuclei isolated from animals infused with P by various steroids follows the same pattern as the inhibition of [^{3}H]-P binding in cytosol. This, coupled with the observation that little or no receptor could be demonstrated in nuclei from animals not subjected to P infusion, supports our contention that the binding of P in the cytosol, and R5020 in the nuclei, is to the same receptor.

The optimum incubation conditions required for equilibrium were similar for the uterine and oviductal nuclear receptor (Fig. 7). Unlike recent studies in the rat [20, 21], we did not observe maximum exchange at 0–4°C. This may represent species variation and suggests that the nuclear receptor in the reproductive tract of the cat is less sensitive to heat degradation than that in the rat. The K_D values we observed for the nuclear binding of [^{3}H]-R5020 were $6–8.2 \times 10^{-9}$ M which is consistent with that reported for the rat uterus [20, 21].

Cytosols and nuclei, prepared from the uteri and oviducts of the same animals, were treated identically in all experiments described in this study. We did not observe any statistically significant differences between the uterus and oviduct in any of the parameters tested for either the characterization of the P binding macromolecule(s) or in the determination of the optimum conditions for incubation. These data suggest that the P receptors in the uterus and oviduct of the cat are identical.

We have demonstrated that the principles of the exchange assay can be used to quantitate the specific

P binding macromolecule present in the reproductive tract of the cat. These techniques can now be used in additional studies to correlate plasma steroid levels and subcellular compartmentalization of the P receptor with specific morphological alterations in uterine and oviduct tissue. These studies should prove useful as we continue to clarify our understanding of the mechanism of P action in the reproductive tract.

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