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CYTOSOL AND NUCLEAR ESTROGEN AND PROGESTERONE RECEPTORS IN THE RABBIT ENDOCERVIX

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SUMMARY

This report describes the measurement of estrogen and progesterone receptors in cytosols and nuclear fractions from endocervical tissue components. Unoccupied cytosol estrogen receptor levels as determined by Scatchard analysis of [^{3}H]-estradiol binding data indicated a single class of high affinity binding sites for the epithelial–stromal complex ($K_D = 0.74 \times 10^{-9}$ M). Binding was specific for estrogen (estradiol > estriol > estrone) and unaffected by desoxycorticosterone, dihydrotestosterone and progesterone. Assays for total estrogen receptor verified that 71.6 ± 5.3% of this 8S estrogen receptor is in the epithelial–stromal complex while the remaining approximately 28% is localized in the stroma and fibromuscular wall, with the cells of the complex containing the highest receptor concentration. In 5-day pseudopregnant and ovariectomized rabbits compared to estrous rabbits there was a 50% decrease in the cytosol estrogen receptor in the epithelial–stromal complex and a 30% decrease in the concentration of nuclear receptor. Cytosol and nuclear progesterone receptors were measured as an indicator of estrogen action in the rabbit endocervix. Cytosol progesterone receptor concentrations (fmol/mg DNA) in 5-day pseudopregnant and ovariectomized animals were reduced to approximately 35% of the concentration in estrous animals. Nuclear progesterone receptor concentrations decreased 65% in 5-day pseudopregnant and 90% in ovariectomized animals suggesting decreased receptor synthesis. Collectively these data support the concept that the rabbit endocervix may be directly regulated by estrogens.

INTRODUCTION

Target cell response to steroid hormones involves gene expression and cytodifferentiation. This is well documented for a variety of mammalian reproductive tract tissues [1]. However, in the human endocervix, cyclical changes in cervical morphology have not been unambiguously defined. It is known that during the menstrual cycle estrogen stimulates the production of a thin, elastic watery mucus while progesterone dominance and/or estrogen deprivation results in a dry, scant, viscous mucus [2, 3]. Sanborn *et al.*[4, 5] report cytoplasmic estrogen binding activity in the human endocervix implying that endocervical events are under hormonal control even in the absence of obvious morphological changes. However, these authors were unable to demonstrate a significant difference in the levels of estrogen binding activity when tissues from the follicular and luteal phases were compared.

We have recently established that estradiol-17β modulates cell morphology in rabbit endocervical epithelium [6] and is required for glycoprotein biosynthesis [7]. Fifteen weeks after ovariectomy endocervical cells do not contain histochemically distinguishable neutral and acidic glycoproteins and incorporation of the glycoprotein precursor, [^{14}C]-*N*-acetyl-D-glucosamine, by these cells is negligible [7]. Administration of estradiol-17β to ovariectomized animals restores cells with histochemical and biosynthetic characteristics comparable to those from estrous animals. Since it is probable that the effects of estradiol-17β in the endocervix are mediated by an estrogen receptor through the two step translocation mechanism, we first characterized cytosol and nuclear estrogen receptor in tissue components of endocervices from rabbits in different hormonal states. Second, cytosol and nuclear progesterone receptor were measured as an indicator of estrogen action in the rabbit endocervix since it is generally accepted that estrogen stimulates the synthesis of progesterone receptor in the mammalian uterus [8, 9]. Two preliminary reports of this work have already been presented [10, 11].

MATERIALS AND METHODS

Reagents and buffers

Reagents were purchased from the following sources: [6,7-^{3}H(*N*)]-estradiol ([^{3}H]-E, SA 52.0 Ci/mmol), 17α-methyl-[^{3}H]-promegestone ([^{3}H]-R5020; SA 87.0 Ci/mmol) and radioinert R5020 from New England Nuclear Corp. (Boston, MA); 4-pregnen-3,20-dione (progesterone), 4-pregnen-21-ol-3,20-dione

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(desoxycorticosterone), diethylstilbestrol (DES), Norit A and bovine serum albumin (BSA) from Sigma Chemical Co. (St. Louis, MO); 5α-androstan-17β-ol-3-one (dihydrotestosterone), 1,3,5(10)-estratrien-3-ol-17-one (estrone) and 1,3,5(10)-estratrien-3-16α,17β-triol (estriol) from Steraloids, Inc. (Wilton, NH); Dextran-70 from Pharmacia Fine Chemicals, Inc. (Piscataway, NY); scintillation cocktail was Ready-Solv EP from Beckman Instruments, Inc. (Fullerton, CA); Hanks' balanced salt solution (HBSS) from Gibco (Grand Island, NY); DNA grade Bio-Gel HTP hydroxylapatite from Bio-Rad Laboratories (Richmond, CA).

Tris–HCl buffers, pH 7.5, containing potassium chloride (K), or disodium ethylenediamine tetraacetic acid (E), dithiothreitol (D) or monothioglycerol (M) were designated according to the millimolarity of their components, i.e. $T_{50}E_1M_{12}$ = 50 mM Tris-HCl/1 mM disodium ethylenediamine tetraacetic acid/12 mM monothioglycerol. Glycerol was added on a vol./vol. basis. Linear 10–30% sucrose gradients were prepared in $T_{10}E_1D_{10}$. Hydroxylapatite slurry (wet hydroxylapatite:buffer approximately 0.5 (vol./vol.): pH 7.4) was prepared according to Garola and McGuire[12, 13]. The dextran-charcoal solution was prepared by suspending 0.5 g Norit A and 50 mg Dextran-70 in 100 ml $T_{10}E_1M_{12}$.

Tissue preparation

Adult (6–8 month old), virgin New Zealand White rabbits were caged individually for 3 weeks to insure against poor health at the time of experimentation. Animals were either estrous, pseudopregnant or ovariectomized. Estrous rabbits displayed a characteristic lordosis behavior, vaginal redness and a total of 8–12 mature follicles were present in the ovaries at the time of laparotomy. Pseudopregnancy was induced, 5 days before sacrifice, with an ear vein injection of 15–20 IU of human chorionic gonadotropin followed by cervical stimulation resulting in 8–12 corpora lutea at the time of laparatomy. Rabbits were bilaterally ovariectomized through a mid-ventral incision and maintained for 16–36 weeks prior to experimentation. All experiments were done in duplicate and 2–3 animals were used in each experiment. Animals were sacrificed with an ear vein injection of 70% ethanol and cervices were removed within 5 min of death. Cervices were rinsed free of blood in HBSS and cut open longitudinally to reveal 5–8 prominent longitudinal folds [14]. For one group of estrous animals, endocervical folds were microdissected from intact cervices [15] and stroma was scraped from the underlying fibromuscular wall using a scalpel blade. Histological evaluation of hematoxylin and eosin-stained tissue sections (not shown) revealed that folds (epithelial–stromal complex) contained mucociliary epithelial crypts plus some underlying stroma. Scraped stroma and fibromuscular wall contained, respectively, stromal cells with minimal epithelial contamination and fibromuscular elements. Pieces of tissue were uniformly cut from the center of each endocervix and checked histologically to insure that all three tissue components were present in what was termed whole tissue. For all other studies, using animals in different hormonal states, only the microdissected epithelial–stromal complexes and whole tissue were assayed for receptor content.

Assay of estrogen and progesterone receptor in cytosol

Tissue (intact cervices, folds, stroma and fibromuscular wall) were weighed and homogenized (100 mg/ml) in ice cold $T_{10}E_1D_3$ containing 10% glycerol with a Polytron Pt-10 homogenizer (Brinkman Instruments, Westbury, NY). Aliquots (100 μl) were reserved for DNA assay. The cytosol fractions were prepared by centrifugation of each tissue homogenate at 40,000 *g* for 40 min.

Unoccupied estrogen receptor and progesterone receptor concentrations were determined by Scatchard analysis of specific binding data [16]. Aliquots (100 μl) of cytosol were incubated in a final volume of 300 μl with increasing concentrations of [^{3}H]-E (0.3–15.0 nM) or [^{3}H]-R5020 (0.6–45.0 nM). Parallel samples were incubated with 100-fold excess of radioinert DES or radioinert R5020 for evaluation of nonspecific binding of radioactive-ligand. Samples were incubated at 4°C for 2–3 h. Following incubation, receptor bound steroid was separated from unbound steroid using procedures described by Garola and McGuire[12, 13]. In summary, after the addition of 250 μl of hydroxylapatite slurry samples were kept at 4°C for an additional 30 min and mixed at 5–10 min intervals. They were then centrifuged at 2400 *g* for 10 min and the resulting pellets washed three times with 1.5 ml Tris-phosphate buffer (50 mM Tris–HCl, 10 mM potassium phosphate; pH 7.2). Bound steroid was extracted from each pellet by incubation in 1.0 ml ethanol at room temperature for 30 min, with mixing every 5–10 min. Samples were centrifuged at 2400 *g* for 10 min. Radioactivity was quantitated in 4 ml scintillation cocktail.

At the time that nuclear fractions were assayed, cytosol progesterone and total estrogen receptor concentrations were determined using a single saturating dose assay. Endocervical folds were homogenized (100 mg/ml) in $T_{50}E_1M_{12}$ containing 30% glycerol using a Polytron Pt-10 homogenizer. A 100 μl aliquot was removed for the DNA assay. The remaining tissue homogenates were centrifuged at 1200 *g* for 20 min to obtain a nuclear fraction. Further processing of the nuclear fractions is described in the next section. Cytosol samples (200 μl) prepared as described above were incubated in a total volume of 400 μl with a final concentration of 15 nM [^{3}H]-R5020 ± 1.5 μM radioinert R5020 or 5 nM [^{3}H]-E ± 0.5 μM DES. Samples were then incubated at 4°C for 18–20 h to measure total progesterone receptor and at 30°C for 3 h to measure total estrogen receptor. Optimum time for exchange was determined by incubating samples for 0.5, 1.0, 1.5, 4, 6, 18, 21 and 24 h for progesterone

receptor and 0.5, 1.0, 1.5, 2, 3, 4 and 5 h for estrogen receptor. Following incubation bound steroid was separated from free using hydroxylapatite as described above.

Extraction of nuclear receptor and assay protocol

The methodology of Chen and Leavitt[17] and Evans *et al.*[18] was used with minor modification for the extraction of nuclear estrogen and progesterone receptors from the nuclear-fibrillar suspensions prepared from tissue components of the rabbit endocervix. The nuclear pellet was washed twice by resuspension in T_{10} containing 10% glycerol (vol./vol.) and centrifuged at 1500 *g* for 10 min. Nuclear receptors were extracted by resuspension in $T_{50}E_1M_{12}$ containing 30% glycerol and 500 mM KCl. Nuclei were incubated for 2 h at 4°C with mixing every 10 min. Nuclear debris was removed by centrifugation at 40,000 *g* for 30 min. The resultant supernatant was assayed for nuclear estrogen and progesterone receptor content.

Receptor concentrations were measured by Scatchard analysis [16] of specific binding data. Nuclear extract (300 μl) was incubated in a total volume of 500 μl with increasing concentrations of [^{3}H]-R5020 (3.0–60.0 nM) and [^{3}H]-E (1.5–60.0 nM). Identical samples were incubated in 100-fold excess radioinert R5020 or DES for quantitation of non-specific binding. Samples were incubated at 4°C for 18–20 h to determine total nuclear progesterone receptor. Total nuclear estrogen receptor was measured by incubation at 30°C for 3 h. Unbound steroid was removed by incubation with 500 μl dextran-charcoal solution (30 s for progesterone receptor; 10 min for estrogen receptor), followed by centrifugation at 2400 *g* for 10 min. Radioactivity was quantitated in a 500 μl aliquot of the supernatant using 4 ml of scintillation cocktail. Optimum time for exchange was established as described for estrogen and progesterone receptors in the cytosol fraction. Having established optimal conditions we then used a single saturating dose assay. Nuclear extract (300 μl) was incubated in a total volume of 500 μl with a final concentration of 12 nM [^{3}H]-R5020 ± 1.2 μM radioinert R5020 or 4 nM [^{3}H]-E ± 4 μM radioinert DES. Samples were processed as described above.

Competition assay

Cytosols wee prepared from epithelial–stromal complexes of estrous rabbits and 100 μl aliquots were incubated in a final volume of 300 μl. The assay mixture contained 15 nM [^{3}H]-E and increasing concentrations (0.015–1.5 μm) of the following radioinert competitors: progesterone, desoxycorticosterone, dihydrotestosterone, estrone, estriol and estradiol. Each assay was incubated and processed according to the protocol described for unoccupied estrogen receptor.

Sedimentation analysis

Aliquots (200 μl) of cytosol prepared from epithelial–stromal complexes homogenized in $T_{10}E_1D_{10}$ were incubated in a final volume of 300 μl with 40 nm [^{3}H]-E ± 4.0 μM DES for 2–3 h at 4°C. Samples (200 μl) from each incubation were then layered on 5.0 ml 10–30% sucrose gradients in $T_{10}E_1D_{10}$ pH 7.4. Gradients were centrifuged at 0°C for 16 h at 300,000 *g* in an SW 50.1 Beckman Rotor. Successive 125 μl fractions were collected from the bottom of each tube and radioactivity was quantitated in scintillation cocktail. Internal markers of ^{14}C labeled ovalbumin (3.6S), rabbit IgG (6.6S) and catalase (11S) were used to calibrate the gradients. Specific binding was then plotted against the fraction number.

Radioimmunoassay for steroids

Blood samples were collected from the marginal ear vein of 5-day pseudopregnant and estrous animals ($n = 15$) just before ovariectomy and 4–15 weeks following ovariectomy. All blood samples were allowed to clot at room temperature for 20–30 min. Serum (approximately 15 ml) was collected and stored frozen (−20°C) for steroid assay. Each sample was thawed and extracted with diethyl ether for estrogen assay. Total estrogens were measured on 2–3 ml samples by radioimmunoassay [19] and expressed as pg/ml ± SEM. Sensitivity of this assay was 10 pg. The percentage recovery of [^{3}H]-estradiol in the total assay was 87%. The degree of cross reactivity of the antiserum to estradiol used in this radioimmunoassay is 100% for estradiol and 80% for estrone.

Serum samples were also used for progesterone radioimmunoassay [20]. Sensitivity of this assay was 50 pg. The degree of cross reactivity of the antiserum to progesterone used in this assay is 100% for progesterone, 15% for 5α-pregnan-3,20-dione, 6% for 5β-pregnane-3,20-dione, 5% for pregnenolone, and only 1% for both 20α-hydroxypreg-nen-4-en-3-one and 17β-hydroxyprogesterone. Results were expressed as ng/ml steroid ± SEM.

Protein and DNA assays

Protein concentrations were measured in cytosol fractions according to the method of Lowry *et al.*[21] using BSA as the standard. The procedure of Burton [22] was used to determine DNA content using calf thymus DNA as the standard.

Data analysis

Standard parametric statistics and the Student *t*-test were used throughout.

RESULTS

Using estrous rabbits the determination of unoccupied estrogen receptor concentrations by Scatchard analysis of [^{3}H]-E binding data indicated a $K_D = 0.74 \times 10^{-9}$ M for epithelial–stromal complex as obtained from our isolation procedure, and a $K_D = 0.84 \times 10^{-9}$ M for intact cervices. In addition the linearity ($r > 0.97$) indicated a single class of high

affinity binding site. The observed K_D values fell within the range of those for the estrogen binding protein in the human endocervix [4] and the estrogen receptor in the rat uterus [23]. Binding capacity was 5034 fmol/mg DNA (750 fmol/mg protein) for epithelial–stromal complex compared to 1907 fmol/mg DNA (360 fmol/mg protein) for intact tissue. The optimum specific binding of [^{3}H]-E in epithelial–stromal complex of estrous rabbits occurred after 60 min and remained constant for 5 h at temperatures of 4° and 30°C. Therefore we selected a time interval of 2–3 h for subsequent assays.

Assays for total estrogen receptor demonstrated that 71.6 ± 5.3% of the total endocervical estrogen receptor was localized in the epithelial–stromal complex with the remaining 28% equally distributed in the stroma and fibromuscular wall. The specific binding (mean ± SEM) was 4125 ± 154 fmol/mg DNA for epithelial–stromal complex, 5309 ± 1352 fmol/mg DNA for stroma, and 2013 ± 138 fmol/mg DNA for fibromuscular wall. Scatchard analysis of [^{3}H]-E binding to nuclear estrogen receptor indicated a $K_D = 2.43 \times 10^{-9}$ M and $r = 0.93$ when samples were incubated for 2–3 h. The concentration of estrogen receptor complexes that were translocated and retained in the nuclei of cells from endocervical tissue components and whole tissue are listed in Table 1. Nuclear estrogen receptor levels were similar in epithelial–stromal complex and stroma from estrous rabbits. There was however a significant ($P < 0.05$) decrease in the total number of estrogen receptors in the cytosol of epithelial–stromal complex (2107 ± 246 fmol/mg DNA) and a concomitant 30% decrease in the concentration of the nuclear receptor in progesterone dominated (5-day pseudopregnant) rabbits (Table 1). Values for whole tissue are also listed for comparison.

Since concentrations of total and unoccupied cytosol estrogen receptor from target tissues of ovariectomized animals are theoretically identical, values were pooled and included in Table 1. As expected, the concentration of cytosol estrogen receptor in the epithelial–stromal complex was higher for ovariectomized rabbits than in estrous controls, while the concentration of nuclear estrogen receptor in the epithelial–stromal complex from ovariectomized animals was minimal (Table 1).

Epithelial–stromal complexes from estrous and 5-day pseudopregnant animals were used to study the sedimentation properties of cytosol estrogen receptor. Cytosols contained saturable estrogen receptor peaks with 8S sedimentation coefficients. Thus the sedimentation properties of cytosol estrogen receptors from rabbits in contrasting hormonal states are identical to the sedimentation characteristics of estrogen receptors from other target tissues such as the rat uterus.

The analysis of estrogen–cytosol binding specificity demonstrated that estrogens (estradiol > estriol > estrone) were effective competitors, whereas desoxycorticosterone, dihydrotestosterone and progesterone did not compete to any great extent.

Since it is generally accepted that estrogen stimulates the synthesis of progesterone receptor in the mammalian uterus, this receptor was measured as an indicator of estrogen-regulated gene expression in the rabbit endocervix. Optimum exchange for cytosol progesterone receptor occurred after 6 h and continued for 24 h at 4°C. Assays for progesterone receptor demonstrated that 64.3 ± 2.5% of the total endocervical progesterone receptor was localized in the epithelial–stromal complex of estrous rabbits, with 11.0 ± 4.3% in the stroma and 24.7 ± 1.8% in the fibromuscular wall. Scatchard analysis of [^{3}H]-R5020 binding data in the epithelial–stromal complex cytosol indicated the K_D values were the same for estrous ($K_D = 0.21 \pm 10^{-9}$ M; $r = 0.98$) and pseudopregnant ($K_D = 0.26 \times 10^{-9}$ M; $r = 0.97$) rabbits. The binding capacity was 5468 ± 1009 fmol/mg DNA for the epithelial–stromal complex from estrous rabbits, whereas for pseudopregnant rabbits it was dramatically reduced to 1823 ± 318 fmol/mg DNA, a value comparable to that obtained for ovarian hormone-deprived rabbits (Table 2). The reduction in progesterone receptor might be due to a decrease in receptor synthesis caused by estrogen withdrawal or

Table 1. Distribution of estrogen receptor* in tissue components of the rabbit endocervix

Animals	Total cytosol (fmol/mg protein)	Total cytosol (fmol/mg DNA)	Nuclear (fmol/mg DNA)
Estrous			
Epithelial–stromal complex	516 ± 13	4125 ± 154	356 ± 25
Stroma	524 ± 30	5309 ± 1352	354 ± 105
Fibromuscular wall	246 ± 13	2013 ± 138	557 ± 68
Whole tissue	226 ± 58	1746 ± 323	223 ± 78
5-Day pseudopregnant			
Epithelial–stromal complex	286 ± 36	2107 ± 246	225 ± 53
Whole tissue	84 ± 2	597 ± 37	202 ± 38
Ovariectomized			
Epithelial–stromal complex	940 ± 353	2442 ± 674	130†*
Whole tissue	1072 ± 422	1983 ± 318	5†*

* Values expressed as means ± SEM. †* Values from a single assay using tissue from two animals.

Table 2. Distribution of progesterone receptor* in tissue components of the rabbit endocervix

Animals	Total cytosol		Nuclear
	fmol/mg Protein	fmol/mg DNA	(fmol/mg DNA)
Estrous			
Epithelial–stromal complex	839 ± 189	5468 ± 1009	392 ± 62
Stroma	550 ± 2	5768 ± 1737	359 ± 37
Fibromuscular wall	781 ± 101	6358 ± 717	1580 ± 256
5-Day pseudopregnant			
Epithelial-stromal complex	307 ± 60	1823 ± 318	145 ± 87
Ovariectomized			
Epithelial-stromal complex	504 ± 182	1434 ± 229	37†*

* Values expressed as means ± SEM. †* Values from a single assay using tissue from two animals.

to increased nuclear translocation in response to elevated endogenous progesterone levels. However, this last possibility does not appear to be likely. In fact, the measurement of progesterone receptor in KCl extracts of nuclear fractions resulted in values of 392 ± 62 fmol/mg DNA for estrous, 146 ± 87 fmol/mg DNA for pseudopregnant rabbits and 37 fmol/mg DNA in ovarian hormone-deprived rabbits. Optimum exchange conditions for measuring bound specific radioactivity in the KCl extracts of nuclear suspensions were the same as for cytosol. Scatchard analysis of [^{3}H]-R5020 binding in the KCl extracts of nuclear suspensions from estrous animals indicated the K_D = 3.85×10^{-9} M ($r = 0.99$) is of the order reported for the rabbit uterus from estrus through day 8 of pregnancy [24].

As previously reported [7] serum concentrations (mean ± SEM) for total estrogens was 26.3 ± 2.5 pg/ml and for progesterone was 1.44 ± 0.70 ng/ml in estrous rabbits. Following ovariectomy, there was no significant change in total estrogen levels (28.3 ± 2.3 pg/ml), but progesterone levels were significantly ($P < 0.01$) reduced to 0.21 ± 0.04 ng/ml. Overstrom and Black[25] have reported similar findings. In contrast, values increased significantly ($P < 0.01$) to 55.13 ± 11.1 pg/ml for total estrogens and 9.21 ± 1.7 for progesterone in 5-day pseudopregnant rabbits.

DISCUSSION

Alterations in cervical mucus secretions are correlated with cyclic changes in estrogen levels in the human [2, 3]. Odor[26] and Riches *et al.*[14] reported a decrease in the number and type of secretory granules in rabbit endocervical cells after ovariectomy and their restoration after the administration of estrogen. Recently we have used the rabbit as an experimental model to demonstrate that estradiol-17β directly affects endocervical cytodifferentiation and is required for glycoprotein biosynthesis by specific epithelial cell types [6, 7]. Molecular mechanisms of steroid action predict the presence of specific steroid receptors. The experiments described here characterize the estrogen receptor in cytosol and nuclear fractions prepared from endocervices of rabbits in contrasting hormonal states.

The mean serum levels for total estrogens and for progesterone in estrous rabbits are comparable to values published by others [27]. In these animals both cytosol and nuclear estrogen receptors are typical of those described for other target tissues [4, 23, 28] and both are abundant in the endocervical epithelial–stromal complex which contains stromal, and epithelial cell populations maximally active in glycoprotein biosynthesis. An estrogen binding protein has also been identified in the epithelial–stromal complex of the human endocervix [4] and Stumpf and Sar[29] have presened autoradiographic evidence that estrogen and progesterone accumulate in the epithelial–stromal complex of the guinea-pig cervix. Previous studies from this laboratory [6] have demonstrated that in 5-day pseudopregnant rabbits the number of glycoprotein containing epithelial cells in the rabbit endocervix is reduced by approximately 50% compared to estrous rabbits. The present study verifies that there is a concomitant 50% decrease in the total number of cytoplasmic estrogen receptors accompanied by a 30% decrease in the concentration of nuclear receptors. Since progesterone is known to inhibit estrogen receptor replenishment in the rat uterus [30] these results suggest that the reduction in estrogen receptor might be due to the 6-fold increase in serum progesterone levels by day 5 of pseudopregnancy, an increase comparable to that reported for both pregnant [31, 32, 33] and pseudopregnant [27] rabbits. We attempted to correlate this suggestion by experiments with ovariectomized rabbits only to find that while concentrations of cytosol estrogen receptor are comparable to values for progesterone dominated animals, serum progesterone levels have plummeted and serum estrogen levels are not significantly different from values for estrous animals. These data are difficult to reconcile. Batra *et al.*[34] have demonstrated a considerable decrease in combined uterine and cervical estradiol levels in 6-day pseudopregnant rabbits compared with estrous controls, in spite of a relatively constant level of plasma estradiol. This leads us to speculate that peripheral steroid levels

may not reflect their local, i.e. endocervical, availability.

It is known that estrogen stimulates the synthesis of progesterone receptor in the mammalian uterus [8, 9], while progesterone reduces progesterone receptor levels [8]. Values for cytosol and nuclear progesterone receptors in 5-day pseudopregnant animals are reduced to approximately 35 and 65% of the values for estrous animals. These data indicate that the low levels of cytosol progesterone receptor may be due to decreased receptor synthesis rather than increased nuclear translocation. Further experiments are required to characterize the kinetics of nuclear clearance especially since we know that a single injection of progesterone causes nuclear translocation of receptors [24, 35] while prolonged exposure results in a decline in nuclear receptor in the rabbit uterus [36].

The concentrations of estrogen receptor for whole estrous tissue (Table 1) are lower than the receptor concentrations in the tissue components. Assaying receptor content of separate tissue components (epithelial–stromal complex, stroma, fibromuscular wall) of the endocervix, rather than of whole tissue homogenates, is a more sophisticated approach to define target cell response to steroid hormones. This approach has shown that 71.6 ± 5.3% of the cytosol estrogen receptor is present in the epithelial–stromal complex, although these cells and the stromal cells have an equivalent receptor concentration when values are expressed as fmol/mg DNA. Similarly, approximately 64% of the total cytosol progesterone receptor is localized in the epithelial–stromal complex, while all three endocervical tissue components contain equivalent concentrations of receptor. McCormack and Glasser[23] have reported that while epithelia and stroma of immature rat uteri have the highest concentrations of cytosol estrogen receptor, stromal and epithelial cells contain only 5–10% of the receptor. In contrast, the uterine myometrium contains the lowest estrogen receptor concentrations but accounts for 84% of the total uterine estrogen receptor. The contrasting distribution of steroid receptors in the rabbit and the immature rat may be due to interspecies variability. Alternatively it may be due to a greater muscle mass in the rat uterus compared to the rabbit endocervix or perhaps it is due to the possibility that the myometrium of the rat uterus is more responsive to ovarian steroids than the fibromuscular wall of the rabbit endocervix. However, the presence of different cell populations may also account for these results. For instance, the cellular composition of the uterine stroma is dynamic and changes in protein synthetic events are probably under hormonal control [37]. In addition, we know that the rabbit endocervical epithelium is a composite of four morphologically distinct cell populations, two of which are actively involved in glycoprotein biosynthesis [6, 7]. The variations demonstrated in the study in endocervical receptor levels from animals in contrasting hormonal states may reflect changes in receptor levels in one or more of these cell types. We are currently investigating this possibility by monitoring hormone-related receptor concentrations in endocervical cell populations as isolated by unit gravity sedimentation.

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