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HORMONAL ACTION IN HUMAN CERVIX—II SPECIFIC PROGESTOGEN BINDING PROTEINS IN HUMAN CERVIX

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SUMMARY

Progesterone binding activity has been detected in cytosols prepared from human cervical tissue. Cytosols showed high affinity (K_a 0.2–1 nM^{-1}) for progesterone and synthetic progestogens but not for corticosteroids or other steroids (norethindrone, medroxyprogesterone acetate > chlormadinone acetate, progesterone > 5α-pregnane-3,20-dione > norethisterone acetate ≫ 17α-hydroxyprogesterone ≫ cortisol, estradiol). Addition of 49 nM cortisol to the assay buffer permitted measurement of high affinity progesterone binding sites in those cytosols with high concentrations of CBG-like contaminants. The hormone dissociated from the complex with a dissociation rate constant of $6.9 \times 10^{-5}\ s^{-1}$ (0°C, 18% glycerol).

Binding sites exhibited similar K_a values throughout the cervix and were found in highest concentration in the region encompassing the columnar epithelium when expressed per mg protein and per g. The concentrations of sites per mg DNA were similar in the columnar epithelium and in the stroma but higher than in the region of the squamous epithelium.

Endometrial tissue exhibited higher concentrations of progesterone binding sites than did the corresponding cervix. In both the endometrium and cervix, the mean concentrations expressed per mg protein were significantly higher for proliferative phase than for secretory phase tissues. The corresponding differences in concentration per mg DNA were significant only for cervical cytosols.

INTRODUCTION

Both the chemical content and physical properties of human cervical secretions are known to be influenced by sex steroids. While these changes can be followed throughout the menstrual cycle [1], they are most readily demonstrated in patients taking sequential estrogen and progestogen oral contraceptives [2]. In these cases, an increase in mucus viscosity and in the concentration of soluble proteins follows the onset of progestogen administration. Use of estrogen–progestogen combination preparations usually results in production of the high viscosity mucus characteristic of the secretory phase [3].

Since estradiol and progesterone appear to influence cervical mucus production, the current dogma on steroid hormone action would predict that the corresponding hormone receptors would be found in human cervical tissue. Properties of specific estrogen binding activity in human cervix have previously been reported [4]. While specific progesterone binding proteins have been found and purified from human endometrium and myometrium [5–15], there has been no detailed study in human cervical tissue. Following injection of ^{3}H-progesterone into the guinea-pig, label has been localized in the cervix [16]. Progesterone receptors have recently been studied in guinea-pig cervical cytosol [17].

This paper describes some properties of specific progestogen binding activity in human cervix and follows the fluctuation in the level of binding activity throughout the menstrual cycle.§

MATERIALS AND METHODS

Cytosol preparation. Patients ranged in age from 26–48 and were undergoing hysterectomy for benign conditions such as the presence of leiomyomas or pelvic relaxation. Postmenopausal women or women with cervical carcinoma were not included. When a patient was using oral contraceptive medication for two or more months immediately prior to surgery, this is indicated.

Hysterectomy specimens were rapidly transported to the pathology department following removal from the patient. After examination by the pathologist, the remaining cervix and endometrial tissue were immediately placed in cold Buffer A (0.01 M Tris–HCl, 1 mM EDTA, 0.25 M sucrose, pH 8.0) and transported to the laboratory. All subsequent manipulations were performed at 0–4°C.

The tissues were washed as free of blood and mucus as possible, minced and homogenized with three volumes of Buffer A plus 1.7 mM β-mercaptoethanol

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with a Polytron PT 10 homogenizer utilizing short bursts (20 s, setting 5) with intermittent cooling, and centrifuged (10,000 *g*, 10 min; 142,000 *g*, 90 min). Glycerol was added to the resulting supernatants to a final concentration of 10% (v/v). Aliquots were rapidly frozen and stored at −190°C.

Cycle stage was assessed on the basis of three criteria. First, histological sections of the endometrium were examined and dated with the aid of a pathologist. Second, the plasma estradiol and progesterone levels in a blood sample drawn immediately prior to surgery were measured. Third, the patient history and date of the onset of the last menses were obtained. Using the information from these three sources it was often possible to date the endometrium ± 2 days even when the patient had an irregular menstrual history. Specimens with irregular endometrial development or grossly anomalous plasma hormone levels were excluded from the study.

Measurement of binding sites. Two methods were employed to determine the number of progestogen binding sites. The first made use of Scatchard [18] plots. Aliquots (150 μl cervical cytosol or 50 μl endometrial cytosol) were incubated (3 h, 10°C) with 1 nM ^{3}H-progesterone ([1,2,6,7-^{3}H]-progesterone, 81 Ci/mmol, Amersham–Searle) plus 2–40 nM unlabeled progesterone in Buffer B (0.01 M tris–HCl, 1 mM EDTA, 30% glycerol [v/v], pH 8.0) ± 49 nM cortisol as indicated in a total vol. of 0.325 ml. Low affinity binding was assessed separately by incubation in the presence of labeled hormone plus 1.3 μM unlabeled hormone. The free steroid was removed with 1.0 ml of a 0.25% carbon (Norite A, Fisher, Lot 722893) 0.025% Dextran (Pharmacia, T-70) suspension (agitated and exposed for 10 min, 0°C; centrifuged for 10 min, 2500 rev/min, 4°C, International PR 6, 256 rotor). The separation conditions were optimized for cervical progestogen binding. Total bound counts declined rapidly during the first 10 min in the presence of charcoal and then more slowly. In contrast, specifically bound counts (as defined above) remained essentially constant for 5–30 min under the same conditions (3233, 2597, and 2245 c.p.m. at 5, 15, and 30 min, respectively, in a specific example). This behavior is consistent with the dissociation half-time of 2.8 h determined for specific binding (*vide infra*). The bound hormone in the supernatant was counted in RPI-toluene (Research Products International, PPO-dimethyl-POPOP) fluor at 56% efficiency. This system was validated for completeness of extraction and absence of quench. Individual data points represent the mean of triplicates corrected for low affinity binding. Scatchard plots were analyzed by the method of least squares.

In the saturation assay aliquots of cytosol (usually 50, 100, and 150 μl) were incubated in Buffer B (3 h, 10°C, total vol. 225 μl) with a saturating concentration (15 nM) of ^{3}H-progesterone ± 2.0 μM unlabeled hormone to correct for low affinity binding. Samples were treated with charcoal as described. All determinations were preformed in triplicate and data are reported as the mean ± S.E. of the determinations at the three concentrations where applicable. The estimations of the number of binding sites by Scatchard analysis and the saturation method were equivalent, within experimental error.

Cytosol protein was determined as described by Layne [19] using BSA as standard. DNA was determined by the method of Burton [20] using calf thymus DNA as standard. Enzyme studies utilized pronase (Calbiochem), trypsin (Worthington), and collagenase (Nutritional Biochemicals Corp.)

Relative affinity constants. The unlabeled steroids under discussion were dissolved in alcohol and pipetted into tubes. The solvent was evaporated under vacuum and the steroids* redissolved in 2.5 μl ethanol. ^{3}H-progesterone and Buffer B were added with mixing, followed by cytosol. The tubes were incubated (0°C, 16 h) and free hormone was removed by the charcoal procedure. Relative affinity constants were calculated as described by Korenman [21], according to the equation: RAC = [R (RA)]/[R + 1-RA] where RAC = relative affinity constant, RA = the ratio of the concentrations of unlabeled steroid to progesterone required to reduce the specifically bound label by 50%, and R = the free/bound ^{3}H-progesterone ratio at 50% competition.

RESULTS

Specificity of binding. Examination of the relative affinity of a series of steroids for progesterone binding sites revealed cytosols with varying degrees of contamination with CBG-like components. Some cytosols (Group 1) showed high affinity for progesterone (K ~ 0.2–1 nM^{-1}) and synthetic progestogens but low affinity for cortisol (relative affinity constant ~ 0.001). Figure 1 illustrates a typical pattern of competition for ^{3}H-progesterone binding sites using such a cytosol. Table 1 summarizes the relative affinity constants calculated for this type of cytosol.

Other cytosols (Group 2) exhibited the behavior shown in Fig. 2. Progesterone was much less potent as an inhibitor of ^{3}H-progesterone binding and cortisol was relatively more potent (relative affinity constant ~ 0.02). It appeared that in these cytosols the contamination with CBG-like components which

* Abbreviations used: Norethindrone: 19-Nor-17α-ethinyl-4-androsten-17β-ol-3-one; Medroxyprogesterone acetate: 6α-methyl-17α-hydroxyprogesterone acetate; Chlormadinone acetate: 6-chloro-17α-hydroxy-4,6-pregnadiene-3,20-dione; Norethisterone acetate: 19-nor-17α-ethinyl-4-androsten-17β-ol-3-one acetate; Norgestrel: 13-ethyl-17-hydroxy-18, 19 nor-17α-pregn-4-en-20yn-3-one; Ethynodiol diacetate: 17α-ethinyl-4-estrene-3β,17β-diol diacetate; Norethynodrel: 17α-ethinyl-17β-hydroxy-5(10)-estrene-3-one; R5020: 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione; Cyproterone acetate: 1,2α-methylene-6-chloro-4,6-pregnadiene-17α-ol-3,20-dione-17-acetate; CBG: corticosteroid binding globulin; RAC: relative affinity constant.

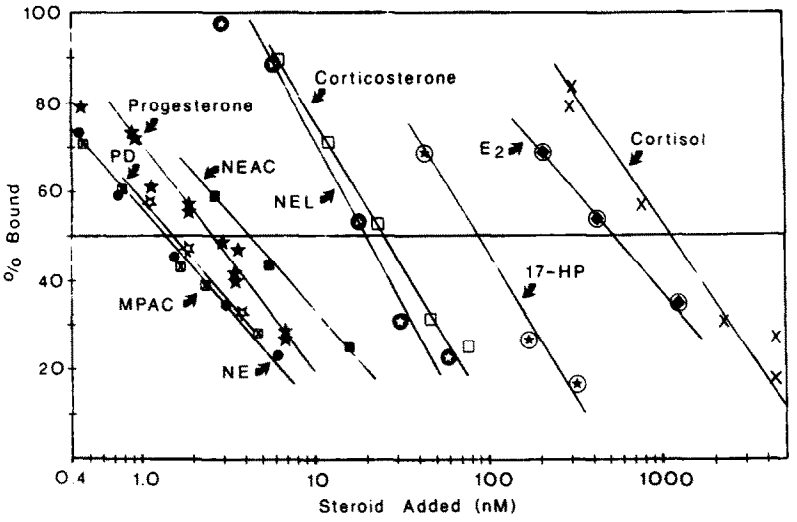

Fig. 1. The ability of unlabeled steroids to compete with ^{3}H-progesterone for binding sites in a cytosol containing little contamination with CBG-like components (Group 1). Free/bound hormone at 50% displacement was 5.0. Steroids used were medroxyprogesterone acetate (MPAC, ✿), norethindrone (NE, ●), 5α-pregnane-3,20 dione (PD, ✲), progesterone (★), norethisterone acetate (NEAC, ■), norethynodrel (NEL, ✪), corticosterone (□), 17α-hydroxyprogesterone, (17-HP ⊛), 17β-estradiol (E_2, ◈), and cortisol (×).

bound both progesterone and cortisol was high relative to specific progesterone binding sites. The degree of contamination did not appear to correlate with cycle stage. A similar study was conducted using serum from one of the patients. Unlabeled cortisol, corticosterone, and progesterone did not compete with ^{3}H-progesterone at 1–10 nM; all three effected a 10% competition at 30 nM. This behavior is in marked contrast to the data in Fig. 1.

Effect of cortisol on ^{3}H-progesterone binding. Critical to this study was the measurement of only specific progesterone binding sites. It can be inferred from

Table 1. Relative affinity constants of steroids for progesterone binding sites in human cervical and uterine cytosols

Steroid	Cervix, RAC[a]		Literature Values			
	Group 1 Cytosols	Group 2 Cytosols	RA (13)	RAC (9)	RA (8)	RA (11,15)
Progesterone	1.0	1.0	1.0	1.0	1.0	1.0
Norethindrone	1.9	1.4 (1.6, 1.3)	1.1	0.36	1.0	1.5
Medroxyprogesterone acetate	1.5	1.7	2.0	0.98	0.83	0.90
Chlormadinone acetate	0.91	1.5 ± 0.4	1.2	–	–	0.50
R5020	–	1.3	–	–	0.77	–
Cyproterone acetate	–	0.39	–	–	–	–
5α-pregnane -3,20-dione	0.67	0.29 ± 0.05	0.30	–	–	0.12
Norethisterone acetate	0.58	0.22 (0.15, 0.30)	0.21	0.05	–	–
Norgestrel	0.31	0.83 ± 0.09	1.6	–	–	–
Norethynodrel	0.14	–	0.22	0.33	0.04	–
5β-Dihydrotestosterone	–	0.046	–	–	–	–
5β-pregnane -3,20-dione	0.034	0.038 (0.026, 0.049)	0.12	–	–	0.06
Ethynodiol diacetate	–	0.066 (0.078, 0.054)	–	0.003	–	–
Testosterone	–	<0.046	–	<0.001	0.025	0.02
17α-hydroxyprogesterone	0.025	–	0.007	<0.001	0.058	0.03
Cortisol	0.001 (0.0014, 0.0005)	–	<0.001	<0.001	0.0001	<0.01
Corticosterone	0.064 (0.089, 0.039)	–	–	<0.001	–	0.05
Estradiol, 17β	0.0041	–	0.008	–	0.0001	<0.01

[a] Data from the present study. Single numbers represent the results of one or more determinations using the same cytosol. Means determined using two cytosols are listed with the individual values in parentheses. Means ± standard errors are given for values determined in three or more cytosols. Group 2 cytosols contained much higher contamination with CBG-like components than Group 1 cytosols and were assayed with 49 nM cortisol in the buffer as described elsewhere. RAC = relative affinity constant; RA = ratio of affinities [21].

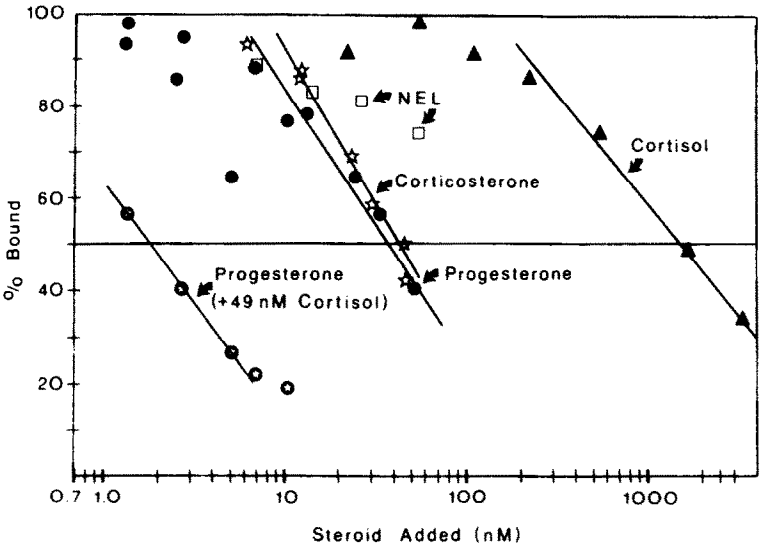

Fig. 2. The ability of unlabeled steroids to compete with ^{3}H-progesterone for binding sites in a cytosol containing a relatively high concentration of a CBG-like component (Group 2). Free/bound hormone at 50% displacement was 5.1. Steroids used were progesterone (●), corticosterone (✡), norethynodrel (NEL, □), and cortisol, (▲). The progesterone competition curve when 49 nM cortisol is included in the incubation buffer is also shown.

the data in Fig. 2 that, in the presence of a CBG-like component, the total ^{3}H-progesterone bound would be spuriously high. Furthermore, the binding in the presence of a 100-fold excess of unlabeled progesterone would not necessarily serve to delineate binding to a single class of sites. If the CBG-like sites, which presumably would have a higher affinity for cortisol than the "receptor" sites, were occupied with unlabeled cortisol, the ^{3}H-progesterone should bind predominantly to progesterone "receptor" sites. A concentration of 49 nM cortisol was chosen because it did not interfere with the specific progesterone sites under the conditions in Fig. 1. Figure 3A shows the Scatchard plots for progesterone binding to the cytosol used in Fig. 2 in the absence and presence of cortisol. The affinity was increased and the number of binding sites decreased as would be predicted. Figure 3B illustrates by a different type of plot that the behavior was not competitive, *i.e.*, that cortisol did not compete for all of the sites that bound progesterone. Similar data were obtained from several different cytosols. The affinity constants determined in the presence of cortisol using the Group 2 cytosols contaminated with CBG-like substances (0.35–1.1 nM^{-1}) were in the same range as those determined for the less contaminated Group 1 cytosols in the absence of cortisol (0.2–1 nM^{-1}). The relative affinities of steroids for progesterone binding sites in Group 2 cytosols deter-

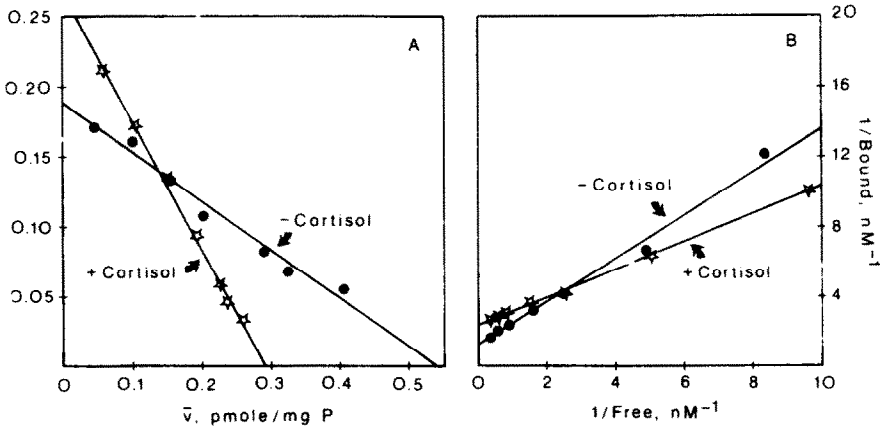

Fig. 3. The effect of 49 nM cortisol on the Scatchard plot (A) and a double reciprocal plot (B) of ^{3}H-progesterone binding data. A cytosol containing a relatively high concentration of a CBG-like component (Group 2) was incubated in Buffer B in the absence (●) and presence (✡) of 49 nM cortisol containing 0.53 nM ^{3}H-progesterone and 0.59–8.8 nM unlabeled progesterone ± 1.2 μM unlabeled progesterone to assess low affinity binding.

mined in the presence of cortisol were similar to those determined in Group 1 cytosols in the absence of cortisol (Table 1).

Preliminary experiments using ^{3}H-medroxyprogesterone acetate as radioligand offer promise. In the cytosol studied, 49 nM cortisol depressed specific ^{3}H-progesterone binding (defined by competition with 67-fold excess unlabeled progesterone) from 71,276 c.p.m. to 22,031 c.p.m. but had no effect on specific ^{3}H-medroxyprogesterone binding defined vs progesterone. In addition, use of unlabeled medroxyprogesterone acetate as competitor to define specific binding of ^{3}H-progesterone resulted in 15,502 c.p.m. and 17,595 c.p.m. bound in the absence and presence of cortisol, respectively. Thus ^{3}H-medroxyprogesterone acetate does not appear to interact with the CBG-like component of this cytosol and has potential use in the assay of cervical progestogen binding sites.

Properties of the binding interaction. Labeled steroid could be quantitatively extracted with organic solvents, indicating the non-covalent nature of the binding interaction. The binding of ^{3}H-progesterone in the presence of 49 nM cortisol was reduced, relative to the value of 0°C, by subsequent incubation for 0.5 h at 37°C (73% binding remaining) or 60°C (14%) or for 2 h at 23°C with 89 μg/ml pronase (9%) or trypsin (84%) but not collagenase (100%).

The dissociation rate constant was evaluated by adding 100-fold excess unlabeled progesterone to a ^{3}H-progesterone–receptor complex at equilibrium and following dissociation of the label. A dissociation rate constant of 6.9×10^{-5} s^{-1} at 0°C in the presence of 18% glycerol ($t_{1/2}$ = 2.8 h) was calculated from the data in Fig. 4. At the incubation temperature employed for assay (10°C), the dissociation rate constant was increased ($t_{1/2}$ < 1 h). Therefore, under the incubation conditions employed (3 h, 10°C) complete exchange of endogenous and labeled hormone takes place and total binding sites are measured.*

Distribution of binding sites. Table 2 summarizes data on the distribution of binding sites within the cervical tissue. Progesterone binding sites were highest per mg protein and per g in the region encompassing the columnar epithelium and approximately equivalent in the region associated with the columnar epithelium and in the remaining stroma when expressed per mg DNA. When expressed as per cent of total binding in the cervical tissue, the stroma contained the largest portion of activity. Thus it was considered important to measure the concentration of binding sites in the total tissue for the purposes of the present study.

The affinity constants for progesterone binding in the various cervical regions were in general similar within a given cervix (Fig. 5). K_a values ranged from 0.12–1.1 nM^{-1} for the segmented cervical regions and from 0.65–2.9 nM^{-1} for the corresponding endometria from three separate specimens.

Concentration of binding sites as a function of cycle stage. Table 3 summarizes the data available at the present time on the progestogen binding site concentration in endometrial and cervical cytosols as a function of the stage of the menstrual cycle. The level of binding was higher in the endometrial tissue than in the corresponding cervical tissue when expressed per mg protein, per g, or per mg DNA. In endometrial cytosols, the mean level of progesterone binding in proliferative phase tissues was significantly higher than the mean for the secretory phase tissue only when expressed per mg protein. The corresponding proliferative phase means for cervical cytosols were significantly higher than the secretory phase means, expressed per mg protein or per mg DNA.

Progesterone binding site concentrations in cervical cytosols from six women taking combination type oral contraceptives were within or somewhat higher than the range found in Table 3 when expressed as pmoles per mg protein (0.16–0.51), per g (4.1–15), and per mg DNA (2.4–4.5).

DISCUSSION

The data presented demonstrate that specific progestogen activity can be measured in human cervical tissue. The concentration of binding sites is 1/3 to 1/6 as high as in corresponding endometrial tissue.

The majority of the cervical cytosols contained a CBG-like component, judging from a 10–60% inhibition of ^{3}H-progesterone binding in the saturation

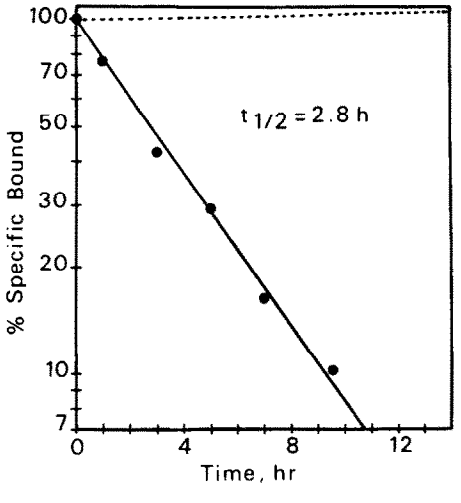

Fig. 4. The dissociation rate of the ^{3}H-progesterone complex at 0°C. Cervical cytosol was incubated in Buffer B plus 49 nM cortisol (final concentration of glycerol was 18%) with 5.8 nM ^{3}H-progesterone ± 2.0 μM unlabeled progesterone (16 h, 0°C). The dissociation reaction was initiated with 71 μM unlabeled progesterone. Data obtained at 20 h were omitted from the figure but were consistent with the line drawn. The hormone–binding protein complex was stable over this incubation period as demonstrated by the dashed line.

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Table 2. Distribution of progesterone binding sites in the cervix

Day of Cycle	Anatomical Region	Pmoles Per mg Protein	Pmoles Per g Tissue	Pmoles Per mg DNA
d 9	Endometrium	1.25	30.4	10.6
	Columnar Epith.	0.53	10.6	3.2
	Squamous Epith.	0.06	1.5	0.8
	Remaining Stroma	0.16	3.5	1.3
d 15	Endometrium	1.98	54.4	11.6
	Columnar Epith.	0.82	23.3	3.9
	Squamous & Stroma	0.22	9.6	3.3
d 17	Endometrium	0.60	21.6	9.0
	Columnar Epith.	0.22	4.9	1.4
	Squamous Epith.	0.06	1.9	1.0
	Remaining Stroma	0.14	3.2	1.3
d 22	Endometrium	0.72	26.0	5.6
	Columnar Epith.	0.44	8.4	2.2
	Squamous & Stroma	0.19	4.2	1.3
d 24	Endometrium	0.68	22.5	6.9
	Columnar Epith.	0.22	4.4	1.8
	Squamous Epith.	0.07	2.0	1.9
	Remaining Stroma	0.17	2.9	1.8

assay in the presence of 49 nM cortisol. CBG or corticosteroid binding globulin exhibits high affinity for cortisol, corticosterone, and progesterone [22]. Alternatively, the contaminant could be an intracellular cervical corticosteroid receptor with some affinity for progesterone [23]. The presence of this type of contaminant has been recognized in human endometrial and myometrial preparations as well as in lower species. Measurements of specific progestogen binding have employed either 0.1 μM [8], 1 μM [11] or 1000-fold molar excess [9] of unlabeled cortisol or albumin [8] in the buffer, ammonium sulfate precipitation prior to assay [6, 13] or, most recently, labeled progestogens which did not bind to CBG [10]. For the purposes of the present study, the method of choice had to be quantitative and simple to perform

Table 3. The concentration of total progesterone binding sites expressed in pmoles in human endometrium and cervix as a function of cycle stage

Day of Cycle	Age	Endometrium Per mg P	Endometrium Per g	Endometrium Per mg DNA	Cervix Per mg P	Cervix Per g	Cervix Per mg DNA
Proliferative Phase							
4	45	–	–		0.19	2.0	4.9
4	48	0.29	2.4	8.2	0.35	2.6	9.1
5	35	1.92	13.3	20.4	0.50	2.8	13.6
6	34	0.97	12.8	26.9	0.28	2.9	8.1
8	44	1.71	–	39.7	0.39	–	7.0
9	28	1.25	30.4	10.6	0.17	–	1.4
9	39	1.93	54.8	27.7	0.17	4.8	2.9
10	34	–	–	–	0.36	3.7	11.9
11	36	–	–	–	0.49	5.0	15.7
13	31	3.00	46.9	11.1	0.23	6.3	2.4
		1.58	26.8	20.7	0.31	3.8	7.7
		±0.32**	±8.5	±4.4	±0.039**	±0.46	±1.6**
Secretory Phase							
15	37	0.19	4.2	1.27	0.29	7.50	2.6
16	37	0.55	21.0	4.56	0.14	3.61	1.4
16	27	1.03	9.6	35.3	0.16	1.19	4.1
16	46	0.60	9.0	21.6	0.13	1.25	3.1
17	30	–	–		0.12	0.78	3.0
17	45	1.14	7.5	24.0	0.34	2.35	7.0
21	27	0.44	14.3	5.22	0.13	3.45	1.2
21	36	0.72	26.0	5.57	0.21	4.68	1.4
22	40	0.48	2.6	14.3	0.15	1.71	2.8
24	46	0.34	2.4	10.0	0.15	0.69	3.5
26	26	–	–	–	0.08	–	1.8
27	28	0.68	22.5	6.90	0.12	2.76	1.8
		0.62	11.9	12.9	0.17	2.7	2.8
		±0.09**	±2.7	±3.4	±0.02**	±0.61	±0.46**

All assays (3 h, 10°C) performed with 49 nM cortisol in the buffer. Significant differences between proliferative and secretory values indicated as $P < 0.05$ (*) and $P < 0.01$ (**).

on a large number of samples. Studies with cytosols relatively free of the CBG-like component revealed that a cortisol concentration of approximately 50 nM would not interfere with specific progesterone binding defined as that binding which was reduced in the presence of potent synthetic progestogens (Fig. 1). Use of this concentration of cortisol is based on the assumption that the conditions of Fig. 1 exist for each assay, *i.e.* that the concentrations of binding sites (n) are in the same range. This is necessary because the relative affinity (as contrasted with the relative affinity constant) is dependent upon the degree of saturation of n [21]. The assumptions made appear valid as evidenced (a) from the relatively small range of n actually measured in the assay and (b) from the linear relationship between binding site concentration and cytosol protein in the saturation assay.

The relative specificities of steroids for progesterone binding sites in cervical cytosols are in general agreement with other reports in the literature (Table 1) and are clearly different from those for CBG [22]. The relative abilities of synthetic progestogens to compete for binding sites are in general consistent with their relative biological potencies [11, 13]. Cyproterone acetate, a potent antiandrogen with progestational activity, exhibited a relative affinity constant of 0.39. On the other hand, neither testosterone nor dihydrotestosterone competed significantly with ^{3}H-progesterone for binding sites. Therefore, the binding measured using this particular labeled ligand probably does not include binding to an androgen receptor. The preliminary data obtained using ^{3}H-medroxyprogesterone acetate indicate that use of this probe may eliminate the need for the inclusion of 49 nM cortisol in the assay buffer. However, since androgen receptors are known to bind certain progestins, including medroxyprogesterone acetate [24], it will be important to evaluate the relative specificity of each labeled progestin used in order to rule out any contribution of androgen binding sites to the measurements. Data in this laboratory indicate that while the total number of binding sites for ^{3}H-progesterone and ^{3}H-R5020 are equivalent, the total number of binding sites for ^{3}H-medroxyprogesterone acetate is larger*. Further study of these systems is in progress.

The affinity constants for progesterone binding activity in the different anatomical regions of the cervix were similar (Fig. 5), indicating the same type of binding protein in these areas. Affinity constants obtained for cervical cytosols (0.2–1.1 nM^{-1}) were also similar to those found in human uterine cytosols by others [6, 8, 9, 12–14]. Concentration of binding sites in the region including and immediately adjacent to the columnar epithelium might be anticipated owing to the presence of the mucus-secreting cells. However, when expressed per mg DNA (*i.e.*, per cell), columnar epithelium and stroma contained equivalent concentrations with few exceptions (Table 2). One cannot exclude the possibility that the stromal tissue contained crypts of mucus-secreting glands; however, it is unlikely that such a contribution could compensate for total absence of binding activity in the stroma. Autoradiographic evidence in the guinea-pig localized label from injected ^{3}H-progesterone in the nuclei of the epithelium of the cervical canal, basal epithelial cells of the tubular glands, connective tissue cells and smooth muscle cells, but not in the mucus-secreting cells themselves [16]. The distribution of progestogen binding sites in human cervical tissue was similar to that previously observed for estrogen binding sites [4].

The dissociation half-time for the cervical progestogen binding activity (2.8 h at 0°C in 18% glycerol) is considerably faster than that for the estrogen binding activity ($t_{1/2}$ = 260 h at 0°C) [4]. Feil *et al.* [25] reported that glycerol markedly increased the dissociation half-time for the mouse uterine progesterone receptor–steroid complex. Similarly, Young and Cleary [12] found that the $t_{1/2}$ for the complex derived from human endometrial cytosol increased from 20 to 40 min in the presence of glycerol.

Considerable evidence has been accumulated in lower species suggesting that estrogen increases and progesterone decreases the level of cytoplasmic uterine progesterone receptor. These conclusions are derived from comparisons of the concentration of sites in immature, hormone-primed immature, and adult animals [17, 25–29] as well as from studies of the fluctuation of binding sites throughout the estrous cycle [30, 31].

In the human, Haukkamaa *et al.* [32] reported an increase in relative progesterone binding by endometrial homogenates from the late proliferative and early secretory phases, in agreement with the concept of positive estrogen regulation. They later reported mean concentrations of 1.9 pmol sites/mg cytosol protein in the proliferative phase and 2.3 pmol/mg pro-

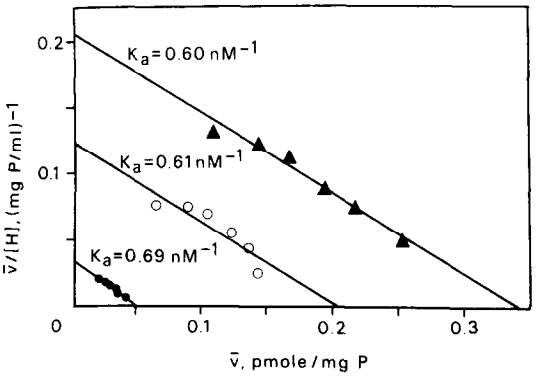

Fig. 5. Scatchard plots of ^{3}H-progesterone binding activity in the cytosols from human (day 22 of the cycle) cervical regions: ▲, columnar epithelium +5 mm; ●, squamous epithelium +5 mm; ○, remaining muscular stroma. Maximum bound/free ratios were 0.59 for columnar epithelium, 0.51 for stroma, and 0.13 for squamous epithelium.

* Sanborn B. M. and Kuo H. S.: unpublished observations.

tein in the secretory phase [8]. Hyperplastic endometria had 4.3 pmol sites/mg protein. Pollow *et al.* [14] found low levels (~0.01 pmol/mg protein) of progesterone binding in day 4–10 endometrial cytosols and slightly higher levels (~0.03 pmol/mg protein) in day 11–12 cytosol. Rao *et al.* [9] found 13 pmol/mg protein for proliferative and 12 pmol/mg protein for secretory myometrium.

In the present study, both endometrial and cervical tissues showed statistically higher concentrations of progesterone binding per mg protein when grouped into proliferative and secretory tissues (Table 3). While the differences in pmoles per mg DNA were significant only in the cervical tissues, the means were higher in proliferative than in secretory endometria. The absolute values obtained for endometrium (~1 pmol/mg protein) agree well with the majority of previously reported concentrations. Cervical concentrations (1.8–16 pmol/mg DNA) are in the range reported for guinea-pig cervix (1–8 pmol/mg DNA) by Atger *et al.* [17].

In the present study, total binding sites were measured [3]. Cervical concentrations of progesterone binding sites in tissue obtained from women taking combination type oral contraceptives for numerous cycles and for 0–16 days of the last cycle were equal to or above the mean for the secretory phase. This may reflect a balance between the positive effect of a continuous presence of estrogen and the proposed negative effect of progesterone. Cervical mucus in combined-type contraceptive users has been reported to be similar to early secretory phase mucus [3].

In contrast to the variation with cycle stage of the progestogen binding, estrogen binding per mg protein did not vary significantly in cervical tissue [4].

While it is tempting to equate specific macromolecular-bound progestogen binding activity with a progestogen receptor, the functional aspects of this activity (*i.e.* transport to nuclear fractions) are as yet not established. Such work is in progress.

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