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ESTROGEN AND PROGESTOGEN BINDING SITE CONCENTRATIONS IN HUMAN ENDOMETRIUM AND CERVIX THROUGHOUT THE MENSTRUAL CYCLE AND IN TISSUE FROM WOMEN TAKING ORAL CONTRACEPTIVES*

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SUMMARY

Estrogen and progestogen binding site concentrations in human cervix and endometrium were measured throughout the menstrual cycle in tissue from cycling women and women taking oral contraceptives. The estrogen binding site concentrations in endometrium were higher in the proliferative and early secretory phase (days 1–17) when compared to the later secretory phase (days 18–28), while the cervical concentrations remained constant throughout the cycle. In contrast, both endometrial and cervical levels of progestogen binding sites were higher in the proliferative than in the secretory phase. Cervices from women taking oral contraceptives exhibited estrogen and progestogen binding site concentrations in the range found for proliferative phase tissues. The data from this and previous studies support the suggestion that the regulation of female sex hormone-mediated events in the human cervix occurs via receptors similar in many aspects to those found in other target tissues.

INTRODUCTION

Cervical tissue responds to the influence of female sex hormones in a characteristic cyclical pattern with production of cervical mucus with varying properties [1,2]. Human cervical estrogen and progestogen binding proteins exhibiting the properties of intracellular receptors have been described by this laboratory [3,4]. These early studies indicated that the concentration of these binding sites fluctuated throughout the menstrual cycle in a manner similar to that found in the corresponding endometrial tissue. The present report represents the findings obtained on a larger patient population and shows that cervical estrogen binding site levels remained constant throughout the cycle while progestogen binding site levels fluctuated. Included also are data on endometrial and cervical binding site levels in women taking oral contraceptives.

MATERIALS

Cytosol preparation

Patients were undergoing hysterectomy for benign conditions such as the presence of leiomyomas or pelvic relaxation. Patients using oral contraceptive medication for two or more months immediately prior to surgery were grouped in a separate category. Cycle stage was assessed from histological dating of the endometrium, plasma estradiol and progesterone levels, and patient history. Cytosols (142,000 *g* supernatants) were prepared as described previously [3,4].

Chemicals

[^{3}H]-Progesterone‖ (1,2,6,7-^{3}H, 81 Ci/mmol, Amersham Searle), [^{3}H]-estradiol (2,4,6,7-^{3}H, 100 Ci/mmol, Amersham Searle) and ^{3}H-R5020 (5,6-^{3}H, 56.5 Ci/mmol, Roussel-Uclaf) were 95% pure as judged by thin layer chromatography. Unlabeled steroids were purchased from Steraloids and used without further purification.

Measurement of total binding sites

Estrogen binding sites were measured by the saturation assay previously described [3], except that cytosol was incubated with [^{3}H]-steroid under conditions which permitted exchange of endogenous and exogenous steroid and allowed the measurement of total binding sites [5,6,7]. Incubation for 24 h at 26°C as suggested by Katzenellenbogen *et al.* [6] for rat cytosol resulted in an average 65% loss of specific binding activity in the human cervical cytosols when compared to incubation at 0°C. However, incubation for 4 h at 26°C allowed sufficient time for exchange without degradation of receptor or steroid (the latter judged by thin layer chromatography). Completeness of exchange was evaluated by: (a) Scatchard analysis

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‖ Abbreviations used: estradiol: 17β derivative; R5020: 17,21-dimethyl-19-nor-4,-pregnadiene-3,20-dione; CBG: corticosteroid binding globulin.

of [^{3}H]-estradiol binding to cytosol ± preincubation with unlabeled estradiol and subsequent demonstration of equivalent numbers of binding sites [5,7]; and (b) measurement of the approach to equilibrium after addition of [^{3}H]-estradiol to cytosol preincubated with unlabeled estradiol [6]. Cytosol estradiol concentrations ranged from nondetectable levels to 4 nM.

Similarly, total progestogen binding site concentration was measured by the saturation assay described previously (4) employing an incubation period of 3 h at 10°C. In this case also, completeness of exchange was assessed by the criteria stated above. [^{3}H]-Progesterone was not degraded during the course of the incubation as judged by thin layer chromatography. Cytosol progesterone concentrations ranged from nondetectable levels to 6 nM. Binding site concentrations measured using the radioligand [^{3}H]-R5020, a compound which binds to progesterone receptors but not CBG [8], were equivalent to or slightly higher than the values obtained using [^{3}H]-progesterone in the presence of 49 nM cortisol [4] in 8 out of 11 cases. These data indicate that the method employed in this study provides a reasonable estimate of progestogen binding even in the presence of CBG-like compounds. Since [^{3}H]-R5020 became available only after a considerable amount of data had been obtained, the decision was made to continue to use [^{3}H]-progesterone as ligand for the remainder of the study. Protein was determined by the method of Lowry [9] and DNA by the method of Burton [10].

RESULTS

Concentrations of binding sites in endometrial and cervical tissue

Figures 1 and 2 show the patterns of estradiol and progesterone binding capacity in the cytosols from cycling women. These data represent an expanded sampling when compared to the previous reports [3,4]. The mean values per mg DNA, per mg protein, and per g pooled arbitrarily at day [1–14] and [15–28] intervals and also pooled according to the pattern seen in Figs 1 and 2, *i.e.* day (1–17) and (18–28), are given in Table 1. For the most part, the means were similar to those reported previously except that the mean cervical estrogen receptor level in the proliferative phase was no longer statistically different from the mean in the secretory phase. When the region associated with the columnar epithelium was studied separately, the absolute concentrations of sites were higher than those found in cervical tissue as expected [3,4], but the patterns were similar to those seen in Figs 1 and 2.

Table 2 shows the data collected from tissue of women taking oral contraceptives. Values were higher than secretory levels in both endometrium and cervix throughout the cycle, except for a drop in the endometrial levels coinciding approximately with cessation

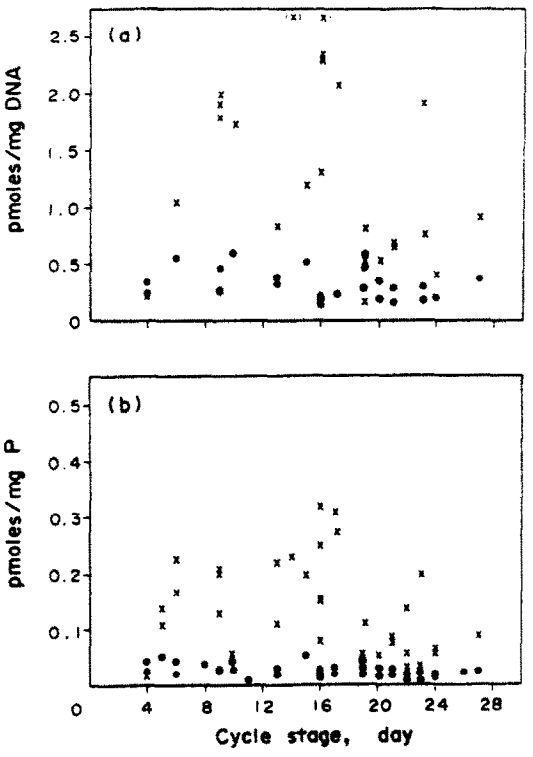

Fig. 1. Concentrations of [^{3}H]-estradiol binding sites in human endometrium (×) and cervix (●) from cycling women, expressed as pmol/mg DNA (A) or pmol/mg protein (B).

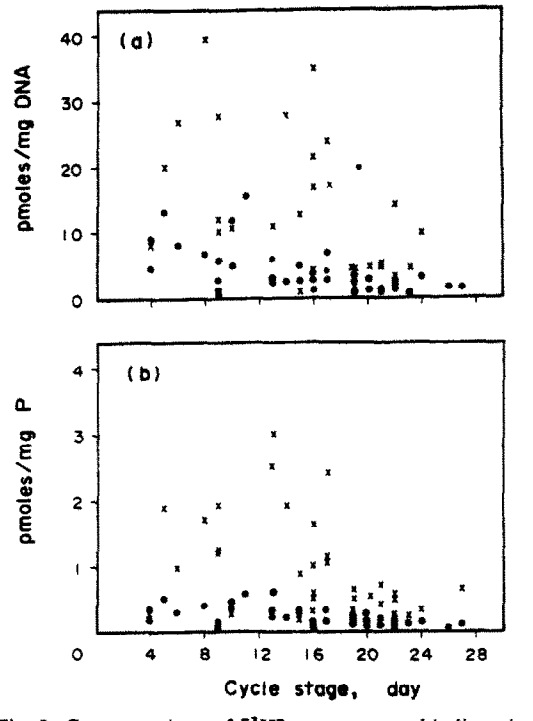

Fig. 2. Concentrations of [^{3}H]-progesterone binding sites in human endometrium (×) and cervix (●) from cycling women, expressed as pmol/mg DNA (A) or pmol/mg protein (B).

Table 1. The mean concentrations of total estradiol and progesterone binding sites expressed in pmol in human endometrium and cervix

		Endometrium			Cervix		
Steroid	$\bar{x} \pm$ S.E.	Per mg P	Per g	Per mg DNA	Per mg P	Per g	Per mg DNA
Estradiol	Proliferative (Day 1–14)	0.15 ± 0.02(11)	4.3 ± 0.7(11)	1.9 ± 0.3(10)	0.030 ± 0.003(15)	0.76 ± 0.06(15)	0.31 ± 0.03(13)
	Secretory (Day 15–28)	0.13 ± 0.02(21)	4.2 ± 0.6(21)	1.2 ± 0.2(21)	0.026 ± 0.002(23)	0.60 ± 0.06(23)	0.31 ± 0.05(22)
	Day 1–17	0.18 ± 0.02**(19)	5.4 ± 0.5**(19)	2.0 ± 0.20**(18)	0.029 ± 0.002(24)	0.72 ± 0.06(24)	0.28 ± 0.03(22)
	Day 18–28	0.08 ± 0.01**(13)	2.5 ± 0.5**(13)	0.70 ± 0.12**(13)	0.026 ± 0.002(14)	0.57 ± 0.06(14)	0.36 ± 0.08(13)
Progesterone	Proliferative (Day 1–14)	1.5 ± 0.25**(11)	37 ± 7**(10)	23 ± 4.5*(11)	0.32 ± 0.04**(15)	4.7 ± 0.9(14)	6.4 ± 1.2**(15)
	Secretory (Day 15–28)	0.69 ± 0.13**(19)	17 ± 3**(19)	11 ± 2.1*(19)	0.18 ± 0.02**(23)	3.6 ± 0.5(22)	2.5 ± 0.3**(23)
	Day 1–17	1.3 ± 0.18**(19)	30 ± 5*(18)	20 ± 3**(19)	0.28 ± 0.03**(24)	4.5 ± 0.7(23)	5.3 ± 0.8**(24)
	Day 18–28	0.42 ± 0.05**(11)	12 ± 3*(11)	6.3 1.1**(11)	0.15 ± 0.02**(14)	3.3 ± 0.5(13)	1.9 ± 0.2**(14)

** $P < 0.01$; * $P < 0.05$; Number in parentheses denotes sample size.

Table 2. Hormone binding sites in tissues from women taking birth control preparations

Tissue	Days since onset of menses	Oral preparation	Estradiol binding sites pmol per Mg P	g	Mg DNA	Progesterone binding sites Pmol per Mg P	g	Mg DNA
Endometrium	7	Ovulen-21	0.21	4.2	0.84	1.5	31	6.1
Cervix			0.056	1.6	0.54	0.44	12	4.3
Endometrium	7	Ovulen-21	0.091	1.2	1.6	1.1	14	20
Cervix			0.030	0.66	0.5	0.29	6.5	4.9
Endometrium	7	Ortho-Novum	0.16	1.7	2.6	0.68	7.6	11
Cervix			0.066	1.6	0.52	0.48	11	3.8
Cervix	9	Norinyl 1-80	0.050	1.1	0.42	0.39	8.1	3.2
Cervix	9	Ortho-Novum	0.056	1.5	0.52	0.29	7.5	2.7
Endometrium	10	Norinyl 1-80	0.15	3.4	1.3	1.2	27	9.9
Cervix			0.033	0.97	0.51	0.18	5.2	2.7
Cervix	10	Ovulen-21	0.05	1.5	0.57	0.48	15	5.5
Endometrium	11	Ovulen-21	0.11	3.5	1.4	1.7	56	22.0
Cervix			0.035	0.8	0.39	0.44	9.9	4.9
Endometrium	12	Ovulen-21	0.20	4.3	1.4	1.4	52	16
Cervix			0.042	1.2	0.37	0.51	15	4.5
Endometrium	14	INA	0.17	5.3	1.0	0.58	18	3.6
Cervix			0.047	1.3	0.55	0.40	11	4.7
Endometrium	16	Ovulen-21	0.12	2.6	0.93	1.5	34	12
Cervix			0.046	1.2	0.53	0.32	8.2	3.7
Endometrium	20	Ovral	0.037	0.49	0.13	0.34	4.4	1.1
Cervix			0.020	0.51	0.18	0.11	2.8	1.0
Endometrium	21	Ovulen-21	0.10	2.9	0.27	1.2	33	3.2
Cervix			0.025	0.68	0.26	0.22	6.1	2.4
Cervix	21	Ovulen-21	0.032	0.73	0.58	0.38	8.6	6.8
Endometrium	21	Norinyl 1-80	0.11	0.51	0.97	0.43	2.0	3.4
Cervix			0.028	0.70	0.22	0.26	6.6	2.0
Cervix	21	Ovulen-21	0.030	0.57	0.39	0.25	4.8	3.3
Endometrium	25	Ortho-Novum	—	—	—	0.43	13	5.2
Cervix			0.054	1.2	0.25	0.18	3.9	0.8
Cervix	28	Norinyl 1-80	0.034	0.78	0.29	0.18	4.2	1.6
Endometrium	7–21	Mean ± S.E.	0.13 ± 0.01	2.9 ± 0.47	1.1 ± 0.20	1.1 ± 0.13	27 ± 5.2	9.8 ± 2.2
Cervix	7–21	Mean ± S.E.	0.042 ± 0.003	1.1 ± 0.095	0.44 ± 0.034	0.32 ± 0.029	8.1 ± 0.85	3.6 ± 0.37

INA-information not available.

of ingestion of hormonally active tablets. A similar trend was noted in the cervical levels, but considering the magnitude of the variability, this may not be significant.

DISCUSSION

Evidence has been presented for positive control by estrogens and negative control by progestogens of the level of cytoplasmic estrogen and progesterone receptors in target tissues [11,12]. In general, measurements of endometrial binding sites as a function of cycle stage in the human have been consistent with this interpretation. Estrogen receptor levels are reported to be higher in the proliferative than in the secretory phase, although the details of the patterns vary with the study [12–16]. The pattern of endometrial binding site levels throughout the menstrual cycle seen in Fig. 1 supports this concept of hormonal control and is closest to that reported by Trams *et al.* [14]. In contrast to the earlier, less extensive study [3], the data demonstrate that the cervical concentration of estrogen binding sites does not vary significantly throughout the menstrual cycle.

Reports of progestogen binding site fluctuations have been quite variable. Some papers describe similar levels in proliferative and secretory phase tissues when expressed as means [17,18] while others note higher levels in proliferative than in secretory phase tissues [19,20] and still others report a peak near mid cycle [21]. The data in Table 1 and Fig. 2 show that in the present study both endometrial and cervical concentrations of receptor sites were higher in the proliferative phase of the cycle.

The data on binding site levels in endometrial and cervical tissue from women taking oral contraceptives (Table 2) indicate that under the influence of the synthetic steroids, estrogen and progestogen receptor levels were maintained at levels seen in normal proliferative phase. Endometrial levels dropped off at the end of the cycle, coincident with cessation of ingestion of hormonally active tablets. Comparable changes in the cervical regions were less obvious. These data suggest that the balance between estrogenic and progestogenic influence in the cervix favors preservation of estrogen and progestogen receptors (an estrogenic effect). The presence of sufficient levels of progestogen receptors would facilitate the expression of progesta-

tional effects in the form of the secretory-type cervical mucus seen in women taking such oral preparations [1,2].

The time course and temperature dependence of the accumulation of radioactivity into the nuclear fraction from cervical tissue after incubation of the tissue with [^{3}H]-estradiol is consistent with a receptor-mediated event (Sanborn *et al.*, unpublished observations). Similarly, the physical properties and specificity of the cervical binding protein [3] suggest that it serves a receptor function. Similarly, the temperature-dependence of nuclear [^{3}H]-R5020 accumulation and the persistence of nuclear binding while cytosol binding is declining (Sanborn *et al.*, unpublished observations), the physical properties and specificity previously described [4], and the fact that [^{3}H]-R5020 binds to receptors but not to CBG-like proteins [8] support the suggestion that the binding activity serves a receptor function.

The human cervix has long been recognized to be responsive to estrogens and progestogens. The data presented in this and previous papers [3,4] indicate that macromolecules with the properties of receptors exist in this tissue and, at least in the case of progestogen binding, appear to be regulated in a manner similar to that existing in the endometrium. Thus the cervix may be considered a target tissue for female sex hormones in the generally accepted use of that term [11].

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