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PROGESTERONE-MEDIATED SUPPRESSION OF ESTRADIOL RECEPTORS IN CYNOMOLGUS MACAQUE CERVIX, ENDOMETRIUM AND OVIDUCT DURING SEQUENTIAL ESTRADIOL-PROGESTERONE TREATMENT

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Summary—We used sequential treatment with implants of estradiol (E_2) and progesterone (P) to create varied hormonal states in a group of spayed cynomolgus macaques. The reproductive tracts were removed, and nuclear and cytosolic estrogen receptors were analyzed in the cervical mucosa, endometrium, and oviducts. Nuclear receptor quantities were greater in tissues of E_2-treated monkeys than in tissues of spayed animals. Sequential P treatment, even in the presence of continuous E_2, decreased the amounts of nuclear and cytosolic E_2 receptors. In the oviduct and endometrium, the P-mediated suppression of receptors occurred within 1 or 2 days. In the cervix, suppression occurred only if the serum P:E_2 ratio was elevated to twice the amount (~100:1) usually found during the luteal phase of the menstrual cycle (~50:1) in this species. Of these three reproductive tract tissues, the cervix had the highest threshold for suppression by P of E_2 receptors in the presence of E_2.

INTRODUCTION

Primate menstrual cycles are regulated by fluctuations in the quantities of a complex array of steroid and protein hormones. The steroid hormones act on tissues of the reproductive tract through interactions with souble receptors in cells. These receptors in turn are regulated by the steroids [1–3].

In previous reports, we described fluctuations in estrogen receptor levels in the oviducts of rhesus macaques during induced cycles [4] and in both the oviducts and the endometrium of cynomolgus macaques during natural cycles [5]. In the study reported here, we determined estrogen receptor levels in the cervices, endometrium and oviducts of spayed cynomolgus macaques treated with various regimens of estradiol (E_2) and progesterone (P) through insertion and removal of hormone-filled Silastic implants. A major goal was to compare receptor regulation in the cervix with that in other regions of the macaque reproductive tract.

EXPERIMENTAL

Steroids

Radiolabeled Moxestrol ([^{3}H]R2858; 11β-methoxy-17-ethynyl-1,3,5[10]-estratriene-3,17-diol; 79–87 Ci/mM) and radioinert Moxestrol were purchased from New England Nuclear (Boston, MA). The other crystalline radioinert steroids were obtained from Steraloids, Inc. (Wilton, NH).

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Animals

Female cynomolgus macaques (*Macaca fascicularis*) were spayed at least 6 weeks before use. Most animals received implants of Silastic tubing filled with either E_2 (2 cm) or P (6 cm) and sealed at both ends [6]. Such capsules can deliver physiologically active amounts of steroids when implanted subcutaneously in macaques [6, 7]. The levels of E_2 and P in blood serum obtained at the time of surgery were determined by radioimmunoassay (RIA) as previously described [8, 9], except that different antisera for E_2 [10] and P [11] were used.

Animal treatments

Initially, seven treatment groups were established. The code name for each group is presented in parentheses after each treatment, as follows: (a) no implant (spayed); (b) one E_2 implant in place for 14 days (E_2); (c) one E_2 implant in place for 28 days and one P implant in place for the last 14 days (14d E_2 plus P); (d) one E_2 implant in place for 35 days and one P implant in place for the last 21 days (21d E_2 plus P); (e) one E_2 implant in place for 28 days and two P implants in place for the last 14 days (14d E_2 plus PP); (f) one P implant in place for 14 days (P); and (g) one E_2 implant in place for 14 days (then removed) and one P implant in place for the next 14 days (E_2, P). The number of monkeys in each group is presented in Tables 1 and 2. Subsequently, we examined the kinetics of the decline in E_2 receptors induced by P in oviducts and endometrium. Tissues were removed after 14 days of E_2 treatment or after 14 days of E_2 treatment followed sequentially by 1–14 days of E_2

plus P treatment. Numbers of animals at each time point are presented in Fig. 2.

The oviducts and entire uterus and cervix were removed by hysterectomy from macaques that were under halothane anesthesia. The monkeys were housed, fed, operated upon, and postoperatively monitored by procedures approved by the Animal Care Committee of the Oregon Regional Research Center. The fresh tissue was placed in Hanks' solution, and within a few minutes the oviduct, uterus, and cervix were separated. The uterus and cervix were split in quarters longitudinally, and the entire endometrium and cervical mucosa, respectively, were separated from their underlying tissues with iris scissors and the aid of a dissecting microscope.

Sucrose gradient studies

The cervical mucosa was weighed and homogenized (200 mg/ml) in TED buffer—10 mM Tris-(hydroxymethyl)aminomethane HCl (Tris–HCl) (pH 7.4), 1.5 mM ethylenedinitrilotetraacetic acid (EDTA), and 1 mM dithiothreitol (DTT)—in an ice bath by means of Duall tissue grinders, size 22 (Kontes Glass Company, Vineland, NJ). A similar buffer, TE, lacked the DTT. All subsequent procedures were carried out at 0°C. The homogenate was centrifuged at 1000 *g* for 10 min to separate the cytosols and crude nuclear pellets. Cytosols were mixed with [^{3}H]Moxestrol (20 nM, final concentration) in the absence or presence of 2 μM radioinert Moxestrol, E_2, P, diethylstilbestrol (DES), 5α-dihydrotestosterone (5α-DHT), or Promegestone (R5020), and were incubated for 3 h at 0°C.

The cytosols were then added to charcoal pellets obtained by centrifugation from 1 ml of a Dextran-charcoal suspension (1.25% charcoal and 0.625% Dextran in TE buffer). The cytosols were mixed gently with the charcoal for 10 min and then centrifuged at 8000 *g* for 10 min. The supernatants were then drawn off from the charcoal pellets, layered on 5–20% sucrose gradients, and ultracentrifuged as previously described [4]. Then twenty-five drop fractions were mixed with 5 ml of Atomlite scintillation solution (New England Nuclear Corp.), and the radioactivity was determined.

Other aliquots of the charcoal-treated cytosol (previously incubated with [^{3}H]Moxestrol with or without 100-fold radioinert Moxestrol) were layered on a similar gradient containing 0.5 M KCl. Another aliquot of the radiolabeled, charcoal-treated cytosol was mixed with 76 μg of D547Spγ, a monoclonal antibody prepared against the extranuclear MCF-7 estrogen receptor (antiestrophilin) [12, 13]. After 2.5 h at 0°C, the sample was layered on a gradient containing 0.5 M KCl and assayed as described above.

Binding assays

Nuclear and cytosolic E_2 receptors (in tissues from the seven treatment groups already described) were quantified in exchange and binding assays, respectively [14–16].

We optimized the steroid concentration, incubation times, and temperatures for [^{3}H]Moxestrol binding to the cervical estrogen receptor. Aliquots (300 μl each) of cytosols, prepared as described above, were mixed with 23 nM [^{3}H]Moxestrol (A) or 23 nM [^{3}H]Moxestrol containing 100-fold radioinert Moxestrol (B). The crude nuclear pellets, prepared as described above, were washed three times with 2 ml of TE buffer each time and by centrifugation at 1000 *g* for 10 min. Aliquots (300 μl) of washed nuclear resuspensions were mixed with [^{3}H]Moxestrol as above. Trial incubation conditions varied from 10 min to 20 h, and from 0 to 37°C. The maximal nuclear receptor was found in the 1 h, 37°C incubation; the maximal cytosol receptor was found in the 3 h, 0°C incubation (data not shown). This cytosol association time was shorter than that found by Ojasso and Raynaud[17], but similar to that which we had previously found [18]. After incubation, the cytosols were treated with charcoal and counted as described above.

After incubation, nuclei were washed four times: first with 1 ml of 0.5% Triton X-100 in TE buffer, then three times with 1 ml of TE. The washed nuclear pellets were solubilized with 1 ml of Soluene 100 (Packard Instrument Company, Downers Grove, IL) at 70°C for 1 h. Five milliliters of Atomlite solution were added, and the radioactivity was determined as above. The "specific receptor" was defined as the difference (A-B) and expressed as femtomoles per milligram of protein [19] and of DNA [20] for the cytosols, and femtomoles per milligram of DNA for the nuclei. We found that inclusion of DTT in the homogenization buffer decreased the viscosity induced by cervical mucus in the homogenate. With DTT, the separation of nuclear and charcoal pellets from the cytosol was easier. Once the viscosity had been reduced, DTT was no longer needed in the nuclear preparation.

Endometrium was assayed for receptors in the same way as the cervix, except that DTT was not included in the homogenization buffer.

Samples for the kinetic study were assayed on a separate group of animals at a different time. Tissues were weighed and homogenized (all subsequent steps on ice) in 100 mg/ml TG buffer (10 mM Tris–HCl, 30% glycerol, pH 7.4) with Duall tissue grinders. The TG buffer facilitated the simultaneous determination of P receptors on the same homogenates (data to be reported elsewhere). The homogenates were centrifuged at 1000 *g* for 10 min, and the supernatants (cytosols) were removed. The pellets (nuclei) were washed three times with 2 ml of TG buffer (vortexed, then centrifugation at 1000 *g* for 10 min); the crude nuclear pellets were resuspended in the original homogenization volume of TG. Aliquots (300 μl) of cytosols and nuclear suspensions were mixed with 17 nM [^{3}H]Moxestrol, in the absence (A)

or presence (B) of 100-fold radioinert Moxestrol. Cytosolic and nuclear samples were incubated at 0°C for 3 h and at 37°C for 1 h, respectively. The cytosols were mixed with Dextran-coated charcoal for 10 min at 4°C, then centrifuged at 8000 *g* for 10 min. The supernatants were mixed with 1 ml of H_2O and 15 ml of Instagel (Packard Instruments), and the radioactivity was determined. After incubation, the nuclei were washed four times by centrifugation (1000 *g*, 10 min); once with 0.5% Triton X-100 in TG buffer and then three times with TG. The washed nuclear pellets were solubilized with 1 ml of Soluene 100 at 70°C. Radioactivity was determined with 15 ml of scintillation cocktail containing 25% Triton X-114 (Rohm and Haas, Philadelphia, PA) in xylene with 3% (w/v) Omnifluor (New England Nuclear). Specific binding was defined as the difference between A (specific plus nonspecific) and B (nonspecific) and expressed as femtomoles per milligram of DNA or protein. All data are presented as means ± standard error of the mean.

RESULTS

The serum levels of steroids in the various treatment groups are shown in Tables 1 and 2 and in the legend of Fig. 2. E_2 and P levels produced by the E_2 and P implants, except for those of the spayed and the 14d E_2 plus PP groups, were similar to those found in cynomolgus macaques during natural menstrual cycles [21, 22].

Cervix

The hormone specificity of the estrogen receptor from the cervical mucosa was documented by the use of several criteria. On low-salt sucrose gradients, a single 8S peak of radioactivity was routinely detected, and this peak was completely abolished when 100-fold radioinert E_2 or DES, but not P or the synthetic progestin R5020, was included in the incubation mixture (Fig. 1A). The androgen 5α-DHT was a partial competitor for the estrogen receptor (Fig. 1A), as has been reported elsewhere [23–27].

When an aliquot of the charcoal-treated cytosol incubated with [^{3}H]Moxestrol was analyzed on a similar gradient containing 0.5 M KCl, a 4S peak was seen (Fig. 1B). This peak was abolished in a parallel gradient that included 100-fold radioinert Moxestrol (Fig. 1B). The monoclonal antiestrophilin D547Spγ recognized the [^{3}H]Moxestrol-bound macromolecule and caused a shift in the gradient to a higher density (Fig. 1B). Thus, the cervical estrogen binder meets these qualitative criteria for a true receptor.

The entire cervical mucosa from each of 40 macaques in seven treatment groups was analyzed for nuclear and cytoplasmic E_2 receptors in the binding-exchange assay (Table 1). Estrogen receptor levels in the cervices of spayed animals were generally increased by E_2 treatment though the only statistically significant increase was in the level of nuclear estrogen receptor. The lack of a statistically significant increase in cytosolic (fmol/mg DNA) or total receptor was presumably due to the great degree of animal variability, as indicated by the large standard error of the mean in Table 1 for the E_2 group. When we treated estrogenized animals for either 14 or 21 days with E_2 plus P, neither nuclear nor total estrogen receptor was significantly decreased from the level found in the estrogenized cervices. However, when we elevated the serum level of P to approx. 14 ng/ml by implanting two P-filled Silastic capsules, there was a statistically significant decrease in the amount of nuclear and total estrogen receptor in the cervix within 14 days of treatment. Each of the E_2 plus P treatments led to a significant decrease in concentration of cytosolic receptor (fmol/mg protein), and when the data from all the E_2 plus P groups were pooled, significant decreases in the amount of total and cytosolic receptor per cell (fmol/mg DNA) became evident.

When we treated spayed animals with P alone, the results were highly variable and there were no indications that P had any significant effect on estrogen receptor levels in cervices of spayed animals. When we treated estrogenized animals for 14 days with P alone after removing the E_2 capsule (E_2, P) there were declines in the mean amounts of estrogen receptors in all compartments but none of these declines were statistically significant.

Endometrium

The endometrium from each of 32 macaques in seven treatment groups was analyzed for nuclear and cytosolic E_2 receptors in the binding-exchange assay (Table 2). Cytosolic (fmol/mg DNA) nuclear, and total receptors were significantly increased over spayed-monkey levels by E_2 treatment. E_2 treatment led to a numerical decrease in the concentration of cytosolic receptors (fmol/mg protein) but this decline was not a statistically significant one. Each of the three E_2 plus P treatments of estrogenized animals produced significant declines in all measures of endometrial estrogen receptor, whether cytosolic, nuclear or total. Moreover, total and cytosolic receptors in the endometria of the E_2 plus P groups were significantly lower than in the endometria of spayed untreated animals ($P < 0.05$ or less for every comparison). There were only 2 spayed animals treated with P alone that had adequate endometria to sample. In these, total receptor levels were generally low, but the sample was too small for statistical comparisons.

The data in Fig. 2 show that a significant suppression by P of nuclear and cytosolic (fmol/mg DNA) endometrial E_2 receptors occurs within 6 days of E_2 plus P treatment. These data on individual monkeys indicate that suppression may be complete after only 1–3 days of E_2 plus P exposure. We also have preliminary data on *Macaca nemestrina* that indicate nuclear E_2 receptors may be suppressed within a few hours of P exposure.

The absolute amounts of endometrial nuclear receptor in the two 14d E_2 groups (Fig. 2; Table 2) probably differ because of the disparate buffers used (see Experimental Procedures). The percentages of decline in receptors induced by sequential P treatment were similar regardless of method.

Oviducts

Nuclear and cytosolic (fmol/mg DNA) E_2 receptors were significantly decreased by P within 6 days of E_2 plus P treatment (Fig. 2). The individual-animal data suggest that after 1–3 days of E_2 plus P treatment, suppression of receptors is essentially complete.

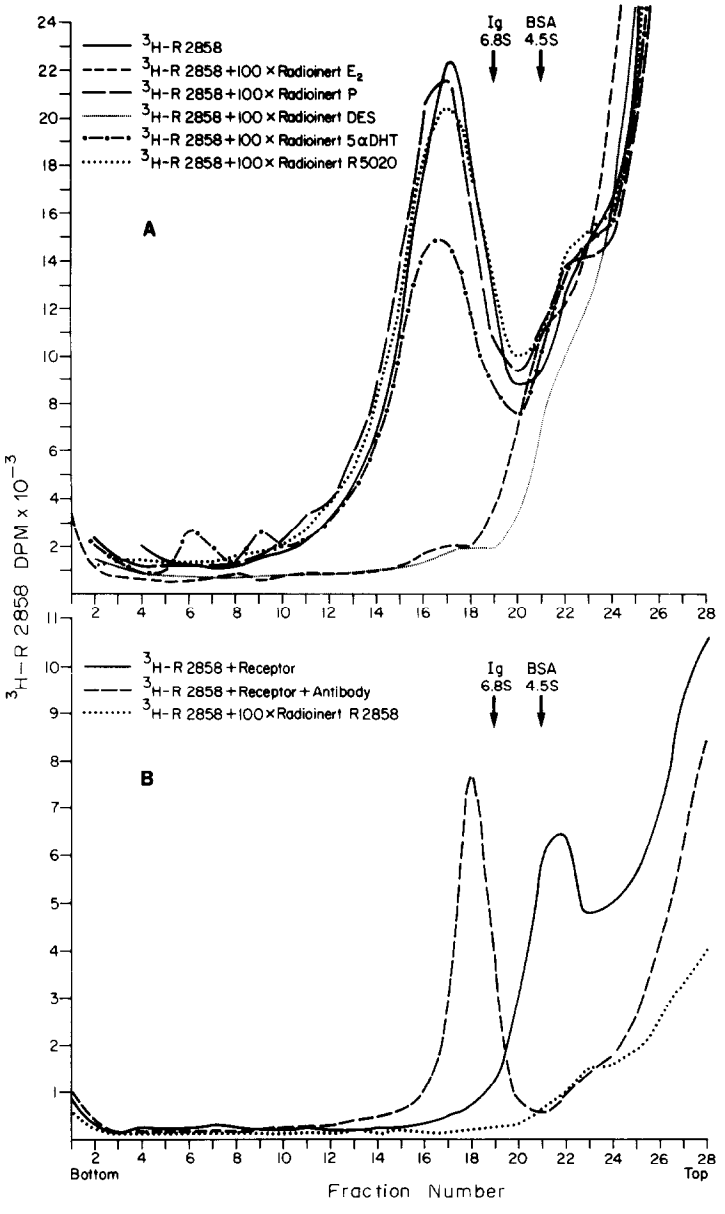

Fig. 1. Sucrose density gradient profiles of [^{3}H]R2858 (Moxestrol) binding to cytosols prepared from macaque cervix. A. Cytosols were incubated for 3 h at 0°C with 20 nM [^{3}H]R2858 only or in the presence of 100-fold concentrations of various radioinert ligands. Samples were charcoal-treated, layered on 5–20% sucrose in TE buffer, and centrifuged for 18 h at 286,000 *g*. The positions of human immunoglobulin (Ig) and bovine serum albumin (BSA) marker proteins are indicated. B. Interaction between monoclonal antibody to human breast cancer (MCF-7) estrophilin and estrogen receptor of macaque cervix. The [^{3}H]R2858 (20 nM) was mixed with cervical cytosol and incubated for 2 h at 0°C. After charcoal treatment, 76 μg of D547Spγ antibody or 100-fold radioinert R2858 were added to aliquots of cytosol and incubated for 2.5 h at 0°C. The samples were layered on 5–20% sucrose in TE buffer with 0.5 M KCl added, and centrifuged for 18 h at 286,000 *g*. The positions of human Ig and BSA marker proteins are indicated.

Table 1. Nuclear and cytosolic estrogen receptors in cervices from seven treatment groups of cynomolgus macaques

	Treatment Groups							
	Spayed	E_2	14d E_2 + P	21d E_2 + P	14d E_2 + PP	P	E_2, P	Pool of all E_2 + P groups
Number of monkeys	(4)	(12)	(7)	(4)	(7)§	(3)	(3)	(18)§
Serum E_2 (pg/ml)	5.8 ± 0.8	174 ± 74	133 ± 12	203 ± 10	133 ± 18	12.7 ± 4.3	22 ± 3.2	149 ± 9.4
Serum P (ng/ml)	0.14 ± 0.04	0.17 ± 0.08	6.46 ± 0.99	6.84 ± 1.1	14.3 ± 2.2	6.37 ± 1.25	4.89 ± 0.19	9.3 ± 1.1
Cytosol receptor								
fmol/mg protein	173 ± 68	209 ± 34	77 ± 6**	57 ± 11***	57 ± 8**	143 ± 48	122 ± 44	65 ± 5*
fmol/mg DNA	936 ± 151	4914 ± 1606	1219 ± 252	1102 ± 101	1078 ± 166	3590 ± 2446	1353 ± 277	1138 ± 115**
Nuclear receptor								
fmol/mg DNA	472 ± 212***	1201 ± 167	1053 ± 392	1187 ± 309	510 ± 99**	475 ± 172	512 ± 150	872 ± 177
Total receptor								
fmol/mg DNA	1408 ± 210	6115 ± 1607	2268 ± 577	2289 ± 211	1588 ± 230***	4065 ± 2425	1864 ± 378	2008 ± 248**

The blood levels of E_2 and P were produced by implantation of Silastic capsules of crystalline steroids subcutaneously into spayed *Macaca fascicularis*. All data are presented as mean ± standard error of the mean. Receptor values of groups significantly different (Student's *t*-test) from the 14d E_2 group are indicated (* = $P < 0.001$; ** = $P < 0.01$; *** = $P < 0.05$; § = number of monkeys 1 less for serum values only).

Table 2. Nuclear and cytosolic estrogen receptors in endometrium from the seven groups of cynomolgus macaques

	Treatment Groups							
	Spayed	E_2	14d E_2 + P	21d E_2 + P	14d E_2 + PP	P	E_2, P	Pool of all E_2 + P groups
Number of animals	(4)	(5)	(6)	(4)	(8)§	(2)	(3)	(18)§
Serum E_2 (pg/ml)	5 ± 0	180 ± 37	130 ± 14	203 ± 10	141 ± 27	17	22 ± 3.2	152 ± 9.7
Serum P (ng/ml)	0.15 ± 0.03	0.20 ± 0.04	6.78 ± 1.12	6.84 ± 1.11	13.9 ± 2.3	5.65	4.89 ± 0.19	9.7 ± 1.0
Cytosol receptor								
fmol/mg protein	1083 ± 291	512 ± 61	57 ± 6*	45 ± 8*	47 ± 9*	261	103 ± 25**	50 ± 5*
fmol/mg DNA	4387 ± 1166***	7634 ± 657	924 ± 112*	921 ± 161*	813 ± 68*	2127	1908 ± 341*	874 ± 57*
Nuclear receptor								
fmol/mg DNA	86 ± 20*	1582 ± 233	352 ± 184**	362 ± 162**	430 ± 122*	569	283 ± 139**	389 ± 85*
Total receptor								
fmol/mg DNA	4473 ± 1178**	9218 ± 699	1276 ± 266*	1283 ± 229*	1310 ± 106*	2696	2172 ± 223*	1293 ± 105*

The blood levels of E_2 and P were produced by implantation of Silastic capsules of crystalline steroids subcutaneously into spayed *Macaca fascicularis*. All data are presented as means ± standard error of the mean. Receptor values of groups significantly different (Student's *t*-test) from the 14d E_2 treated group are indicated (* = $P < 0.001$; ** = $P < 0.01$; *** = $P < 0.05$; § = number of monkeys 1 less for serum values only) (The data on endometrial estrogen receptors in the E_2 and 14-day E_2 plus P groups have previously appeared in another publication [43].)

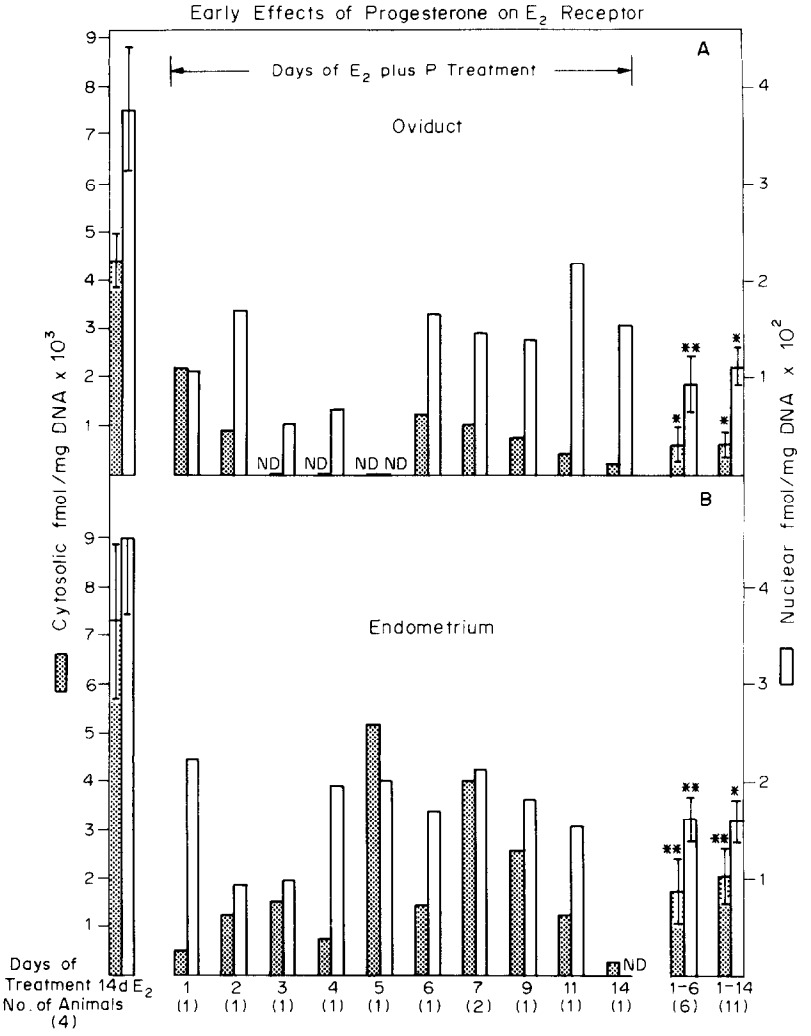

Fig. 2. Early effects of progesterone on estrogen receptor. Fifteen spayed cynomolgus macaques were treated with E_2 implants. After 14 days, oviducts and uteri were removed from 4 animals to establish initial levels of E_2 receptors. A P implant was then added to 11 animals, and oviducts and uteri were removed from them at 1, 2, 3, 4, 5, 6, 7, 9, 11 and 14 days later. The serum steroid levels for these monkeys were: treatment with 14d E_2; 178 ± 35 (SEM) pg/ml E_2 and 0.16 ± 0.06 ng/ml P: treatment with 1–6d E_2 plus P; 131 ± 24 pg/ml E_2 and 7.1 ± 0.66 ng/ml P: treatment with 1–14d E_2 plus P; 128 ± 13 pg/ml and 5.8 ± 0.7 ng/ml P. Nuclear and cytosolic E_2 receptor amounts in the oviducts (A) and endometrium (B) were determined as described in the text. N.D. = nondetectable.

DISCUSSION

The previously published data on cyclic changes in primate cervix E_2 receptor are contradictory. Sanborn *et al.*[28] initially observed that levels of E_2 receptors (picomoles per wet-weight gram) in cytosols prepared from human ($n = 14$) cervical tissue were higher in the follicular than in the luteal phase of the menstrual cycle. When E_2 receptor data from 24 additional women were added to the series, there no longer was any statistically significant difference (picomoles per wet-weight gram) between follicular and luteal samples [29]. In neither of these studies was there a significant difference between the follicular- and luteal-phase E_2 receptor contents when the data were expressed as "picomoles per milligram of protein or DNA" [28, 29]. Holt *et al.*[30] found no significant difference in cytosolic E_2 receptor (femtomoles per wet-weight gram) in cytosols prepared from cervices of women in the midfollicular, midcycle, or luteal phase. Ochiai[31] found that cytoplasmic E_2 receptors (femtomoles per milligram of protein) in women's cervices were significantly more numerous in the proliferative phase than in the secretory phase, but Cao *et al.*[32] found the converse. A progestin, medroxyprogesterone acetate, significantly reduced the quantity (femtomoles per milligram of protein) of cytoplasmic E_2 receptors in the cervical tissues of women [33]. Elsner *et al.*[34] treated spayed rhesus macaques for 2 weeks with E_2 in Silastic capsules or with E_2 for 3 weeks with a P capsule added for the last week. When cervical tissue

was assayed for nuclear and cytoplasmic E_2 receptor, no difference was found in nuclear receptor content (picomoles per milligram of DNA), but there were significantly more cytoplasmic E_2 receptors (femtomoles per milligram of protein) in the E_2 group than in the E_2 plus P group [34, 35]. Two-week E_2 treatment increased the levels of nuclear but not cytoplasmic receptor over quantities detected in control (spayed-primate) cervices [34, 35].

Our data indicate that the cervical estrogen receptor in cynomolgus macaques exhibits the usual steroid specificity, behavior on sucrose gradients and reactivity with a monoclonal antiestrophilin typical of other well studied estrogen receptors. However, the ability of progesterone to suppress estrogen receptor levels is much less dramatic in the cervix compared to the oviduct and endometrium. Until we elevated the serum P:E_2 ratio to approximately double that found during the normal menstrual cycle we found no significant suppression of the level of nuclear estrogen receptor in the cervix. Only by one measure (fmol/mg protein) was cytosolic estrogen receptor significantly suppressed by any of the E_2 plus P treatments. In many of the same animals, serum P:E_2 ratios typical of the normal luteal phase brought about significant declines in all measures of estrogen receptor in oviduct and endometrium. Also, although E_2 treatment of spayed animals produced a significant rise in cervical nuclear estrogen receptor, animal variability precluded demonstration of a statistically significant elevation in cytosolic receptors. In contrast, the endometrium of many of the same E_2-treated animals showed significant increases above spayed levels in all the measures of estrogen receptor that were expressed on a DNA basis.

In the case of the endometrium, published reports on E_2 receptor fluctuations during the menstrual cycles of monkeys and women consistently indicate that levels of both nuclear and cytosolic receptors are higher in the proliferative phase than during the secretory phase [5]. However, published receptor data on hormone-treated, spayed macaques are not so consistent. Elsner *et al.*[34] found a significant decrease in cytoplasmic but not nuclear E_2 receptors when they compared levels after 14 days of E_2 treatment to levels after 14 days of E_2 followed by 7 days of E_2 plus P treatment. Kreitmann-Gimbal *et al.*[36] found that 12 but not 6 days of E_2 plus P treatment significantly reduced nuclear and cytosolic receptors from the levels found in spayed monkeys treated for 12 days with E_2. We found a significant decrease in endometrial receptors in both cellular compartments by day 6 (Fig 2) of sequential E_2 plus P treatment that continued for 14 days (Table 2; Fig. 2). We also found that 14 days of E_2 treatment increased receptor amounts (per milligram of DNA) over spayed-monkey levels, which had previously been unexamined.

Reports on oviductal receptor levels throughout the human menstrual cycle are also contradictory [5]. We originally used sucrose density gradients to show, in oviducts of spayed rhesus macaques, that sequential P treatment by injection suppresses cytoplasmic E_2 receptors even in the presence of continuous E_2 treatment [4]. Also, the tissue content of E_2, as determined by RIA, is significantly greater in oviducts from spayed rhesus macaques treated with E_2 alone than in oviducts from spayed rhesus macaques given E_2 plus sequential P [37]. The data reported here show that nuclear and cytosolic receptors in macaque oviducts are suppressed by P within 6 days of E_2 plus P treatment, and that levels remain low for 14 days.

The reasons for discrepancies in the published data on E_2 receptor changes throughout the reproductive tract during natural and artificial menstrual cycles are difficult to pinpoint. In studies on natural menstrual cycles, it is often difficult to accurately define the stage of the cycle. We developed a technique of oviductal profile analysis to help accomplish staging in the cynomolgus macaque [21]. The simulated (artificial) menstrual cycles created by Kreitmann-Gimbal *et al.*[36] included an E_2 peak near "mid-cycle," a feature absent in our study. The main technical difference between this study and others on hormone-treated, spayed macaques is that we used Moxestrol instead of E_2 as the assay radioligand. We have already justified our choice of ligand [5]. Differences in implants used to deliver steroids may affect receptor quantities. During the early luteal phase of the natural menstrual cycle, when receptors are significantly suppressed, the mean serum P:E_2 ratios in cynomolgus [5] and rhesus [22] macaques are 42:1 and 60:1, respectively. Studies on artificial menstrual cycles have used ratios of ~27:1 [36] or ~67:1 [34]. In the study reported here, one and two P implants resulted, respectively, in average ratios of ~48:1 and 107:1. A P:E ratio of 107:1 is not typical of the luteal phase of the menstrual cycle in macaques, but such ratios may occur during pregnancy [38–42]. The threshold for suppression of the E_2 receptor by P may be higher in the cervix, and such suppression may only occur naturally during pregnancy or when the P:E ratio is substantially greater than usual. Why the estrogen receptor system in the cervix should have a higher threshold for P suppression than in the endometrium or oviduct in cynomolgus macaques is not clear. We hope to examine E_2 receptors in the macaque cervix during the natural menstrual cycle and in pregnancy to help resolve this question.

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