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Hormonal Regulation of Myometrial Estrogen, Progesterone, and Oxytocin Receptors in the Pregnant and Pseudopregnant Hamster*

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ABSTRACT. Estrogen receptor (Re) and progesterone receptor (Rp) concentrations were measured in the myometrium of hamster uterus during pregnancy and pseudopregnancy. Comparison of Re and Rp levels with serum estradiol and progesterone titers revealed that receptor concentration was low when progesterone was elevated during pregnancy and pseudopregnancy. However, Re and Rp levels increased when progesterone levels dropped at the end of each condition. In comparing serum estradiol relative to progesterone at the end of pregnancy and pseudopregnancy, it was discovered that Re and Rp recovery occurred not only when the estradiol to progesterone ratio increased (pseudopregnancy) but also when the ratio did not change (pregnancy). This suggested that serum progesterone was the primary determinant of receptor down-regulation, and this was confirmed by comparing the receptor recovery response to estrogen and progesterone withdrawal in the decidualized hamster uterus. Total Re levels increased to the same extent after progesterone withdrawal whether or not serum estradiol was maintained. When serum estrogen was maintained at a steady state, nuclear Re (nRe) increased within 4 h of progesterone withdrawal, and estrogen-dependent protein responses (Rp and oxytocin receptor) were obtained within 8 h. Thus, progesterone-induced down-regulation of nRe and estrogen-dependent proteins is rapidly reversed upon removal of hormone. The recovery response of Re, Rp, and oxytocin receptor to progesterone withdrawal can be blocked by cycloheximide treatment at 4 h, suggesting that receptor recovery involves protein synthesis. These results are consistent with the hypothesis that progesterone down-regulates the Re system by a selective action on nRe retention. (*Endocrinology* **116:** 1079–1084, 1985)

ESTROGEN and progesterone exert opposing effects on estrogen receptor (Re), progesterone receptor (Rp), and oxytocin receptor (R_{OT}) availability in the uterus (1, 2). Estrogen stimulates macromolecular synthesis leading to the accumulation of Rp, Re, and R_{OT} sites in the uterine target cell (3–5). In contrast, progesterone down-regulates these receptors by processes that are not fully understood (6–8). Correlations of circulating hormone and uterine receptor profiles during the female cycle have established that the following relationships apply. Receptor concentrations are higher during the follicular phase of the cycle than in the luteal phase, presumably reflecting the positive and negative effects of estrogen and progesterone action on receptor synthesis and degradation, respectively (9, 10).

Less is known about the mechanisms controlling uterine receptor levels during pregnancy, but the information available indicates that progesterone down-regulates uterine Re, Rp, and R_{OT} levels until the time of parturition (11, 12). While it has been convenient to correlate serum steroid and uterine receptor levels during pregnancy (13, 14), in fact such correlations fail to establish cause and effect relationships. Therefore, the purpose of the present study was to determine what happens to the number and subcellular distribution of Re, Rp, and R_{OT} when serum steroid levels are varied experimentally within the range normally observed during pregnancy in the hamster.

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Materials and Methods

Chemicals and buffers

[2,4,6,7-^{3}H]Estradiol-17β (90 Ci/mmol) and [1,2,6,7-^{3}H]progesterone (97 Ci/mmol), were obtained from New England Nuclear (Boston, MA) and stored in ethanol (100 μCi/ml) at −10 C. [Tyrosyl-^{3}H]Oxytocin (20 Ci/mmol) was generously supplied by Dr. J. S. Roberts (Worcester Foundation, Shrewsbury, MA). Radioinert steroids and unlabeled oxytocin were from Sigma Chemical Co. (St. Louis, MO). Other chemicals were obtained from commercial sources at reagent grade or better.

Scintillation counting solution was toluene-Triton X-100 (2:1, vol/vol) with 5 g PPO (2,5-diphenyloxazole) and 50 mg POPOP (1,4-bis[2-(5-phenyloxazolyl)]-benzene)/liter. Buffers used in Re and Rp assays were: A_{30} [50 mM Tris-HCl, 1 mM EDTA, 12 mM monothioglycerol, 30% glycerol (vol/vol), pH 7.5]; TG [10 mM Tris-HCl, 10% glycerol (vol/vol), pH 7.5]; B

(10 mM Tris-HCl, 1 mM EDTA, 12 mM monothioglycerol, pH 7.5); buffered saline (10 mM Tris-HCl, 150 mM NaCl); and Dextran-charcoal [0.5 g Norite A (Sigma), 50 mg Dextran-70 (Pharmacia)/100 ml buffer B]. TMG buffer used in the R_{OT} assay was 50 mM Tris-maleate, 5 mM $MnCl_2$, 1% (wt/vol) gelatin, pH 7.6.

Animal preparation

Adult female golden hamsters (Engle Laboratories, Farmersburg, IN) were maintained in a controlled environment with a 14-h light, 10-h dark photoperiod (lights on 0500–1900 h). Estrous cycles were monitored according to the appearance of the postestrous vaginal discharge on cycle day 1. Mating occurred at 2100 h (estrus) on cycle day 4. Males of proven fertility and vasectomized males were used to prepare pregnant and pseudopregnant females, respectively. Day 1 of pregnancy or pseudopregnancy was the day immediately after mating (day 4 of the cycle). Decidualization was induced at 1200 h on pseudopregnancy day 4 under pentobarbital (90 mg/kg BW) anesthesia by inserting a 3-cm length of nylon monofilament into the lumen of each uterine horn. In our experience, this procedure induces a uniform and reproducible deciduomal response in the hamster (15).

Estradiol and progesterone implants were prepared by packing crystalline steroid into 1-cm (estradiol) or 2.5 cm (progesterone) lengths of Silastic tubing (type C, od, 2.5 mm; bore, 1.5 mm; New Brunswick Scientific, New Brunswick, NJ), and the ends were sealed with polymerized Silastic plugs. The implants were soaked in saline for 24 h before being inserted sc at the time of ovariectomy on pseudopregnancy day 4. Progesterone withdrawal occurred on pseudopregnancy day 8 when progesterone implants were removed under ether anesthesia. Control animals were subjected to a sham procedure on day 8.

Animals were killed by decapitation, and trunk blood was collected for measurement of serum steroids by RIA as described elsewhere (16). Serum estradiol was 111 ± 7.5 pg/ml (n = 26), and progesterone dropped from 6.3 ng/ml to nondetectable (<0.3 ng/ml) levels within 8 h of progesterone withdrawal. Uteri were removed rapidly, chilled on a cold plate, stripped of fat and mesentery, and slit longitudinally. The myometrium was isolated by gently scraping away attached tissues with a spatula, weighed, and placed in ice-cold buffered saline for receptor analysis.

Receptor assays

Myometrial tissue was minced and homogenized in buffer A_{30} (1:8, wt/vol) with a Polytron Pt-10 (Brinkman Instruments, Westbury, NY). Cytoplasmic and nuclear fractions were separated by centrifugation of the homogenate at 800 × *g* for 10 min. The low speed cytoplasmic fraction was centrifuged at high speed (170,000 × *g*) for 30 min to prepare cytosol and a high speed pellet. The high speed pellet containing the membrane fraction was homogenized in TMG buffer for R_{OT} assay as described by Soloff (5) and modified by Roberts *et al.* (17). Cytosol and nuclear KCl extract were prepared for Re and Rp assay as detailed elsewhere (16). These assays measured unoccupied cytosol Re (cRe), total cytosol Rp (cRp), and total nuclear Re (nRe) and Rp (nRp). Protein and DNA were determined according to Lowry *et al.* (18) and Burton (19), respectively, using BSA and calf thymus DNA as standards. Statistical treatment of the results was by analysis of variance and the Student-Newman-Keuls test (20).

Results

In agreement with previous reports (21, 22), serum estradiol and progesterone increased gradually during pregnancy, and then both hormones declined significantly before term on day 16 (Fig. 1). However, the estradiol to progesterone ratio did not change appreciably

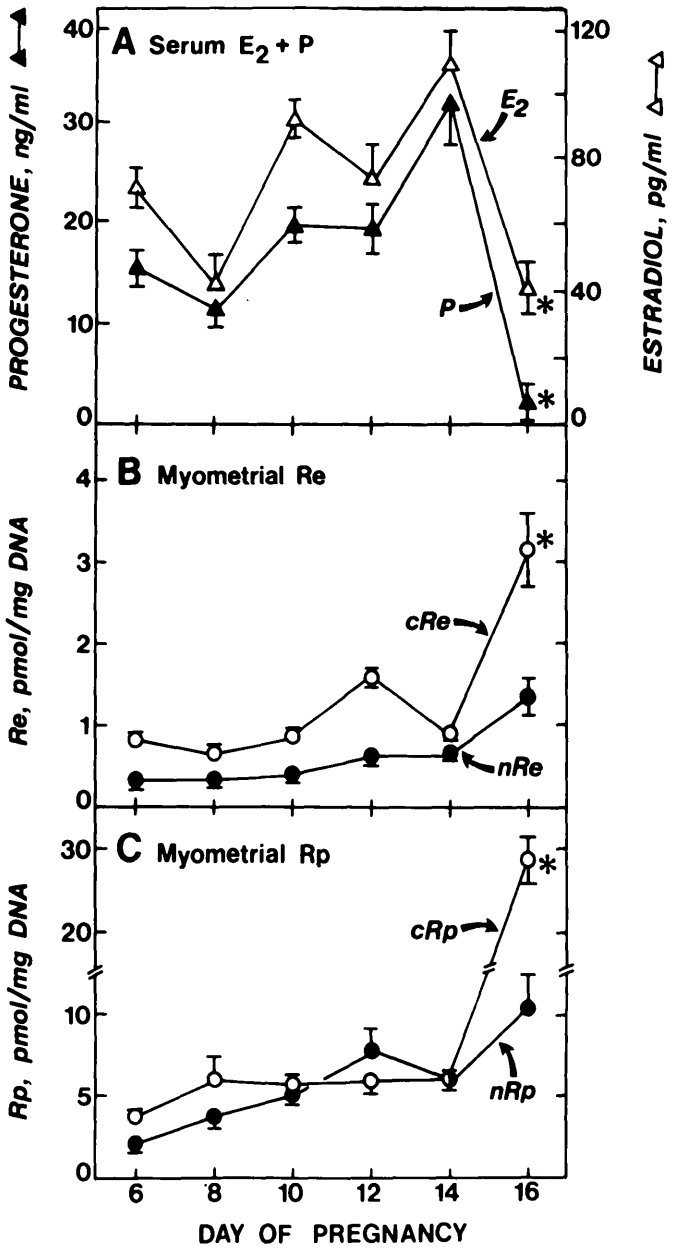

FIG. 1. Serum steroid-myometrial receptor relationships during pregnancy in the hamster. Parturition occurs in the morning of day 16. E_2, estradiol-17β; P, progesterone. (n = 6–10 per *point.*) *, Significantly different ($P < 0.05$) from preceding value.

from day 14 (0.004) to day 16 (0.008). Myometrial receptors during pregnancy are expressed on a DNA basis in Fig. 1. From day 6 through day 14 of pregnancy, myometrial Re and Rp remained nearly constant in cytosol and nuclear fractions, and on pregnancy day 16, cRe and cRp increased before parturition (Fig. 1). Re and Rp levels in cytosol and nuclei were low during gestation as compared with uterine receptor levels during the estrogen-dominated phase of the hamster estrous cycle. For example, at proestrus, myometrial receptor levels (picomoles per mg DNA) were: cRp, 38.5 ± 3.8; nRp, 5.2 ± 1.2; cRe, 2.02 ± 0.08; nRe, 1.66 ± 0.06 (mean ± SE), and receptor recovery at term tended to approach the proestrous values. Thus, prepartum Re and Rp recovery occurred when serum progesterone and estradiol were both declining.

The hormonal profile observed at the end of pseudopregnancy (Fig. 2) differed from that seen at the end of pregnancy (Fig. 1) in that estradiol rose as progesterone dropped. Thus, the estradiol to progesterone ratio increased progressively from day 7 (0.006) to day 8 (0.011) to day 9 (0.055). Myometrial receptors were suppressed during pseudopregnancy as compared with proestrous values (see above), and there was a significant recovery of nRe, nRp, and cRp on day 9 after serum steroids had shifted in favor of estradiol (Fig. 2).

The serum steroid-myometrial receptor relationships for pregnancy and pseudopregnancy indicated not only that Re and Rp levels were suppressed by progesterone action but also that receptor recovery during progesterone withdrawal occurred whether estrogen secretion was sustained (pseudopregnancy) or not (pregnancy). The possibility that Re recovery did not depend on estrogen was tested experimentally by subjecting pseudopregnant animals to various combinations of estrogen and progesterone withdrawal. Indeed, the results presented in Fig. 3 establish that the myometrial Re concentration is inhibited by progesterone in the presence or absence of estrogen. It is pertinent that total Re recovery to progesterone withdrawal occurred to the same extent whether or not serum estrogen was maintained. In progesterone-withdrawn groups, estrogen exposure increased nRe and cRp levels above those obtained with estrogen withdrawal. Thus, Re recovers in both cases when progesterone inhibition is removed, but not unexpectedly, estrogen-dependent responses such as cRp synthesis appear to require Re binding in the nucleus.

To examine the recovery of estrogen-dependent responses in more detail, ovariectomized pseudopregnant hamsters bearing progesterone and estradiol implants were subjected to progesterone withdrawal at time zero, and myometrial Re, Rp, and R_{OT} responses measured at 4 h, 8 h, and 16 h (Table 1). At 4 h after removal of progesterone implants, myometrial Re levels increased significantly in cytosol and nuclear fractions (Table 1).

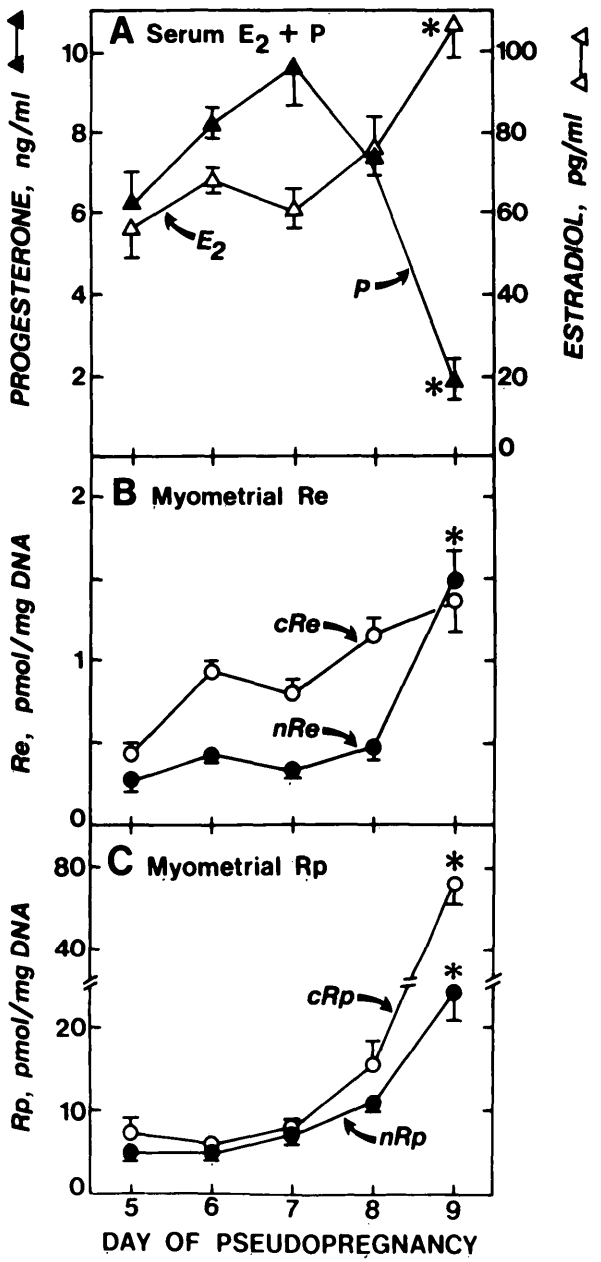

FIG. 2. Serum steroid-myometrial receptor relationships during pseudopregnancy in the hamster. Decidualization was induced on day 4, and deciduomal involution occurred on day 9. Abbreviations are defined in Fig. 1. (n = 8–12 per *point*.) *, Significantly different ($P < 0.05$) from preceding value.

This was followed by an elevation of the estrogen-induced proteins, Rp and R_{OT}, at 8 h and 16 h (Fig. 4). Re and Rp responses appeared to plateau between 8 h and 16 h, while the R_{OT} response was still increasing at 16 h. This experiment confirms that the Re system can recover rapidly after progesterone withdrawal and that this is followed by the restoration of estrogen-dependent responses.

In order to approach the question of whether myometrial receptor recovery was dependent on protein synthe-

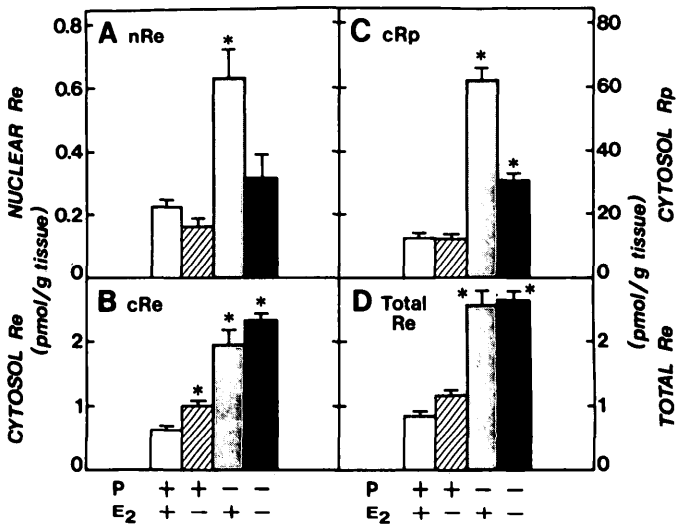

FIG. 3. Myometrial receptor response to estrogen and progesterone withdrawal in the decidualized hamster uterus. Animals were ovariectomized and given estradiol and progesterone implants on pseudopregnancy day 4. Hormone implants were removed on day 8, and receptor responses were measured 8 h later. +, Implant maintained; −, implant removed; n = 6 per treatment group. *, Significantly different ($P < 0.05$) *vs.* P+, E_2 + control.

TABLE 1. Response of Re, Rp, and R_{OT} in the myometrium of the decidualized hamster uterus to progesterone withdrawal

Treatment	n	Re (fmol/g tissue)		Rp (pmol/g tissue)	R_{OT} (dpm/mg protein)
		Nuclear	Cytosol		
4 h					
Control	12	222 ± 12	618 ± 29	8 ± 0.4	1,523 ± 90
−P	12	337 ± 21[a]	795 ± 52[a]	11 ± 0.5	1,781 ± 161
8 h					
Control	6	206 ± 14	582 ± 35	16 ± 1.1	1,547 ± 41
−P	9	556 ± 19[a]	1,951 ± 92[a]	45 ± 2.8[a]	2,778 ± 139[a]
16 h					
Control	6	150 ± 18	848 ± 85	11 ± 0.5	3,051 ± 362
−P	11	617 ± 50[a]	1,729 ± 198[a]	37 ± 1.7[a]	12,317 ± 1,222[a]

Pseudopregnant hamsters were ovariectomized at the time of deciduomal induction (day 4), and Silastic pellets of estradiol and progesterone were given sc. Progesterone withdrawal occurred at time zero on day 8. Controls were subjected to a sham procedure at time zero on day 8. −P, Progesterone withdrawal.

[a] $P < 0.05$ *vs.* control.

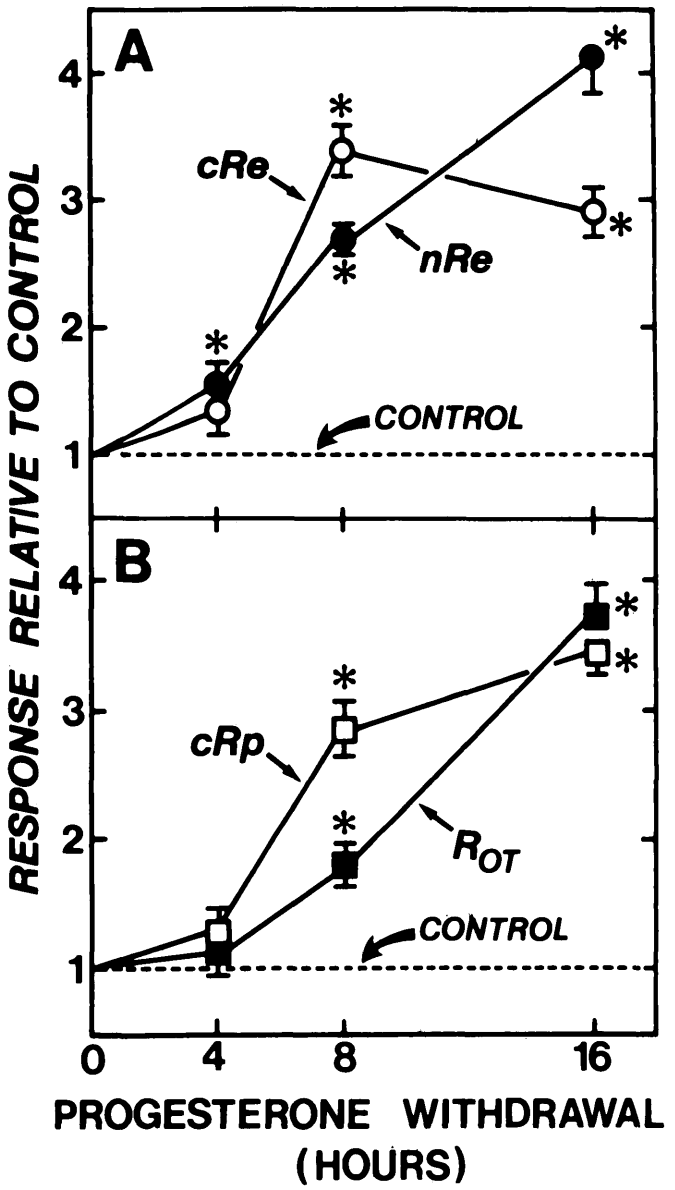

FIG. 4. Myometrial receptor response to progesterone withdrawal in the decidualized hamster uterus. Hamsters were ovariectomized and given estradiol and progesterone implants at the time of deciduomal induction on pseudopregnancy day 4. Progesterone implants were removed on day 8, and receptor responses were measured at the times indicated thereafter. The relative responses were calculated from the results in Table 1. *, Significantly different ($P < 0.05$) *vs.* paired control.

sis, the inhibitor cycloheximide was administered at 4 h after progesterone withdrawal, and receptor responses were measured at 8 h (Fig. 5). Cycloheximide (10 mg/100 g BW) effectively blocked all responses at 8 h, indicating that the receptor recovery responses were all mediated by stimulation of protein synthesis.

Discussion

The present results document the pattern of myometrial Re and Rp in the hamster uterus during pregnancy and pseudopregnancy. As has been reported for other species (12–14), receptor concentrations remain low throughout pregnancy, and Re and Rp levels recover when serum progesterone declines at term. Re and Rp recovery has been attributed to a shift in the ratio of estrogen relative to progesterone, *e.g.* the estrogen-progesterone ratio (11–13). In comparing receptor responses at the end of pregnancy and pseudopregnancy in the hamster a significant discovery was made. In both cases, Re and Rp levels recovered when serum progesterone

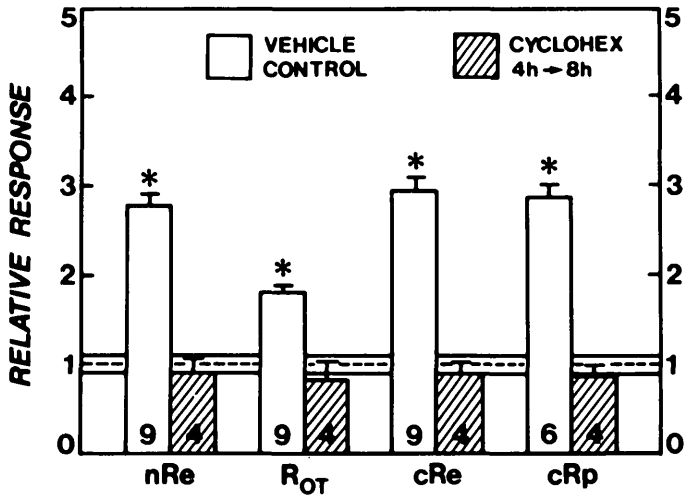

FIG. 5. Effect of cycloheximide on myometrial receptor response to progesterone withdrawal. Pseudopregnant hamsters were prepared as in Fig. 4, and progesterone implants were removed at zero time on day 8. Cycloheximide (10 mg/100 g BW) and vehicle control treatments were given at 4 h, and receptor levels were measured at 8 h after progesterone withdrawal. The *horizontal bar* represents results from sham-withdrawn preparations which served as a control for progesterone withdrawal. The control values were: nRe, 198 ± 12 fmol/g tissue; R_{OT}, 1510 ± 50 dpm/mg protein; cRe, 661 ± 47 fmol/g tissue; cRp, 15 ± 0.82 pmol/g tissue. Cyclohex, Cycloheximide; n is given at the base of each *bar*. *, Significantly different ($P < 0.05$) control *vs.* cycloheximide.

declined. However, in one case (pregnancy) serum estrogen fell and in the other (pseudopregnancy) it rose such that the estrogen-progesterone ratio did not change in the first case and shifted in favor of estradiol in the second. This suggested that the estrogen-progesterone ratio was not as important as the serum progesterone level in determining whether Re and Rp levels were down-regulated or not. This hypothesis was tested in the experiment done to compare the effects of estrogen and progesterone withdrawal on myometrial receptor levels in the decidualized hamster uterus. It is significant that total Re levels increased to the same extent after progesterone withdrawal whether or not serum estradiol was maintained. However, Re distribution between nucleus and cytosol was found to be a function of estrogen exposure. Thus, progesterone is the primary determinant of Re down-regulation, and the serum estrogen level regulates Re distribution.

Since estrogen stimulates the synthesis of Rp (3) and R_{OT} (2, 5) in myometrium, it was of interest to correlate responses in these end points of estrogen action with the recovery of the Re system. Progesterone withdrawal for 8 h enhanced Rp and R_{OT} levels when serum estradiol was maintained. The results demonstrate that recovery of nRe at 4 h is one of the earliest responses that can be detected upon progesterone withdrawal. cRe also increases early, but it is the nRe response which appears to be required for the subsequent recovery of estrogen-dependent proteins such as Rp and R_{OT}. From this, it follows that progesterone action inhibits nRe levels, and the down-regulation of nRe leads to the suppression of estrogen-dependent protein synthesis including the production of Rp, R_{OT}, and perhaps Re itself. That protein synthesis is required for the recovery of myometrial receptors after progesterone withdrawal is indicated by the ability of cycloheximide to block the recovery of all receptors between 4 h and 8 h. This indicates that the recovery of the Re system from suppression by progesterone may be dependent on protein synthesis. However, it remains to be determined whether there is a sequential recovery of nRe followed by Rp and R_{OT} synthesis or a simultaneous recovery of all receptors.

Progesterone has a rapid inhibitory effect on nRe retention in the estrogen-primed hamster and rat uterus (16, 23), and progesterone appears to down-regulate nRe by induction of an Re regulatory factor (24, 25). The present results indicate that progesterone down-regulates Re and inhibits estrogen-induced proteins (Rp and R_{OT}) during pregnancy by a similar mechanism. The hormone withdrawal studies demonstrate that the inhibitory effect of progesterone is readily reversible when hormone is removed from the system. Finally, it is clear that the serum progesterone concentration is more important in down-regulating Re than is the estrogen to progesterone ratio. However, further studies are needed to determine the absolute levels of estrogen and progesterone required for up- and down-regulation of the Re and Rp systems.

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