J. steroid Biochem. Vol. 25, No. 2, pp. 219–224, 1986
Printed in Great Britain.

0022-4731/86 $3.00 + 0.00

TEMPORAL EFFECTS OF PROGESTERONE DOMINATION ON ESTROGEN AND OXYTOCIN RECEPTORS IN HAMSTER UTERUS

AKIHIRO TAKEDA and WENDELL W. LEAVITT*
Department of Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX 79430, U.S.A.

(*Received* 11 *July* 1985)

Summary—The purpose of this study was to determine whether progesterone (P)-induced down regulation of estrogen receptors (Re) and oxytocin receptors (R_{OT}) changes with the time of P exposure. Ovariectomized hamsters were given s.c. Silastic implants of estradiol (E_2) and P for 4, 8 and 16 days. Cytosol and nuclear Re were measured at low temperature with the pyridoxal phosphate exchange assay, and R_{OT} was assayed in the membrane fraction by [^{3}H] oxytocin binding. Nuclear Re and R_{OT} were down regulated throughout the 16-day P exposure period, but cytosol Re (and total Re) increased progressively from 4 to 16 days indicating that the down regulation of cytosol Re escapes P control with time. This conclusion was supported by P withdrawal studies in which P implants were removed for 6 or 12 h. P withdrawal resulted in equivalent recovery responses of nuclear Re and R_{OT} after 4, 8 and 16 days of P exposure. Although cytosol Re recovery to P withdrawal occurred at 4 and 8 days, no response was obtained after 16 days of P exposure. Uterine weight increased during steroid treatment, and morphometric analysis of the P-dominated uterus revealed significant increases in the cross sectional area of the endometrium and myometrium with time of P exposure. Cytological examination of the uterus showed prominent secretory changes in the epithelial compartment on day 16 with accumulation of secretion in the uterine lumen. These results demonstrate that P can chronically down regulate nuclear Re and R_{OT}. However, the control of cytosol Re varies with the time of P exposure, and cytosol Re levels become refractory to P domination by 16 days. The present observations indicate that the escape of cytosol Re from P control may be associated with the proliferation of one of more uterine cell populations such as glandular and luminal epithelial cells.

INTRODUCTION

Estrogen (E) and progesterone (P) exert opposing effects on estrogen receptor (Re) and oxytocin receptor (R_{OT}) availability in the uterus [1, 2]. E up regulates Re and R_{OT} sites in uterine target cells by stimulation of macromolecular synthesis [3, 4], and P down regulates these receptors by processes that need to be clarified [1, 2].

P rapidly inhibits nuclear Re retention in the E-primed hamster and rat uterus [5, 6], and P appears to down regulate nuclear Re by induction of an Re regulatory factor [7–9]. Chronic P treatment suppresses myometrial Re and R_{OT} levels in the presence of steady state E exposure [10], and recent studies with the hamster and sheep have shown that the inhibitory effect of P on uterine Re and R_{OT} is readily reversible when P is withdrawn for a few hours [10–12]. Thus, during pregnancy, the information available indicates that P down regulates uterine receptor levels until the time of parturition when P levels may decline markedly [10, 13, 14]. An important unresolved question which needs to be considered is whether P-induced down regulation of the Re system changes with time of hormone exposure? Therefore, the purpose of the present study was 2-fold. First, we determined whether the down regulation of Re and R_{OT} in hamster uterus changes with the time of P exposure from 4 to 16 days. Second, we tested the receptor recovery response of the uterus to P withdrawal after various intervals of P domination. A preliminary report of these studies has appeared [15].

EXPERIMENTAL

Animal preparation

Adult female golden hamsters (Sasco, Omaha, Neb.) were maintained under a controlled photoperiod (lights on 0500–1900 h). The regularity of the estrous cycle was established according to the appearance of the postestrous vaginal discharge (morning, cycle day 1). Ovariectomies were performed on cycle day 1 under pentobarbital anesthesia (90 mg/kg b.wt). E implants were prepared by packing crystalline 17β-estradiol (E_2) [Sigma, St Louis, Mo.] into 1.0-cm lengths of Silastic tubing (No. 602-235, Dow Corning Co., Midland, Mich.), and P implants contained crystalline P (Sigma, St Louis, Mo.) packed into 2.5-cm lengths of Silastic tubing. At the time of ovariectomy, the E and P implants were placed subcutaneously in the flank region, and hamsters were sacrificed at 4, 8 and 16 days of E plus P treatment. P withdrawal was done by removing the P pellet under light ether anesthesia at 6 or 12 h before sacrifice. E pellets were left in place.

*To whom correspondence should be addressed.

Chemicals and buffers

[6,7-^{3}H] Estradiol-17β ([^{3}H]E_2; 47.4 Ci/mmol) was obtained from New England Nuclear Corp. (Boston, Mass.) and stored in ethanol (100 μCi/ml) at -10°C. [^{3}H]Oxytocin ([^{3}H]OT; 32.5 Ci/mmol) was obtained from Amersham (Arlington Heights, Ill.) and stored at -10°C. Radioinert steroids, pyridoxal 5'-phosphate (PLP) and sodium barbital were purchased from Sigma Chemical Co. (St Louis, Mo.). Pitocin (synthetic oxytocin; 10 U/ml) was obtained from Parke, Davis & Co. (Detroit, Mich.). All other chemicals were obtained from standard commercial sources and were reagent grade or better.

Barbital buffer contained 20 mM sodium barbital and 5 mM dithiothreitol, pH 8.0. Saline was buffered with 10 mM Tris–HCl, pH 7.4. Buffer A_{30} contained 50 mM Tris–HCl, 1 mM EDTA, 12 mM monothioglycerol and 30% glycerol (v/v), pH 7.5. Dextran-coated charcoal contained 0.5 g Norit A (Sigma) and 50 mg Dextran T70 (Pharmacia, Piscataway, N.J.) in 100 ml of 10 mM Tris–HCl and 1 mM EDTA, pH 7.5. Scintillation cocktail was toluene–Triton X-100 (2:1, v/v) with 5 g diphenyloxazole (PPO) and 50 mg 1,4-*bis*-[2,(5-phenyloxazolyl)]benzene (POPOP) per l.

Tissue preparation

Uteri were removed rapidly, stripped of fat and mesentery, slit longitudinally, blotted, weighed and placed in ice-cold buffered saline. Blood was collected from the axillary vein for measurement of E_2 and P levels. All subsequent procedures were carried out at 0°C, unless otherwise noted. Uteri were minced and homogenized in 6 vol (w/v) of barbital buffer with a Polytron Pt-10 homogenizer (Brinkman Instruments, Westbury, N.Y.), as previously described [16]. The homogenate was centrifuged at 170,000 *g* for 1 h to yield the cytosol fraction which was diluted 1:1 (v/v) with barbital buffer plus 20 mM PLP prior to assaying for cytosol Re. The nuclear fraction was washed twice by resuspension in 6 vol (w/v) of barbital buffer and centrifugation at 800 *g* for 10 min. Nuclear Re was extracted from the washed nuclear pellet using 6 vol (w/v) of barbital buffer plus 10 mM PLP. The nuclear suspension was incubated for 1 h, with mixing at 15-min intervals, and centrifuged at 170,000 *g* for 1 h to remove nuclear debris. The resultant supernatant was diluted 1:1 (v/v) with barbital buffer plus 10 mM PLP and used to assay nuclear Re.

Assay of Re in cytosol and nuclear extract

Receptor concentrations were determined by Scatchard plot analysis [17] of specific binding data [18], using the PLP exchange assay for Re at low temperature [16]. Aliquots (300 μl) of cytosol and nuclear extracts were incubated in a total volume of 500 μl with increasing concentrations of [^{3}H]E_2 (0.19–3 nM) for determination of total binding. Identical samples were incubated with an excess amount of unlabeled E_2 (4 μM) for measurement of nonspecific binding. Specific binding was total binding minus nonspecific binding. All samples were incubated at 0°C for 18 h to measure total Re, and all assays were done in duplicate. After overnight incubation, free steroid was removed from each sample by incubation with 500 μl dextran-coated charcoal for 10 min, followed by centrifugation at 1500 *g* for 5 min. Fifty μl of 120 mM sodium borohydride was added to the supernatant to blanch the yellow color of PLP. Four ml of scintillation cocktail was added to each vial and radioactivity was counted at 27% efficiency for cytosol Re and 35% efficiency for nuclear Re using a Beckman LS-230 liquid scintillation counter.

Assay of R_{OT}

Oxytocin receptor was assayed according to Soloff[4] with modification [10]. Tissues were homogenized in 8 vol of A_{30} buffer (w/v). Homogenate was centrifuged at 800 *g* for 10 min, and the supernatant was centrifuged at 105,000 *g* for 30 min. The 105,000 *g* pellet containing membranes was homogenized in 50 mM Tris–maleate, 5 mM $MnCl_2$ and 0.1% gelatin, pH 7.6, and used for R_{OT} assay. Aliquots (200 μl) of the resuspended membrane fraction were incubated with [^{3}H]-OT (3 nM) with or without an excess of unlabeled pitocin (1.5 μM) for 60 min at 22°C. The reaction was stopped by adding 5 ml of Tris–maleate buffer without gelatin, and then the aliquots were filtered on a Millipore manifold (Bedford, Mass.) using an 8.0 μm filter overlying a 0.3 μm filter. After filtration, the 8.0 μm filter was placed in a counting vial and 5 ml of scintillation cocktail was added. Radioactivity was extracted overnight and then counted at 30% counting efficiency.

Histological examination

Representative pieces of uterine tissue from the different treatment groups were fixed in 10% formalin, embedded in paraffin, and paraffin sections stained with hematoxylin and eosin (H and E). For morphometry, uterine tissues were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde and postfixed in 1% OsO_4. Samples were taken from three different (proximal, middle and distal) regions of each uterine horn, and these were dehydrated in ethanol and embedded in LX-112 (Ladd Res. Ind. Inc., Burlington, Vt). One micrometer sections were cut and stained with toluidine blue. For tissue area measurements, photographs of uterine cross sections were taken, and area measurements were made by tracing around the serosal and luminal surfaces and the interface between myometrium and endometrium.

Other methods

Serum E_2 and P were determined by specific RIA as described before [19] using antibodies supplied by

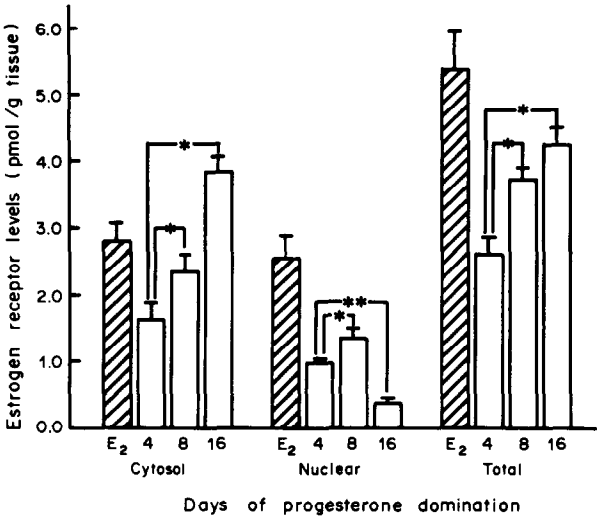

Fig. 1. Temporal effects of P domination on cytosol, nuclear and total (cytosol plus nuclear) uterine Re levels. Ovariectomized hamsters were given either a s.c. E_2 pellet for 4 days (hatched bars) or E_2 and P pellets for 4, 8 and 16 days (open bars). Uterine Re was measured with the PLP exchange assay done at low temperature as described in the text. Each value represents the mean ± SEM ($N = 14$). *Higher than day 4 value, $P < 0.05$. **Lower than day 4 value, $P < 0.05$.

Dr G. D. Niswender, Colorado State University, Fort Collins, Co. Protein concentrations in cytosol were determined by the method of Sedmak and Grossberg[20], using BSA as standard. The protein content of membrane preparations was determined by the Lowry method [21]. DNA was assayed according to the procedure of Burton[22], using calf thymus DNA as standard. Statistical analysis of results was done by analysis of variance and Student's t-test.

RESULTS

Temporal effects of P domination on Re levels

First, we studied uterine Re levels in the ovariectomized hamster given subcutaneous Silastic pellets of P and E_2 for 4, 8 or 16 days (Fig. 1). Serum steroid concentrations in animals bearing these P and E_2 pellets for 4–16 days were as follows: $E_2 = 98 \pm 5$ pg/ml and $P = 10 \pm 2$ ng/ml (mean ± SEM, $N = 12$). Both cytosol Re and nuclear Re were down regulated significantly ($P < 0.05$) in the first 4 days of P treatment as shown by comparing Re values for 4 days of E_2 alone with those for 4 days of E_2 plus P. P action reduced nuclear Re levels below the values for E_2 alone for the entire 16-day treatment period, with small but significant changes observed in nuclear Re on day 8 (increase) and day 16 (decrease) [Fig. 1]. Although cytosol Re was down regulated on day 4 of P exposure, cytosol Re increased by day 8 and continued to recover on day 16 reaching a value 2.3-fold higher than that on day 4. Total Re (cytosol plus nuclear) followed a pattern similar to that observed for cytosol Re, decreasing initially and then recovering progressively from day 4 to day 16 of steroid treatment.

Re response to P withdrawal

Different Re recovery responses to P withdrawal were observed in uterine cytosol and nuclear fractions (Fig. 2). Recovery of cytosol Re occurred on days 4 and 8, and in these two groups, cytosol Re increased with time after P withdrawal, rising 1.5-fold at 12 h after P withdrawal as compared to the P-maintained control. However, on day 16, cytosol Re did not respond to P withdrawal. In contrast, a nuclear Re

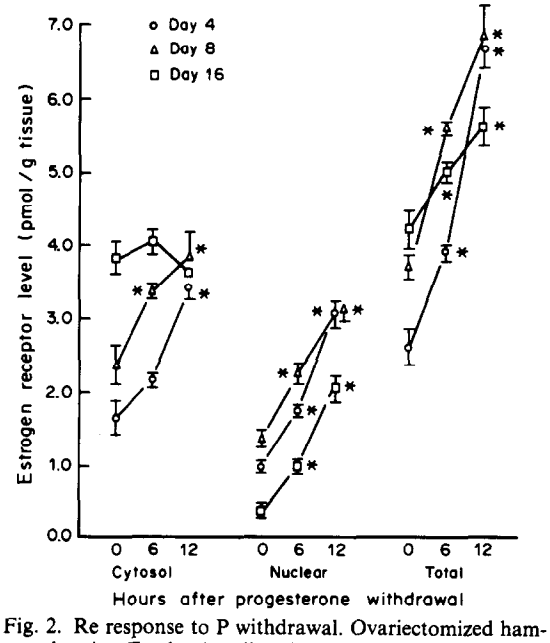

Fig. 2. Re response to P withdrawal. Ovariectomized hamsters bearing E_2 plus P pellets for 4 days (○), 8 days (△), or 16 days (□) had the P pellets removed for 0, 6 and 12 h. Each Re value represents the mean ± SEM ($N = 6$–14). *Significant increase, $P < 0.05$.

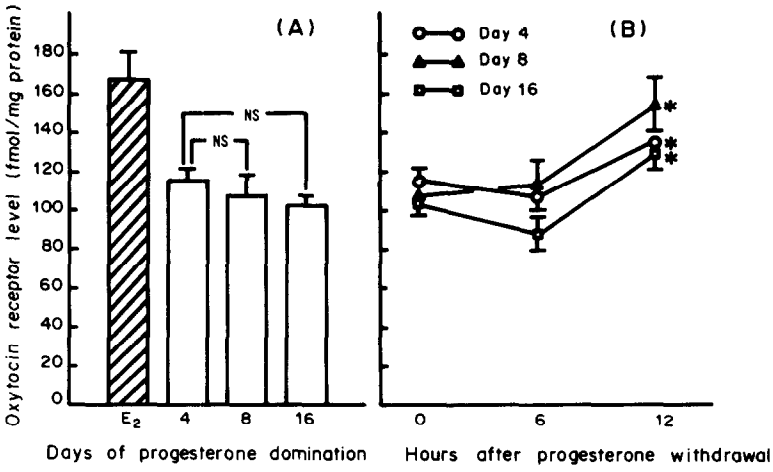

Fig. 3. Effect of P domination (A) and P withdrawal (B) on uterine R_{OT} levels. R_{OT} was assayed in the uterine membrane fraction after 4 days of E_2 (hatched bar) or after E_2 plus P for 4, 8 and 16 days (open bars). The R_{OT} response to P withdrawal for 0, 6 and 12 h was determined on day 4 (○), day 8 (△) and day 16 (□) of P domination. Each value represents the mean ± SEM ($N = 5$–7). NS, not significantly different; * significant increase, $P < 0.05$.

recovery response to P withdrawal was observed at all times studied, e.g. days 4, 8 and 16. Total Re levels increased after P withdrawal in all experimental groups, although the Re recovery on day 16 was reduced somewhat as compared to those obtained on days 4 and 8.

R_{OT} responses

P domination caused a significant ($P < 0.05$) down regulation of R_{OT} on day 4, and this persisted until day 16 (Fig. 3A). There were no significant changes of R_{OT} levels between day 4 and day 16. Although no significant changes in R_{OT} levels were observed at 6 h after P withdrawal, R_{OT} increased significantly at 12 h of P withdrawal on days 4, 8 and 16 (Fig. 3B).

Uterine weight and histology

There were no significant changes in uterine DNA content and cytosol protein content during P treatment (results not shown). However, uterine wet weight increased significantly on day 16 as compared to the value on day 4 (Table 1). In addition, P withdrawal for 12 h caused a significant uterine wet weight response on days 4, 8 and 16 (Table 1).

Histological analysis of P-dominated uteri revealed that striking cytological changes occurred with time of P treatment. The most notable transformations were evident on day 16 when the cross-sectional area of endometrium and myometrium had increased. The increase in endometrial area was due primarily to the hyperplasia and prominent secretory activity of epithelial cells with accumulation of secretion in the uterine lumen (Fig. 4).

Table 1. Uterine wet weight

Day of E_2 + P treatment	Time of P withdrawal (hours)		
	0	6	12
4	471 ± 25	551 ± 8†	626 ± 24†
8	551 ± 35	559 ± 4	842 ± 46†
16	675 ± 30*	658 ± 22	947 ± 70†

Hamsters were ovariectomized on cycle day 1 and given E_2 and P implants. After 4, 8 and 16 days of treatment, P implants were removed for 0, 6 and 12 h. Mean ± SEM ($n = 5$–6). *$P < 0.05$ vs day 4. †$P < 0.05$ vs 0 h.

DISCUSSION

The present study demonstrates that P-induced down regulation of uterine Re changes with the time of P exposure. Nuclear Re was down regulated throughout the 16-day P exposure period, but cytosol Re (and total Re) increased progressively from 4 to 16 days (Fig. 1) indicating that the down regulation of cytosol Re escapes P control with time. This latter interpretation is supported by the results of the P withdrawal studies which showed that cytosol Re recovery responses were obtained at 4 and 8 days but not at 16 days of P exposure (Fig. 2). These results (Fig. 2) suggest that cytosol Re levels may be regulated within certain limits, and cytosol Re appears to increase up to about 4 pmol/g tissue in response to P withdrawal. Thus, on day 16 no further response is obtained perhaps because cytosol Re has already reached this plateau. In contrast, P withdrawal elicited equivalent recovery responses of nuclear Re (and R_{OT}) at all times tested. Thus, P appears to have a differential action on nuclear and cytosol Re.

Recent immunocytochemical evidence [23] and cell fractionation studies [24] suggest that the majority of Re may be localized in the nucleus of intact target cells. Thus, Re distribution between cytosol and nucleus may be an artifact of the cell fractionation procedure. It this is true, then we may be measuring loosely-bound (cytosol) and tightly-bound (nuclear) populations of nuclear Re. Tight binding of the E_2–Re complex is believed to occur with specific

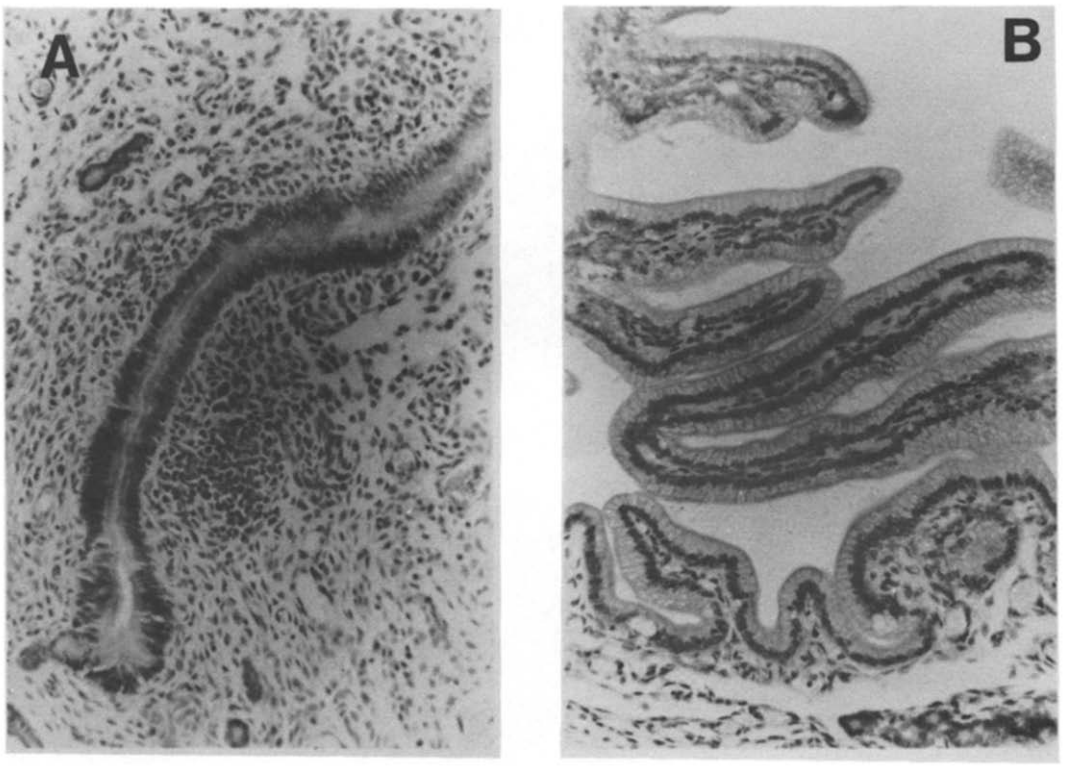

Fig. 4. Uterine morphology during P domination. Histological sections were from the middle portion of the uterine horn: (A) endometrium after 4 days of E_2 plus P; (B) endometrium after 16 days of E_2 plus P. H + E (×200).

acceptor sites located in the nuclear chromatin [25], and the occupation of these nuclear binding sites by the activated form of receptor appears to be responsible for the stimulation of hormone-dependent gene expression [26, 27]. The present results indicate that P antagonism of the uterine Re system and E-dependent responses occurs primarily by down regulation of the tightly-bound population of nuclear Re under conditions of long-term progestin action. This conclusion is supported by the finding that uterine weight and R_{OT} responses were correlated with nuclear Re recovery following P withdrawal at all times tested (Table 1, Figs 2 and 3). Thus chronic suppression of the active form of nuclear Re is seen with long-term P treatment, and this is consistent with our previous studies which showed that P selectively controlled nuclear Re retention in the hamster, rat and sheep uterus [5–12].

Of interest is the time-dependent escape of cytosol Re from P control. In agreement with previous studies with the rat uterus [28], we found that short-term P treatment blocked replenishment of cytosol Re in the hamster uterus (Fig. 1). However, cytosol Re appears to become refractory to long-term P action (Fig. 2). It may be pertinent that uterine growth occurred during steroid treatment for 16 days (Table 1), and morphometric analysis of the P-dominated uterus revealed an enlargement of both endometrium and myometrium. In addition, prominent secretory changes were evident in the epithelium on day 16 with accumulation of secretion in the uterine lumen (Fig. 4). Thus, certain aspects of uterine function change with time of P exposure, and these include various cellular responses occurring especially in the endometrial compartment. The present observations suggest that the escape of cytosol Re from P control may be associated with the proliferation of one or more uterine cell populations such as glandular and luminal epithelial cells. However, it remains to be determined whether these P-associated modifications in uterine cell composition account for the development of a P-independent pool of cytosol Re. It is possible that cytosol Re may increase with time of P exposure simply because certain target cells become refractory to P action. Rabbit endometrial cells appear to become refractory to sustained P action in terms of uteroglobin production [29]. Uterine epithelial cells in the cat undergo distinct secretory changes in response to E and P action, and chronic P treatment is first antiestrogenic and later progestational [30]. Thus, it will be of interest in future studies to determine how cytosol Re is controlled during long-term P action.

Acknowledgements—This work was supported by NIH grants HD18711 and HD18712. We thank Ann Laros and Rebecca Reeves for preparation of the manuscript.

REFERENCES

1. Leavitt W. W., MacDonald R. G. and Okulicz W. C.: Hormonal regulation of estrogen and progesterone receptor systems. In *Biochemical Actions of Hormones* (Edited by G. Litwack). Academic Press, New York, Vol. X (1983) pp. 324–356.
2. Nissenson R., Flouret G. and Hechter O.: Opposing effects of estradiol and progesterone on oxytocin receptors in rabbit uterus. *Proc. natn. Acad. Sci. U.S.A.* **75** (1978) 2044–2048.
3. Sarff M. and Gorski J.: Control of estrogen binding protein under basal conditions and after estrogen administration. *Biochemistry* **10** (1971) 2557–2563.
4. , Soloff M. S.: Uterine receptor for oxytocin: effects of estrogen. *Biochem. biophys. Res. Commun.* **65** (1975) 205–212.
5. Evans R. W. and Leavitt W. W.: Progesterone action in hamster uterus: rapid inhibition of ^{3}H-estradiol retention by the nuclear fraction. *Endocrinology* **107** (1980) 1261–1263.
6. Okulicz W. C., Evans R. W. and Leavitt W. W.: Progesterone regulation of estrogen receptor in the rat uterus. A primary inhibitory influence on the nuclear fraction. *Steroids* **37** (1981) 463–470.
7. Evans R. W. and Leavitt W. W.: Progesterone inhibition of uterine nuclear estrogen receptor: dependence on RNA and protein synthesis. *Proc. natn. Acad. Sci. U.S.A.* **77** (1980) 5856–5960.
8. Okulicz W. C., Evans R. W. and Leavitt W. W.: Progesterone regulates the occupied form of nuclear estrogen receptor. *Science* **213** (1981) 1503–1505.
9. Okulicz W. C., MacDonald R. G. and Leavitt W. W.: Progesterone-induced estrogen receptor-regulatory factor in hamster uterine nuclei: preliminary characterization in a cell-free system. *Endocrinology* **109** (1981) 2273–2275.
10. Leavitt W. W.: Hormonal regulation of myometrial estrogen, progesterone, and oxytocin receptors in the pregnant and pseudopregnant hamster. *Endocrinology* **116** (1985) 1079–1084.
11. Leavitt W. W. and Okulicz W. C.: Progesterone control of nuclear estrogen receptor. Demonstration in hamster uterus during the estrous cycle and pseudopregnancy using a new exchange assay. *J. steroid Biochem.* **22** (1985) 583–588.
12. Leavitt W. W., Okulicz W. C., McCracken J. A., Schramm W. and Robidoux W. F. Jr: Rapid recovery of nuclear estrogen receptor and oxytocin receptor in the ovine uterus following progesterone withdrawal. *J. steroid Biochem.* **22** (1985) 687–691.
13. Alexandrova M. and Soloff M. S.: Oxytocin receptors and parturition. I. Control of oxytocin receptor concentration in the rat myometrium at term. *Endocrinology* **106** (1980) 730–735.
14. Alexandrova M. and Soloff M. S.: Oxytocin receptors and parturition in the guinea pig. *Biol. Reprod.* **22** (1980) 1106–1111.
15. Takeda A. and Leavitt W. W.: Temporal effects of progesterone domination on estrogen and oxytocin receptors in hamster uterus. *Biol. Reprod.* **32** [Suppl. 1] (1985) 194.
16. Okulicz W. C., Boomsma R. A., MacDonald R. G. and Leavitt W. W.: Conditions for the measurement of nuclear estrogen receptor at low temperature. *Biochem. biophys. Acta* **757** (1983) 128–136.
17. Scatchard G.: The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **51** (1949) 660–672.
18. Leavitt W. W., Chen T. J. and Evans R. W.: Regulation and function of estrogen and progesterone receptor systems. In *Steroid Hormone Receptor Systems* (Edited by W. W. Leavitt and J. H. Clark). Plenum Press, New York (1979) pp. 197–222.
19. Evans R. W., Chen T. J., Hendry W. J. and Leavitt W. W.: Progesterone regulation of estrogen receptor in the hamster uterus during the estrous cycle. *Endocrinology* **107** (1980) 383–390.
20. Sedmak J. J. and Grossberg S. E.: A rapid, sensitive and versatile assay for protein using Coomassie brilliant blue. *Analyt. Biochem.* **79** (1977) 544–552.
21. Lowry O. H., Rosebrough N. L., Farr A. L. and Randall R. J.: Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
22. Burton K.: A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62** (1956) 315–323.
23. King W. J. and Greene G. L.: Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. *Nature* **307** (1984) 745–747.
24. Welshons W. V., Lieberman M. E. and Gorski J.: Nuclear localization of unoccupied oestrogen receptors. *Nature* **307** (1984) 747–749.
25. Kon O. L. and Spelsberg T. C.: Nuclear binding of estrogen-receptor complex: receptor specific nuclear acceptor sites. *Endocrinology* **111** (1982) 1925–1935.
26. Taylor R. N. and Smith R. G.: Effects of highly purified estrogen receptors on gene transcription in isolated nuclei. *Biochemistry* **21** (1982) 1781–1787.
27. Spelsberg T. C., Littlefield B. A., Seelke R., Dani G. M., Toyoda H., Boyd-Leinen P., Thrall C. and Kon O. L.: Role of specific chromosomal proteins and DNA sequences in the nuclear binding sites for steroid receptors. *Recent Prog. Horm. Res.* **39** (1983) 463–513.
28. Clark J. H., Hseuh A. J. W. and Peck E. J. Jr: Regulation of estrogen receptor replenishment by progesterone. *Ann. N.Y. Acad. Sci.* **286** (1977) 161–179.
29. Isotalo H.: Regulation of uteroglobin synthesis and conservation of progesterone and estrogen receptors in immature rabbits uterus during progesterone treatment. *Biochem. biophys. Res. Commun.* **115** (1983) 1015–1022.
30. Boomsma R. A. and Verhage H. A.: The uterine progestational response in cats: Ultrastructural changes during chronic administration of progesterone to estradiol-primed and nonprimed animals. *Am. J. Anat.* **164**, (1982) 243–254.