Vol. 135, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 26, 1986 Pages 98-104

PROGESTIN-INDUCED DOWN REGULATION OF NUCLEAR ESTROGEN RECEPTOR IN UTERINE DECIDUAL CELLS: ANALYSIS OF RECEPTOR SYNTHESIS AND TURNOVER BY THE DENSITY-SHIFT METHOD

Akihiro Takeda and Wendell W. Leavitt*

Department of Biochemistry
Texas Tech University Health Sciences Center
Lubbock, Texas 79430

Received December 12, 1985

SUMMARY: The density-shift method was used to study the effect of the synthetic progestin, R5020, (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione) on the turnover and synthesis of nuclear estrogen receptor in hamster decidual cells. Newly-synthesized receptor was labeled with dense [^{2}H, ^{13}C, ^{15}N] amino acids and separated from pre-existing receptor by density-gradient centrifugation. Progestin increased receptor turnover within 3 h of treatment and blocked estradiol-induced receptor synthesis at 6 h and 9 h. Thus, progestin down regulates estrogen receptor by increasing receptor turnover and inhibiting estrogen-induced receptor replenishment.

INTRODUCTION: It is well known that estrogen action is modulated by progesterone in target cells of the female reproductive system (1), but the underlying mechanism responsible for progesterone-induced changes in estrogen action is unknown. It is perhaps pertinent that progesterone down regulates the Re[1] system (2), and the site of progesterone action appears to reside in the target cell nucleus (3,4). Recently, we found that progesterone mediates a selective loss of the occupied form of Re from the uterine cell nucleus (5), and our studies with the estrogen-primed rodent uterus (6,7) suggest that progesterone may promote nuclear Re turnover by a process involving a receptor regulatory factor (ReRF) which causes Re release from nuclear acceptor sites

*To whom correspondence should be addressed. This work was supported by NIH grants HD18711 and HD18712.

[1] ABBREVIATIONS: Re, estrogen receptor; ReRF, estrogen receptor-regulatory factor; R5020, 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione; E_2, 17β-estradiol; [^{125}I]-E_2, [16α-^{125}I]-iodoestradiol; PLP, pyridoxal-5'-phosphate; DCC, dextran-coated charcoal; HBSS, Hanks' balanced salt solution; DME, Dulbecco's modified Eagle's medium; NAA, normal amino acid; DAA, dense amino acid.

0006-291X/86 $1.50

(8). In order to test the hypothesis that progestin controls Re turnover, it is necessary to evaluate both Re synthesis and degradation. Recent studies on the receptors for several hormones (9-13) have demonstrated the utility of the density-shift technique for monitoring receptor protein dynamics. Thus, we have employed the density-shift method to determine whether the synthetic progestin, R5020, controls Re turnover and/or synthesis in monolayer cultures of hamster decidual cells.

Decidualization of the rodent uterus is a progesterone-dominated process in which endometrial stromal cells are converted to decidual cells during early pregnancy. We have recently developed conditions for growing hamster decidual cells in primary culture (14), and these cells contain Re in addition to progesterone receptor when cultured in the absence of steroid hormones (15). In the present study, we show that R5020 down regulates nuclear Re by increasing receptor turnover in decidual cells.

METHODS: Materials. [^{125}I]-E_2 (2000 Ci/mmol) and R5020 were purchased from New England Nuclear (Boston, MA). Algal amino acid mixture enriched in the dense isotopes [^{2}H, ^{13}C, and ^{15}N] (98, 90 and 99%, respectively) was obtained from Merck, Sharp and Dohme (Montreal, Canada). Tissue culture media and horse serum were purchased from Gibco (Grand Island, NY). Other chemicals were of the highest purity available from commercial sources. The tissue culture medium was Ham's F12/DME (1/1, v/v) supplemented with 5% DCC-stripped horse serum, insulin (10 μg/ml), transferrin (10 μg/ml) and selenium (10 ng/ml). NAA medium contained complete HBSS, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, 9.1 mM $NaHCO_3$, non-essential amino acids (Flow Laboratories, McLean, VA), 60 μg/ml L-glutamine, insulin, transferrin, selenium and 5% DCC-treated horse serum as above. Horse serum was dialyzed against HBSS to remove amino acids. DAA medium contained the same ingredients as NAA medium except that non-essential amino acids were replaced with 0.5 mg/ml [^{2}H, ^{13}C, ^{15}N]-amino acids, 30 ng/ml L-cystine and 10 μg/ml L-tryptophan. Barbital buffer was 20 mM barbital, 5 mM dithiothreitol and 10% glycerol, pH 8.0. PTG buffer was 5 mM sodium phosphate, 10 mM monothioglycerol and 10% glycerol, pH 7.4. Saline (0.15 M NaCl) was buffered with 10 mM Tris-HCl (pH 7.4).

Cell-culture Conditions. Decidual cells were isolated from the endometrium of decidualized hamster uteri by collagenase digestion as previously described (14). Dispersed cells were washed, counted and plated at 1.7 X 10^7 cells/dish in #3003 Falcon tissue culture dishes. Two hours later, non-adherent cells were removed by changing the medium, leaving a homogeneous population of decidual cells attached to the dish.

Density Shift Method. Decidual cells were grown in Ham's F12/DME for 2 days. Prior to DAA labeling, cells were pre-treated with NAA medium supplemented with 1 nM E_2 for 1 h at 37°C to charge the unfilled Re sites at the beginning of the experiment. Then, NAA medium was replaced with DAA medium supplemented with either 1 nM E_2 or 1 nM E_2 plus 10 nM R5020 and incubation was continued

for 3, 6 and 9 h. After DAA labeling, cells were washed twice with Tris-saline and scraped from the dish. Cells were collected by centrifugation and homogenized in 300 µl of barbital buffer with a Polytron Pt-10 homogenizer. The homogenate was centrifuged at 800 xg for 10 min, and the nuclear pellet was washed twice with barbital buffer. Nuclear Re was extracted with 200 µl of 10 mM PLP in barbital buffer for 1 h with vortexing every 10 min (16). Then, nuclear Re extract was obtained by centrifugation at 170,000 xg for 1 h. An aliquot (160 µl) of the PLP-extracted nuclear Re was labeled with 5 nM [^{125}I] E_2 for 1 h at 37°C. After removing free [^{125}I] E_2 by DCC treatment (32 µl), an aliquot (130 µl) was layered on a 5-20% sucrose gradient prepared in D_2O with PTG buffer and 0.4 M KCl. The gradient was centrifuged at 368,000 xg for 38 h in a Beckman SW56 rotor. Two-drop fractions were collected from the top of the gradient and counted in a Beckman model 5500 gamma-counter at 74% counting efficiency. The amount of Re was estimated from the peak height after the gradient profile baseline had been corrected for nonspecific binding as determined using parallel Re samples competed with a 100-fold molar excess of unlabeled E_2. DNA was determined according to Burton (17) using calf thymus DNA as standard, and Re is expressed on a DNA basis.

RESULTS: Decidual cells were first cultured in Ham's F12/DME medium without steroid hormones for 2 days and then exposed to 1 nM E_2 for 1 h at 37°C before DAA labeling. Preliminary experiments showed that this E_2 pretreatment caused a significant accumulation of occupied Re in the nuclear fraction, whereas no significant Re peak was observed upon sucrose-density gradient centrifugation without this treatment (data not shown). At time 0, cells were switched to DAA medium supplemented with either E_2 alone or E_2 + progestin. After incubation for 3,6 and 9 h, newly-synthesized nuclear Re labeled with DAA was separated from pre-existing Re of normal density by sucrose gradient centrifugation. At time 0, the pre-existing nuclear Re from decidual cells sedimented as a single 4S peak on sucrose gradients containing 0.4 M KCl (Fig. 1). With time of incubation, the 4S peak of normal density decreased and a second peak of DAA-labeled Re appeared as a faster-sedimenting component with a sedimentation coefficient of 6S (Fig. 1). In E_2 + R5020-treated cells, both normal and heavy Re peaks were smaller than those of E_2-treated cells, and this difference became greater with increasing time of incubation (Fig. 1).

The sum of normal and heavy Re peaks provided an estimate of total nuclear Re at each time of incubation. E_2 treatment had no effect on total nuclear Re during the first 3 h of incubation, but thereafter nuclear Re levels increased significantly ($P<0.05$) from 6 h to 9 h of E_2 treatment (Fig.

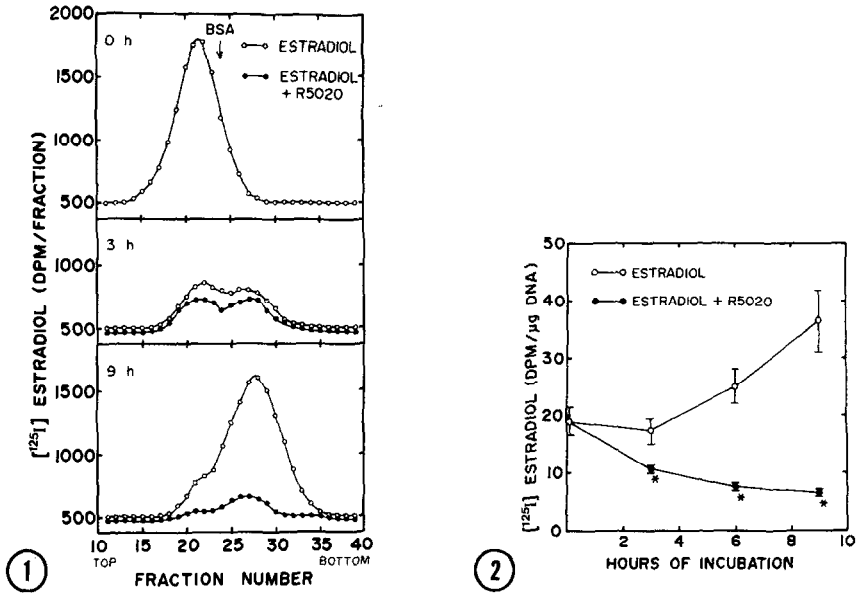

Figure 1: Sedimentation profiles of pre-existing and newly synthesized nuclear Re using the density shift technique. Decidual cells were cultured in Ham's F12/DME medium for 2 days. Prior to DAA labeling, cells were exposed to 1 nM E_2 for 1 h at 37°C to charge the unfilled Re. Then cells were switched to DAA medium supplemented with either 1 nM E_2 (○) or E_2 + 10 nM R5020 (●). After 3, 6 and 9 h of incubation, cells were harvested and nuclear Re was extracted with 10 mM PLP. Nuclear Re was labeled with 5nM [^{125}I]-E_2 and free steroid was removed by DCC treatment. Normal and heavy density forms of nuclear Re were separated by centrifugation in 5-20% sucrose-density gradients (containing 0.4 M KCl in D_2O) at 340,000 xg for 38 h in a SW56 rotor. Bovine serum albumin (BSA) was used as a sedimentation marker (4.6 S). Only the bottom 30 fractions are shown.

Figure 2: Effect of E_2 and E_2 + progestin treatment on nuclear Re levels. Decidual cells were grown in DAA medium with either 1 nM E_2 or E_2 + 10 nM R5020 for 3, 6 and 9 h, and nuclear Re peaks were quantified as shown in Fig. 1. Each point represents the sum of normal and DAA-labeled Re peaks (mean ± SEM). *$P < 0.05$ versus E_2 groups.

2). In contrast, combined treatment with E_2 + R5020 significantly ($P<0.05$) down regulated nuclear Re at 3 h as compared to the E_2 control group, and nuclear Re levels failed to recover during subsequent incubation from 3-9 h.

The disappearance pattern of the normal density Re is shown in Fig. 3A. The half-life ($t_{\frac{1}{2}}$) of pre-existing Re in decidual cells treated with E_2 alone was 3.9 h (n= 16, r = 0.95), and progestin treatment caused a marked shortening of the half-life of nuclear Re from 3.9 to 2.1 h (n= 15, r= 0.97). Statistical analysis of the Re values at each incubation time showed that R5020 started to stimulate the degradation of nuclear Re as early as 3 h after treatment and this effect persisted until 9 h.

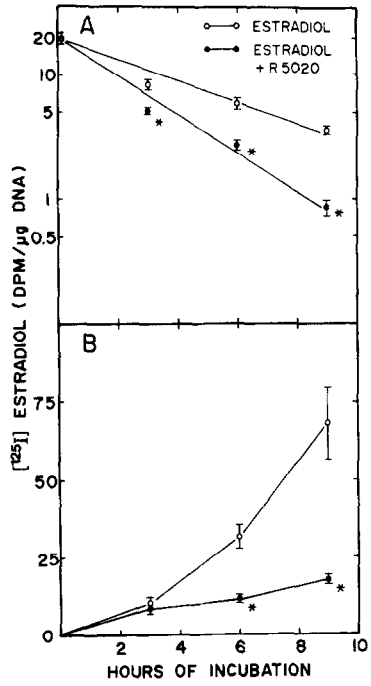

Figure 3: Effect of E_2 and E_2 + progestin treatment on Re turnover and synthesis in decidual cells. A. The half-life of nuclear Re was extrapolated from the lines obtained by linear regression analysis of the values for the normal density peak when cells were cultured with 1 nM E_2 or E_2 + 10 nM R5020. The half-life ($t_{\frac{1}{2}}$) of nuclear Re was 3.9 h (n = 16, r = 0.95) for cells incubated with E_2. R5020 treatment shortened the $t_{\frac{1}{2}}$ of Re to 2.1 h (n = 15, r = 0.97). B. Synthesis of nuclear Re was calculated from the values of the heavy density peak according to the method of Schimke (18) as described in the text. The mean ± SEM Re synthesis (Ks · t) is plotted. Synthesis rates (Ks) are given in Table 1. *P < 0.05 versus E_2 group.

The synthesis of nuclear Re was determined from the height of the heavy Re peak labeled with DAA (Fig. 3B). Since this heavy Re degrades at the same rate as the Re of normal density and R5020 stimulated Re degradation (Fig. 3A), it was necessary to correct Re synthesis for differences in Re degradation (18) using the formula: $Ks = Kd \cdot Rt/(1-e^{-kdt})$, where Ks is the synthesis rate, $K_d = 0.693/t_{\frac{1}{2}}$, and Rt is the Re value (DPM/µg DNA) at time "t". With this approach, the corrected synthesis (Ks · t) of nuclear Re was calculated (Fig. 3B), and there were no significant differences in Re synthesis comparing E_2 and E_2 + progestin treatments at 3 h. Subsequently, from 3 h to 9 h E_2 increased the Re synthesis rate from 2.9 ± 0.2 to 7.9 ± 1.3 DPM/µg DNA/h, respectively (Table I), and R5020 treatment blocked the E_2-induced stimulation of Re synthesis (Fig. 3B).

Table I: Synthesis rates of Re in decidual cells as influenced by E_2 and R5020 treatment

TREATMENT	Synthesis Rate (DPM [^{125}I] estradiol/μg DNA/h)		
	3 h	6 h	9 h
E_2 (control)	2.9 ± 0.2	5.1 ± 0.9	7.9 ± 1.3[a]
E_2 + R5020	2.8 ± 0.1	1.9 ± 0.1[b]	2.0 ± 0.1[b]

The Re synthesis rate (K_s) was calculated from the height of the faster-sedimenting Re peak resulting from incorporation of dense amino acids, using the formula, $K_s = K_d \cdot Rt/(1 - e^{-Kdt})$ as explained in the text. Each value represents the mean ± SEM for 3-5 observations. a) $P<0.05$ versus 3 h value. b) $P<0.05$ versus E_2 control.

DISCUSSION: The present report demonstrates that decidual cells can serve as a useful paradigm for studying how progestin controls the Re system. Other workers have shown recently that steroid hormones influence the turnover of their own receptors. Glucocorticoids (19) and estrogens (13) both enhance the turnover rate of their respective receptors, while androgens have the opposite effect (20). We have shown that the synthetic progestin, R5020, can affect the dynamics of the receptor for estrogen.

Using the density-shift method to measure Re turnover and synthesis in uterine decidual cells, new features of the molecular mechanism responsible for progestin-induced down regulation of nuclear Re were revealed. R5020 markedly stimulated the turnover of preexisting nuclear Re within 3 h, and the synthesis of new Re from DAA was inhibited subsequent to the change in Re turnover. Thus, these results demonstrate there is a rapid primary effect of progestin on nuclear Re processing followed by a secondary inhibition of E_2-induced Re replenishment. We suggest that progestin-induced Re turnover may be responsible for the down regulation of uterine Re under physiological conditions such as pregnancy where progesterone action predominates over that of estrogen. Progesterone not only suppresses Re but also inhibits other E_2-induced uterine proteins including the oxytocin receptor until the time of parturition (21,22). However, nuclear Re and E_2-dependent proteins can recover rapidly upon progesterone withdrawal (22,23) showing that the

inhibitory action of progesterone on nuclear Re retention is readily reversible. Thus, the stimulation of nuclear Re turnover may represent a fundamental mechanism responsible for progesterone modulation of E_2 action.

REFERENCES

1. Leavitt, W.W., MacDonald, R.G. and Okulicz, W.C. (1983) in Biochemical Actions of Hormones, vol. 10. (Litwack, G., ed.) pp. 324-356, Academic Press, New York.
2. Hsueh, A.J.W., Peck, Jr., E.J. and Clark, J.H. (1976) Endocrinology 98, 438-444.
3. Evans, R.W. and Leavitt, W.W. (1980) Proc. Natl. Acad. Sci. 77, 5856-5860.
4. Okulicz, W.C., Evans, R.W. and Leavitt, W.W. (1981) Steroids 37, 463-470.
5. Okulicz, W.C., Evans, R.W. and Leavitt, W.W. (1981) Science 213, 1503-1505.
6. Leavitt, W.W. (1985) in Molecular Mechanism of Steroid Hormone Action (Moudgil, V.K., ed.) in press, Walter de Gruyter & Co., Berlin.
7. MacDonald, R.G., Rosenberg, S.P. and Leavitt, W.W. (1983) Mol. Cell. Endocrinol. 32, 301-313.
8. Spelsberg, T.CV., Littlefield, B.A., Seelke, R., Dani, G.M., Toyoda, H., Boyd-Leinen, P., Thrall, C. and Kon, O.L. (1983) Rec. Prog. Horm. Res., vol. 39 (Greep, R.O., ed.) pp. 463-517, Academic Press, New York.
9. Gardner,J.M. and Fambrough, D.M. (1979) Cell 16, 661-674.
10. Reed, B.C. and Lane, M.D. (1980) Proc. Natl. Acad. Sci. 77, 285-289.
11. Raaka, B.M. and Samuels, H.H. (1981) J. Biol. Chem. 256, 6883-6889.
12. Raaka, B.M. and Samuels, H.H. (1983) J. Biol. Chem. 258, 417-425.
13. Eckert, R.L., Mullick, A., Rorke, E.A. and Katzenellenbogen, B.S. (1984) Endocrinology 114, 629-637.
14. Leavitt, W.W., MacDonald, R.G. and Schwaery, G.T. (1985) Biol. Reprod. 32, 631-643.
15. Leavitt, W.W. (1984) Biol. Reprod. 30 (Suppl. 1), 160.
16. Okulicz, W.C., Boomsma, R.A., MacDonald, R.G. and Leavitt, W.W. (1983) Biochem. Biophys. Acta 757, 128-136.
17. Burton, K. (1956) Biochem. J. 62, 315-323.
18. Schimke, R.T. (1975) in Methods in Enzymology, vol. XL. (O'Malley, B.W. and Hardman, J.G., eds.) pp. 241-266, Academic Press, New York.
19. McIntyre, W.R. and Samuels, H.H. (1985) J. Biol. Chem. 260, 418-427.
20. Syms, A.J., Norris, J.S., Panko, W.B. and Smith, R.G. (1985) J. Biol. Chem. 260, 455-461.
21. Alexandrova, M. and Soloff, M.S. (1980) Endocrinology 106, 730-735.
22. Leavitt, W.W. (1985) Endocrinology 116, 1079-1084.
23. Leavitt, W.W., Okulicz, W.C., McCracken, J.A., Schramm, W. and Robidoux, Jr., W.F. (1985) J. Steroid Biochem. 22, 687-691.