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EFFECTS OF ESTROGEN AND PROGESTERONE ON CYTOPLASMIC ESTROGEN RECEPTOR AND RATES OF PROTEIN SYNTHESIS IN RAT UTERUS*

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SUMMARY

Changes in total cytoplasmic estrogen receptor (E_2-R_C) concentrations were compared to changes in rates of protein synthesis after steroid treatment in uterine tissue of long-term castrate Sprague Dawley rats. Animals were treated with estradiol 17β (E_2) alone or with progesterone (P_4) after 24 h of E_2 "priming". E_2-R_C concentration was "modulated" by E_2 (0.1 μg I.P.) in two distinct temporal phases: (A) Short term depletion with restoration to basal E_2-R_C/mg DNA occurred by 20 h after E_2 treatment. (B) Increases in E_2-R_C above basal levels were seen after 20–24 h. Twenty-four h after E_2 treatment, the administration of P_4 (1.0 mg, s.c.) caused the elevated concentration of total E_2-R_C to decrease to base line levels within 2 h. As determined from E_2-R_C decay experiments, modulation of E_2-R_C by E_2 or P_4 was not caused by activation of some soluble cytoplasmic receptor precursor nor by degradation (through proteolytic enzymes) of functional E_2-R_C. Uterine protein synthesis was monitored by pulse-labeling with [^{35}S]-methionine under the same treatment schedule as used for E_2-R_C quantitation. Both E_2 and P_4 stimulated incorporation of [^{35}S]-methionine into ribosomal, cytoplasmic and nuclear fractions. Increases in rates of [^{35}S]-methionine incorporation were interpreted as a demonstration of "hypertrophy". Since hypertrophy accompanied treatment with both estradiol and progesterone, and since differential modulation of E_2-R_C followed treatment with these steroids, the increase in E_2-R_C concentration following E_2 treatment appears to be a specific event which is distinct from hypertrophy.

It is proposed that estrogen mediated increases in E_2-R_C concentration represent a mechanism of molecular amplification which contributes in part to the cascading growth observed in the uterus. Conversely, progesterone antagonizes estrogen stimulation by depleting E_2-R_C concentration (in some unknown manner) thereby providing a biochemical "brake" to estrogen stimulation.

INTRODUCTION

Uterine tissue contains a high affinity estrogen binding protein which is reported to be involved in the mechanism of steroid hormone action. Following exposure to estradiol 17β (E_2), the E_2 passes across the cell membrane, binds to a specific uterine cytoplasmic protein (E_2-R_C) and eventually moves as a "steroid–receptor" complex into the nucleus [1, 2]. As a result, the concentration of "available" E_2-R_C declines and is gradually restored to the initial level of receptor concentration approximately 20 h after treatment. Such short-term restoration is termed "replenishment" [3–7]. Continuation of replenishment results in E_2-R_C concentrations clearly elevated above basal levels after 24–72 h. These later changes in E_2-R_C concentration are examined in this report. Hence, "modulation" of E_2-R_C concentration denotes short term "replenishment", long term increases above control levels, as well as decreases in E_2-R_C concentration mediated by progesterone [8, 9, 22]. Since several laboratories have shown that progesterone (P_4) antagonizes estrogen stimulation of uterine endometrial cells [10–12] it is important to correlate this antagonism with the steroid–receptor interaction. Because neither competitive inhibition nor a negative cooperative influence on steroid binding [8, 9] can explain the effect of progesterone on the apparent E_2-R_C concentration, this report focuses on those phenomenon which might mediate real changes in E_2-R_C concentration.

In this report steroid mediated modulation of E_2-R_C concentrations over a 48-h period is described. Both E_2-R_C activation and degradation during modulation were examined. Experiments were designed to compare modulation of E_2-R_C concentration with steroid mediated changes in rates of protein synthesis. Pulse labeling with [^{35}S]-methionine was performed in order to establish whether increases in E_2-R_C concentration, mediated by estradiol, occur as part of a wide spectrum of protein synthesis changes or if these increases in E_2-R_C are specific

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phenomena which are selectively distinct from hypertrophy.

MATERIALS AND METHODS

Sprague-Dawley rats (150–180 g) were bilaterally ovariectomized and used four weeks post-operatively. These "long term castrate" females all received an injection of estradiol 17β (Sigma, 0.1 μg/0.5 ml saline) intraperitoneally (i.p.) at $t = 0$. Some animals received progesterone (Sigma, 1.0 mg/0.5 ml propylene glycol) subcutaneously (s.c.) at $t = 24$ h. Animals were killed by cervical dislocation while under light ether anesthesia. Uterine horns were dissected free of oviduct, vagina, fat and connective tissue and placed in Hank's calcium free media (CFM) at 0–2 C. Uteri were diced into 1–2 mm fragments and washed twice in CFM.

Pulse labelling. Uterine fragments were incubated in Hank's minimal essential media (MEM, GIBCO) containing [^{35}S]-methionine (10 μCi/ml, 381.2 Ci/mmol, New England Nuclear Corp.) for 30 min at 37°C under an atmosphere of 95% O_2 and 5% CO_2 (Fig. 1). Incubation was terminated by chilling (0–2°C). Fragments were immediately washed (4×) with Hank's CFM (4 ml) at 0–2°C and homogenized in TEMK buffer containing Tris (pH 7.4, 40 mM), EDTA (1.5 mM), β-mercaptoethanol (14 mM) and KCl (50 mM). A tissue/buffer ratio of 4 uterine horns/ml was maintained. The homogenate was centrifuged at 800 *g* for 15 min (0–2°C) and the nuclear-myofibrillar pellet assayed for DNA [13].

This low speed supernatant was recentrifuged at 106,000 *g* for 90 min in a Beckman fixed angle rotor yielding a ribosomal pellet and a high-speed supernatant or "cytosol" fraction. The ribosomal pellet was washed twice with TEMK buffer in order to reduce potential contamination by soluble protein and free [^{35}S]-methionine. This washing was sufficient to substantially reduce counts due to free [^{35}S]-methionine, [^{35}S]-methionine-tRNA, and aminoacyl transferase

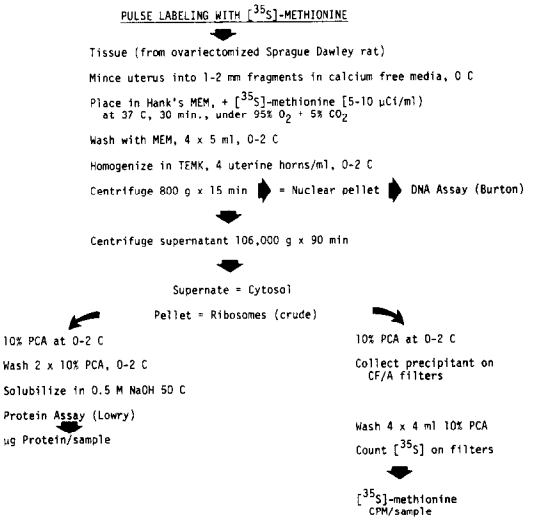

Fig. 1. Flow diagram for pulse labelling experiments with [^{35}S]-methionine.

charged with [^{35}S]-methionine. The washed pellet was solubilized in 0.5 M NaOH at 50°C. Following a 1:1 dilution with TEMK buffer, the ribosomal protein was precipitated with 10% perchloric acid (PCA) onto glass filters (Whatman GF/A) and washed 4 times with 4 ml of 10% PCA. The filters were then transferred into liquid scintillation vials containing 22% Triton X100 (Rohm-Haas) and PPO-POPOP-toluene fluor (2,5 diphenyloxazole [4 g], and 1,4 bis [2-(5 phenyloxazole)] benzene [0.5 gm] in 1 liter toluene).

Preparation of acid insoluble protein. The soluble proteins in the cytosol fraction were precipitated with an equal vol. of 20% PCA at 0–2 C. The precipitates were collected on GF/A glass fiber filters (Whatman Industries), washed four times with 4 ml 10% PCA and counted directly in liquid scintillation vials. Protein determinations were made on samples redissolved in 0.5 M NaOH [14]. Duplicate experiments, in which soluble protein was precipitated with 20% trichloroacetic acid (TCA), washed twice with 20% TCA and three times with ether, gave isotope incorporation data which were indistinguishable from the PCA precipitation. [^{35}S]-methionine could easily be distinguished during scintillation counting from [^{3}H]-estradiol because of its higher energy emission (0.167 and 0.018 MeV respectively).

Preparation of the "nuclear" fraction. The nuclear-myofibrillar pellet, obtained by low speed centrifugation at 800 *g*, was resuspended in 0.5 M PCA and precipitated by centrifugation at 0–2°C. The pellet was then washed 1 × 4 ml with 95% ethanol 1 × 4 ml ethanol–ether (3:1 v/v), and 2 × 4 ml 10% PCA. DNA was hydrolyzed by heating at 80°C for 45 min and assayed according to Burton[13]. The acid insoluble material was resuspended in 0.25 M NaOH and sampled for [^{35}S]-methionine incorporation into protein.

Assay of estrogen cytosolic receptors. High speed cytosol fractions were assayed for E_2-R_C concentrations using the "batch" hydroxylapatite (HTP) method [9, 15]. Aliquots of cytosol (225 μl) were incubated with saturating amounts of [^{3}H]-estradiol 17β(80–100 Ci/mmol, New England Nuclear Corp., 4.0×10^{-12} mol [^{3}H]-E_2 per 225 μl of cytosol). Incubation (0–2°C) was terminated after 10 h by the addition of 1.2 ml of hydroxylapatite suspension [10 g DNA grade hydroxylapatite (BioRad Industries) per 100 ml TEMK]. Adsorption of E_2-R_C to HTP was allowed to proceed at 0–2°C for 45 min. HTP with E_2-R_C adsorbed was then centrifuged, washed 5 × 1 ml with TEMK, the bound [^{3}H]-E_2 extracted 2 × 1 ml with 100% ethanol.

Liquid scintillation counting of the ethanol extract was performed with a tritium counting efficiency of 30–40%. Quench correction was determined for each vial with an external standard. Correction for non-specific binding was routinely made by subtracting [^{3}H]-E_2 bound in the presence of a 1000-fold excess of diethylstilbesterol (DES). All points were run in replicates of four [9].

Rates of decay of estrogen receptors. The three experimental treatment groups contained 18 rats each (Fig. 2). Group A (control) was injected with 0.5 ml saline (i.p.), Group B received 0.1 μg E_2 in 0.5 ml saline (i.p.) at $t = 0$, and Group C received 1000 μg P_4 in 0.5 ml propylene glycol (s.c.) at $t = 24$ h in addition to estradiol at $t = 0$. All animals were killed after 36 h. Mixed groups designated AB, BC and AC were formed by combining equal portions (1:1, v/v) of cytosol fractions from A, B or C respectively. Homogenization was performed in 10 ml of TEMK at 0–2°C followed by centrifugations at 800 *g*, and at 106,000 *g* as described (Fig. 1). The high speed cytosol fraction was diluted to 37 ml with TEMK and delivered into individual assay tubes. Incubation with saturating amounts of [^{3}H]-estradiol 17β (2×10^{-12} mol per 225-μl cytosol) was performed for 12 h at 0–2°C.

The six experimental cytosols were then tested for potential activation or degradation of E_2-R_C at 0°C and at 22°C. Control cytosols were included in the experimental design during all temperatures and times in order to compensate for the expected rate of E_2-R_C decay ($T_{1/2}$ = 5–6 days [3]) during the course of the experiment. All points were run as replicates of four and completed within ± 10 s per experimental time category. A post-experimental period (0–2 C for 12 h) was included to reestablish equilibrium between free steroid and receptor. Therefore, receptor bound activity did not reflect accelerated dissociation caused by elevated temperatures. Lowry assay for protein was performed as described elsewhere [14].

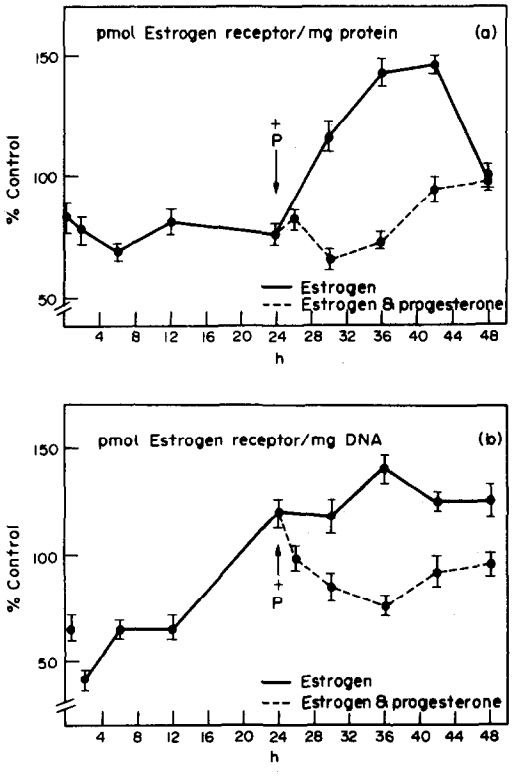

Fig. 2. Changes in estrogen cytoplasmic receptor concentration following treatment with E_2 or E_2 plus P_4. At $t = 0$ long-term castrate rats received 0.1 μg estradiol 17β in saline (solid line). At $t = 24$ h a second group of long-term castrate rats received 1000 μg progesterone (s.c.) in propylene glycol (dashed line) marked by the arrows (+P). Replicates of four were performed for each determination and expressed ± % S.E.M. (a) Shows E_2-R_C per mg protein in the cytosol fraction. Control values (at zero h) approximately 3.5 ± 0.3 pmol E_2-R_C/mg protein. (b) Shows E_2-R_C per mg DNA. Control values (at zero h) were approximately 40.8 ± 3.3 pmol E_2-R_C/mg DNA.

RESULTS

E_2-R_C modulation by estradiol and its opposition by progesterone

Comparisons between the time course of estradiol stimulation and P_4 mediated opposition to these changes in E_2-R_C concentration are presented in Fig. 2(a) and 2(b). Because protein and DNA concentration are changing differentially in the uterus following initial E_2 stimulation, results were normalized for both parameters.

E_2-R_C concentration per mg protein (Fig. 2a) remained below control levels for 24 h in animals treated with estrogen alone and became maximal 36–40 h after E_2 treatment. Protein concentration increased signficantly after 6 h of E_2 treatment, and this increase continued for 48 h. For the first 24 h following E_2 treatment E_2-R_C concentration increased in parallel with protein concentration but following this period E_2-R_C concentration increased more rapidly than protein concentration. Finally 42 h after E_2 treatment, the specific activity of E_2-R_C began to decline due to increases in uterine soluble protein. In animals, "primed" with E_2 and then treated with P_4 at $t = 24$ h, E_2-R_C/mg protein failed to increase.

E_2-R_C per mg DNA in rats injected with E_2 alone rose above control levels within 20 h. After P_4 treatment (at $t = 24$ h) E_2-R_C/mg DNA declined to below control levels within 4 h. This rapid decrease in available E_2-R_C concentration may be due to several factors including (a) decreased E_2-R_C synthesis, (b) decreased E_2-R_C replenishment, (c) decreased activation of a "percursor" molecule, or (e) increased E_2-R_C degradation (Fig. 2b).

Estrogen receptor activation or degradation

Receptor activation might explain long term E_2-R_C increases and E_2-R_C degradation may explain progesterone mediated decreases in E_2-R_C concentration. Estrogen receptor turnover was therefore explored as a process potentially responsible for modulation.

If inactive E_2-R_C precursors exist, cytosols from estrogen treated animals might also be capable of activating these receptor "precursors" in control cytosols. Conversely, cytosols from P_4 treated animals might demonstrate increased degradation of E_2-R_C when mixed with cytosols from control or estrogen treated animals. Hence mixing or "combinational" experiments were performed.

Table 1. Baseline parameters for rat uterine cytosol fractions used in receptor decay determination

In vivo treatment	I Cytosol fraction μg protein/ml ± S.E.M.	II E_2-R_C d.p.m.*/225 μl ± S.E.M.	III S.A. E_2-R_C d.p.m.*/mg protein ± S.E.M.
A. Control	496 ± 16	49,660 ± 1066	419,800 ± 9013
B. Estrogen	885 ± 9	102,300 ± 1617	538,400 ± 8506
C. Estrogen and Progesterone	1024 ± 53	70,080 ± 415	303,900 ± 1798

A. Saline control, 0.5 ml/rat (i.p.), 18 animals/group.
B. Animals were injected with 0.1 μg estradiol 17β at $t = 0$ and sacrificed after 36 h.
C. Animals were injected with 0.1 μg estradiol 17β at $t = 0$, received 1000 μg progesterone at 24 h and were sacrificed at 36 h.
* d.p.m. refers to specifically bound [^{3}H]-estradiol (E_2-R_C) after correction for non-specific binding using 1000 × excess DES: ± S.E.M. = standard error of the mean ($N = 4$).

Based on the information in Fig. 2, an experiment was designed to terminate after 36 h of steroid treatment when differences in E_2-R_C/mg DNA or E_2-R_C/mg protein were greatest. Both E_2 mediated increases as well as P_4 mediated decreases in E_2-R_C (d.p.m./225 μl or d.p.m./mg protein) were apparent (Table 1). Thus modulation was occurring when this system was examined at 36 h.

Cytosol fractions from control (A), estrogen treated (B) and estrogen–progesterone treated (C) animals were examined individually as well as when combined in 1:1 proportions. All fractions were examined for changes in the rate of E_2-R_C decay. Cytosols were either held for varying periods of time at 22°C, rapidly cooled and equilibrium re-established at 0–2°C for 12 h, or cytosols were kept at 0–2°C and assayed for E_2-R_C after varying intervals of time. The results of these experiments were subjected to linear regression analysis and are expressed as the linear equation: $Y = bx + a$, where b = "rate of decay" and a = "initial activity" (Table 2).

Long term decay rates (0°C, 10 h) for E_2-R_C did not differ significantly between cytosols from any of the treatment groups (Y_A, Y_B or Y_C) as judged by least squares regression analysis of the reaction slopes, (Table 2A). In fact, the P_4 treated group showed the slowest rate of E_2-R_C decay ($Y_C = -4.2$ d.p.m./min).

Short term decay rates (22°C, 30 min) of E_2-R_C showed no detectable differences in decay between cytosols from any treatment group. In fact, the least square slope is slightly positive for all groups at 22°C and reflects the fact that no decay was detectable (Table 2).

In the combinational experiments performed at 22°C, no increase in E_2-R_C decay could be detected (Fig. 3). A numerical reduction in least squares slope occurred for all groups at 22°C; however, this value changed the least for "$P_4 + E_2$" (Y_{BC}) and for "control + P_4" (Y_{AC}) combinations. None of the combinations yielded significantly different decay rates (Table 2C, mixtures as judged by t comparison criteria).

These results in Fig. 3 indicate that soluble factors mediating activation or degradation were not functionally detectable. Hence, cytosols from progesterone treated animals did not detectably increase degradation of E_2-R_C in either control or cytosols from E_2 treated animals. Control aliquots maintained 0–2°C for the duration of the experiment showed only small decreases in E_2-R_C which could be explained by the expected rate of receptor decay ($t_{1/2}$ = 5–6 days [3]). Therefore receptor degradation does not appear to be responsible for the rapid decrease in E_2-R_C concentration observed after progesterone treatment.

Table 2. Rate of Decay [d.p.m./min ± S.E.M.]

In vivo treatment	Incubated at 0–2°C ($df = 14$)	Incubated at 22°C ($df = 14$)	Mixtures incubated at 22°C ($df = 14$)
A. Saline control	−11.4 ± 5.44	146.1 ± 93.6	12.7 ± 46.7 (=AB)
t test	A *vs* B = 0.719	A *vs* B = 0.001	AB *vs* BC = 0.286
B. Estrogen	−22.9 ± 15.0	145.4 ± 531.5	46.2 ± 107.2 (=BC)
t test	B *vs* C = 1.205	B *vs* C = 0.155	BC *vs* AC = 0.077
C. Estrogen and progesterone	−4.2 ± 4.1	232.2 ± 174.7	37.2 ± 47.2 (= AC)
t test	A *vs* C = 1.074	A *vs* C = 0.434	AB *vs* AC = 0.368

Treatment groups were the same as in Table 1. Rates of decay were obtained from linear regression analysis of mean d.p.m. specifically bound to receptor at different periods of time. Combinational cytosols are designated AB, BC, AC. Students t comparisons were made as indicated, and no comparison was found to be significantly different at $F \geq 0.90$; $P < 0.1$ (df = degrees of freedom).

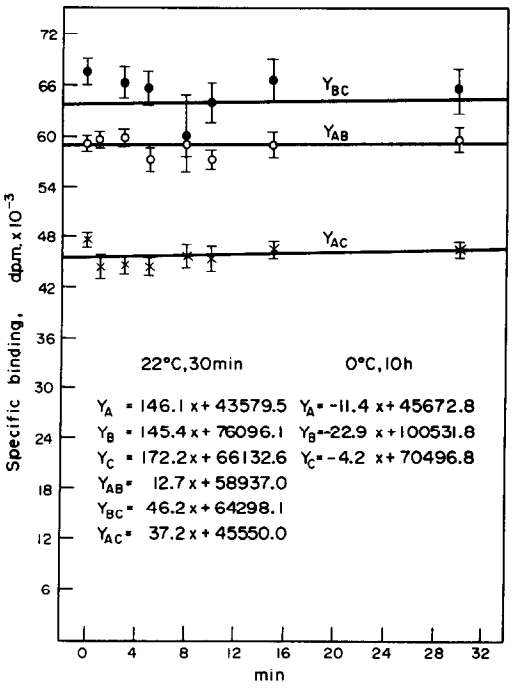

Fig. 3. Receptor decay determination. Decay rates for E_2-R_C following 36 h *in vivo* steroid treatment. Group A received saline only, Group B received 0.1 μg E_2 (i.p.) in saline, and Group C received 0.1 μg E_2 (i.p.) at 0 h and 1000 μg P_4 (s.c.) at 24 h. Groups designated AB (open circles = control + E_2), AC (crosses = control + P_4), BC (solid circles = E_2 + P_4) are 1:1 combinations of Groups A, B, or C at 22°C. Decay was examined at 0°C (data not graphed) as well as 22°C. Equilibrium was re-established following exposure at 22°C for periods of time shown on the *X* axis. Linear regression analysis was performed on all data points and is fitted to the equation shown. Compensation for the normally expected decay of E_2-R_C over the course of the experiment was made by including parallel reference cytosols which were not subject to temperature manipulations. Data is expressed as a mean E_2-R_C d.p.m. × 10^{-3} ± S.E.M. (N = 4).

Effect of estrogen and progesterone treatment on translation

Ribosomal incorporation. Pulse-label incorporation of [^{35}S]-methionine into the ribosomal fraction began increasing 2–4 h after E_2 treatment, continued to a maximum by 24 h, and then declined to control rates by 30 h (Fig. 4). Progesterone treatment prolonged the elevated rate of [^{35}S]-methionine incorporation into the ribosomal fraction. The group treated with E_2 and P_4 showed an extended high level of incorporation and returned to control rates only after 42 h. Changes in synthetic activity very likely reflect changes in numbers of ribosomes, rates of polysome formation, or changes in functional capacity mediated through increased mRNA availability as has already been reported after E_2 treatment [16–19].

These data show that incorporation into the ribosomal fraction increases to a constant maximal level which is maintained for 24 h after E_2 treatment. P_4 treatment at 24 h rapidly prolonged a maximal utilization of the available ribosomal translational components. Since an early lag did occur in response to E_2 treatment but not after P_4, it is likely that all translational components were not initially available for utilization.

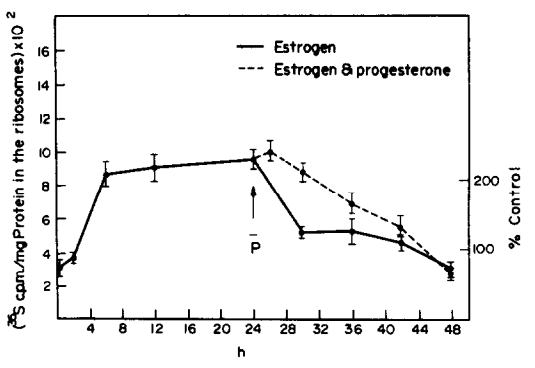

Fig. 4. Rate of incorporation of [^{35}S]-methionine into uterine ribosomal fraction. The *in vivo* animal treatment was described in Fig. 2 for 48 h. Specific incorporation [^{35}S]-methionine into the ribosomal fraction is expressed as c.p.m. ribosomal protein ± S.E.M. (N = 4).

Incorporation into soluble protein. Examination of the rate of incorporation of [^{35}S]-methionine into uterine acid precipitable cytoplasmic protein showed an increase by 8 h after E_2 treatment when normalized per mg protein (Fig. 5a) and also when normalized per mg DNA (Fig. 5b). In addition, for the first

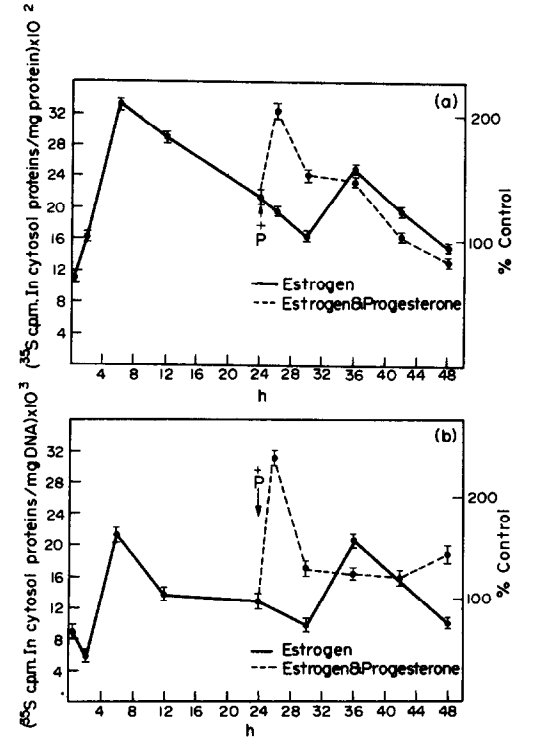

Fig. 5. Rate of incorporation of [^{35}S]-methionine into acid precipitable cytoplasmic uterine proteins. *In vivo* animal treatment was described in Fig. 2. (a) Results were expressed as c.p.m./mg protein ± S.E.M. (N = 4). (b) Results were expressed as c.p.m./mg DNA ± S.E.M. (N = 4).

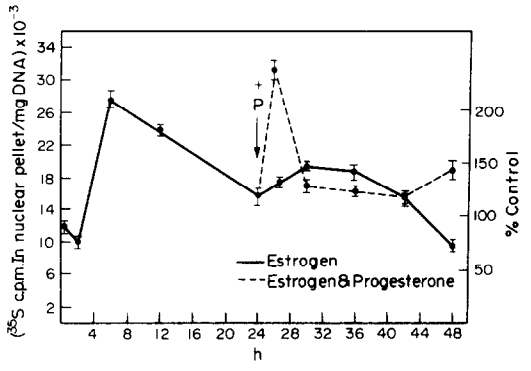

Fig. 6. Rate of incorporation of [^{35}S]-methionine into the "nuclear" fraction. *In vivo* animal treatment was described in Fig. 2. Results are expressed as [^{35}S] c.p.m./mg DNA ± S.E.M. (N = 4).

4 h following estradiol treatment, rates of protein synthesis were below saline control levels (=100%) as has already been reported [20, 21]. This correlates well with an initial fall following E_2 stimulation in the relative rate of synthesis of uterine "induced protein" reported recently by Katzenellenbogen[7].

Following P_4 treatment at 24 h, a sharp increase in the rate of incorporation into cytosol proteins occurred within 2 h. While progesterone decreased E_2-R_C concentration, it concomitantly mediated an increase in the rate of incorporation of labelled [^{35}S]-methionine into soluble protein. Hence, decreases in E_2-R_C concentration which are mediated by P_4 are coordinated with increased rates of protein synthesis.

Incorporation into the "nuclear" fraction. The rate of incorporation of [^{35}S]-methionine into the uterine "nuclear" fraction appeared similar to the incorporation into cytosol protein. Increased incorporation was seen by 8 h after E_2 treatment and within 2 h following the P_4 treatment at 24 h (Fig. 6). This "nuclear" preparation was not purified to homogeneity; however, incorporation can be attributed primarily to proteins associated with the nuclear and membraneous fractions. The effects of E_2 treatment on the rate of synthesis of nuclear proteins are similar to those reported by Means and Hamilton[20].

As with soluble cytosol proteins, the proteins of the particulate fraction showed increased rates of synthesis after either E_2 or P_4 stimulation.

DISCUSSION

Progesterone (P_4) has been shown to decrease or oppose long-term estrogen (E_2) mediated increases in cytoplasmic estrogen receptor concentration in the rat uterus [8, 9, 22, 32, 33]. This opposition could not be attributed to competitive inhibition or to negative cooperative effects on the estrogen binding site [15]. This report describes the temporal sequence of "modulation" of E_2-R_C as well as changes in the rate of uterine protein synthesis monitored *via* pulse-labeling.

Total E_2-R_C concentration during the early replenishment period showed maximum depletion within 2–6 h after E_2 treatment. This was followed by a return to control E_2-R_C/mg DNA levels by approximately 18–20 h (Fig. 2b) which is consistent with the data reported by Sarff and Gorski[3], Cidlowski and Muldoon[5] and Hsueh *et al.*[32]. After 24 h of E_2 stimulation *in vivo* the concentration of E_2-R_C normalized to either protein or DNA (Fig. 2a and 2b) was well above control values. P_4 rapidly opposed these increases in E_2-R_C concentration. The effect of P_4 was particularly convincing since it occurred when concentrations of E_2-R_C were clearly above saline injected control levels.

The modulation of uterine E_2-R_C concentration is apparently comprised of an early restoration phase ("replenishment") as well as a later phase in which E_2-R_C concentration is increased above control levels. The replenishment phase, which occurred in the first 0–20 h after E_2 treatment, has been shown to be sensitive to cycloheximide [3–6]. While work with inhibitors suggests that E_2-R_C synthesis must occur during early replenishment, it is also conceivable that synthesis of some other protein(s) is required to recycle existing receptor already involved in mediation of estrogenic signals [6]. Little information exists as to what extent excess non-radioactive estradiol from the "priming" treatment dilutes the specific activity of the [^{3}H]-estradiol in the assay during the early phases of replenishment. Considering these complexities it is particularly difficult to inquire about molecular events in the early replenishment phase of E_2-R_C modulation. This investigation focused, therefore, on the later phase of modulation where increased concentrations of E_2-R_C were clearly above control levels.

Progesterone might have opposed E_2 mediated increases in E_2-R_C by accelerated degradation of E_2-R_C. Experiments were designed to measure degradation rates of E_2-R_C and also to inquire whether replenishment resulted from activation of existing stores of "receptor precursor". Such a hypothetical activation should not be confused with recycling of existing E_2-R_C which was not studied in this experimental design.

Since E_2-R_C concentrations reached maximal levels by 36 h after initial E_2 treatment, experiments performed at this time point do not implicate receptor turnover in the modulation process. This evidence suggests that E_2 mediated increases in E_2-R_C concentration may primarily reflect increased synthesis of the binding protein. Because E_2 and P_4 both promote hypertrophy in terms of increased rates of protein synthesis, the correlation of P_4 with decreased E_2-R_C concentration supports a model where increases in E_2-R_C concentration are independent of uterine hypertrophy. Hence, increases in E_2-R_C concentrations are not merely part of a spectrum of synthetic events associated with increases in total protein synthesis.

Since cytoplasmic estrogen receptors are specifically increased by estradiol and selectively decreased by progesterone, estrogen mediated modulation may represent a mechanism of molecular amplification which contributes in part to the cascading growth observed in the uterus. It has been suggested that progesterone may mediate its antagonism to E_2 stimulation [2, 11, 12, 23–27, 32, 33] by selectively decreasing the synthesis of the binding protein. However, it is evident that P_4 does not limit all translational (and/or transcriptional) processes since rates of general protein synthesis increase concomitantly with decreases in E_2-R_C concentration.

Pulse labelling studies were performed with [^{35}S]-methionine since it has a high rate of incorporation into uterine tissue [28, 31]. Rates of incorporation into the ribosomal fractions support a model in which both E_2 and P_4 can stimulate a maximal utilization of ribosomes. Our data are consistent with the reports of increased ribosome formation 4–12 h after E_2 treatment by Moore and Hamilton[16] increased polyribosome formation by Teng and Hamilton[17], increased capacity by Greenman and Kenny[18], and increased rate of peptide chain initiation by Suvatte and Hagerman [19] following E_2 treatment.

The fact that synthetic rates in the ribosomal fraction do not mirror synthetic rates in the cytosol or "nuclear" fraction suggests that ribosomes represent a limited capacity component which is maximally utilized and that protein synthesis may be limited by transcriptional controls (Figs. 4 and 5). It also suggests that there is an early uneven distribution of newly synthesized protein between cytosol, ribosomes and nucleus.

An alternative speculation, to decreased synthesis of E_2-R_C mediated by P_4, is that P_4 promotes sequestration of E_2-R_C in the nucleus [29] or elsewhere. However, other researchers have shown that nuclear bound estradiol (E_2R_n) is exponentially reduced *in vivo* to approximately 10% of cytoplasmic estrogen receptor capacity by 16 h after treatment with E_2 [3, 6]. In the present studies P_4 was used at 24 h, well after the 16 h point at which nuclear activity is maximally reduced (Fig. 2b); and in addition [^{3}H]-estradiol was bound *in vitro* to the high speed supernate after removal of the nuclei. If progesterone could prolong nuclear binding or "sequestration" of what little E_2-R_n remained at 24 h, this amount of E_2-R_n would not be sufficient to explain the magnitude of decreased cytoplasmic receptor observed. Under the conditions employed, here a sequestration hypothesis would be more tenable for the trapping of binding protein which is not associated with [^{3}H]-estradiol. To date there is no evidence that P_4 can physically interact with the "uncharged" estrogen binding protein and promote nuclear retention, or that P_4 influences nuclear interaction so that the "uncharged" binding protein is retained in a nuclear position inaccessible to E_2.

Evidence does exist that P_4 promotes binding of E_2-R_n to purified rabbit uterine chromatin [30]. However, the experiments reported here were designed so that levels of nuclear E_2-R_C were well reduced, and the cytoplasmic binding protein was separated from the nuclear fraction prior to *in vitro* equilibrium binding with [^{3}H]-estradiol. While P_4 may enhance nuclear binding parameters for E_2-R_n, this enhancement alone does not explain the P_4 induced depletion of E_2-R_c.

Both E_2 and P_4 increased the rates of [^{35}S]-methionine incorporation in the cytosol fraction (Fig. 5a and b) and in the nuclear fraction (Fig. 6). Incorporation into nuclear protein was consistent with the depression of nuclear protein synthesis (0–2 h after E_2 treatment) and subsequent increase (after 4 h) reported by Means and Hamilton[20].

In summary, the kinetics of amino acid incorporation into uterine soluble protein following E_2 treatment were reconfirmed [20, 21] and extended to include progesterone treatment. Both estrogen and progesterone were shown to stimulate an increase in rates of general protein synthesis; however, E_2-R_C concentration was increased by treatment with estrogen and decreased by treatment with progesterone. Possible changes in the rate of degradation of E_2-R_C by cytoplasmic factors were not found and cannot explain the E_2-R_C depletion observed. While it appears likely that estrogen receptor synthesis may be influenced positively by estradiol and in a negative sense by progesterone, it is now necessary to *directly* determine whether *synthesis* of E_2-R_C is increased by estrogen and reduced by progesterone. Such evidence must be obtained by experiments which pulse-label the estrogen receptor directly.

REFERENCES

1. Jensen E. V., Brecher P. I., Nunata M., Smith S. and De Sombre E. R.: *Methods in Enzymology*, Vol. 36A (1975) pp. 267–274.
2. Katzenellenbogen A. and Gorski J.: Litwack (Ed.), *Biochemical Action of Hormones* (Edited by Litwack). Vol. 3 (1975) pp. 187–238.
3. Sarff M. and Gorski J.: *Biochemistry* **10** (1971) 2557–2563.
4. Jensen E. V., Suzuki T., Nunata M., Smith S. and DeSombre E. R.: *Steroids* **13** (1969) 417–427.
5. Cidlowski J. A. and Muldoon T. G.: *Endocrinology* **95** (1974) 1621–1629.
6. Gorski J., Sarff M. and Clark J.: *Adv. Biosci.* **7** (1970) 5–20.
7. Katzenellenbogen B. S.: *Endocrinology* **96** (1975) 289–297.
8. Pavlik E. J. and Coulson P. B.: *J. cell Biol.* **63** (1974) 260a.
9. Pavlik E. J. and Coulson P. B.: *J. steroid Biochem.* **7** (1976) 357–368.
10. Finn C. A. and Martin L.: *Biol. Reprod.* **8** (1973) 585–588.
11. Kang Y.-H., Anderson W. A. and DeSombre E. R.: *J. cell Biol.* **64** (1975) 682–692.
12. Anderson W. A., Kang Y.-H. and DeSombre E. R.: *J. cell Biol.* **64** (1975) 692–703.

13. Burton K.: *Biochem J.* **62** (1956) 315–323.
14. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: *J. biol. Chem.* **193** (1951) 265–275.
15. Pavlik E. J. and Coulson P. B.: *J. steroid Biochem.* **7** (1976) 369–376.
16. Moore R. J. and Hamilton T. H.: *Proc. Natn. Acad. Sci. U.S.A.* **52** (1964) 439–446.
17. Teng C. S. and Hamilton T. H.: *Biochem. J.* **105** (1967) 1101–1109.
18. Greenman D. L. and Kenney F. T.: *Arch. biochem Biophys.* **107** (1964) 1–6.
19. Suvatte A. B. and Hagermann D. D.: *Endocrinology* **87** (1970) 641–645.
20. Means A. R. and Hamilton T. H.: *Proc. Natn. Acad. Sci. U.S.A.* **56** (1966) 686–693.
21. Barnea A. and Gorski J.: *J. Biochem.* **9** (1970) 1899–1904.
22. Clark J. H., Peck E. J. and Anderson J. N.: *Nature* **251** (1975) 446–448.
23. Martin L. and Finn C. A.: *J. Endocr.* **41** (1968) 363–371.
24. Martin L. and Finn C. A.: *J. Endocr.* **44** (1968) 279–280.
25. Martin L., Das R. M. and Finn C. A.: *J. Endocr.* **57** (1973) 549–554.
26. Clark B. F.: *J. Endocr.* **50** (1971) 527–528.
27. Clark B. F.: *J. Endocr.* **56** (1973) 341–342.
28. Means A. R. and Hamilton T. H.: *Biochem. biophys. Acta* **129** (1966) 432–435.
29. Glasser S. R., Clark J. H.: *Developmental Biology and Reprod.* **33** (1975) 311–345.
30. Chatkoff M. L. and Julian J. A.: *Biochem. biophys. Res. Commun* **51** (1973) 1015–1022.
31. Greenwald T. and Everett J. W.: *Anal. Rec.* **134** (1959) 171–184.
32. Hsueh A. J. W., Peck E. J. and Clark J. H.: *Endocrinology* **98** (1976) 438–444.
33. Brenner R. M., Resko J. A. and West N. B.: *Endocrinology* **95** (1974) 1094.