PROGESTERONE-INDUCED ESTROGEN RECEPTOR-REGULATORY FACTOR IS NOT 17β-HYDROXYSTEROID DEHYDROGENASE

Richard G. MacDonald, Edmund A. Gianferrari, and Wendell W. Leavitt*

Worcester Foundation for Experimental Biology
Shrewsbury, Massaschusetts 01545

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ABSTRACT

These studies were done to determine if the progesterone-induced estrogen receptor-regulatory factor (ReRF) in hamster uterus is 17β-hydroxysteroid dehydrogenase (17β-HSD), i.e. that rapid loss of nuclear estrogen receptor (Re) might be due to enhanced estradiol oxidation to estrone catalyzed by 17β-HSD. Treatment of proestrous hamsters with progesterone (~25 mg/kg BW) for either 2 h or 4 h had no effect on 17β-HSD activity measured as the rate of conversion of [6,7-^{3}H]estradiol to [^{3}H]estrone by whole uterine homogenates at 35°C. During this same time interval, progesterone treatment increased the rate of inactivation of the occupied form of nuclear Re as determined during a 30 min incubation of uterine nuclear extract in vitro at 36°C. Since we previously demonstrated that such in vitro Re-inactivating activity represents ReRF, the present studies show that ReRF is not 17β-HSD or a modifier of that enzyme.

INTRODUCTION

Estrogen effects on the growth of the mammalian uterus are modified by the subsequent action of progesterone (1). We have proposed that progesterone regulates estrogen action in the uterus by rapid inhibition of nuclear estrogen receptor (Re) retention (2). This mechanism is selective for occupied nuclear Re (3), and can be blocked by inhibitors of RNA and protein synthesis (4). Thus, it would appear that progesterone induces the synthesis of a factor, estrogen receptor-regulatory factor (ReRF), which stimulates inactivation of nuclear Re in vivo (2,3). Recent studies employing a cell-free assay for ReRF suggested that progesterone-induced Re inactivation may involve

phosphatase activity (5,6). Another mechanism by which progesterone might alter estrogen action in the uterus is by enhancing conversion of estradiol (E_2) to the less potent estrogen, estrone (E_1). Such a mechanism may operate in epithelial cells of the human endometrium, where it has been demonstrated that progesterone increases E_2 oxidation by eliciting a dramatic increase in 17β-hydroxysteroid dehydrogenase (17β-HSD) activity (7-11). However, studies with the rat uterus indicate that 17β-HSD activity is inducible by estrogen (12-14), and that progesterone suppresses the estrogen-dependent increase in uterine 17β-HSD activity (14,15). The hormones controlling uterine 17β-HSD have not been established for the hamster. In the present study, we tested the hypothesis that progesterone induces rapid loss of nuclear estrogen receptor in the hamster uterus through changes in 17β-HSD activity. The results demonstrate no effect of progesterone on uterine 17β-HSD during the relatively short (2-4 h) time period required for induction of ReRF.

MATERIALS AND METHODS

Materials. [6,7-$^3H_2$]Estradiol ([6,7-^3H]E_2, 53 Ci/mmol) and [2,4,6,7-$^3H_4$]estradiol (97 Ci/mmol) were obtained from New England Nuclear. Radiochemical purity of [6,7-^3H]E_2 as assessed by thin layer chromatography (TLC) at the time of the experiments was >95%. Radioinert steroids were from Sigma. All other chemicals were reagent grade or better and were purchased from standard commercial sources. TLC plates pre-coated with silica gel 60 were from EM Laboratories, Elmsford, NY.

Animals. Adult female golden hamsters (Engle Labs, Farmersburg, IN) were maintained in a controlled environment with a 14:10 photoperiod. The regularity of estrous cycles was monitored by the appearance of the postestrous vaginal discharge (AM, cycle day 1). On the morning of proestrus (day 4), animals were injected subcutaneously with progesterone (5 mg in 0.3 ml corn oil). Control animals were either not treated or injected with oil vehicle. The hamsters were killed by cervical dislocation 2 or 4 h later. For studies involving pregnant animals, females were mated with intact males on the evening of day 4 (estrus) and were sacrificed 2.5 days later (midday on day 3 of pregnancy).

Preparation of Tissue Homogenates and Uterine Nuclear Extracts. Uteri were removed, trimmed, slit longitudinally, blotted and placed in ice-cold buffered saline. The tissues were minced and homogenized for 17β-HSD studies in ten volumes (v/w) of 50 mM Tris-HCl, pH 8.0 as described before (16). Ovaries were excised from pregnant animals, trimmed, pooled in ice-cold saline and homogenized as above. The protein content of each homogenate was estimated by a rapid method (17) and the homogenates were diluted with buffer to give a final protein concentration of about 1 mg/ml. For the in vitro assay of nuclear ReRF activity, uteri were homogenized in 6 volumes (v/w) of buffer A_{30}: 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 12 mM monothioglycerol, 30% (v/v) glycerol. Nuclear KCl extracts were prepared from the low-speed nuclear pellet as described previously (18).

Assay of 17β-Hydroxysteroid Dehydrogenase Activity. Diluted homogenates were assayed for 17β-HSD activity by a modification of the method of Tseng and Gurpide (7). Each incubation mixture contained, in a final volume of 5 ml: 4.5 ml of homogenate, 0.72 mM NAD^+ and [6,7-^{3}H]E_2 (37 μM, ~800,000 cpm/ml). The incubation was conducted at 35°C, and 1.0 ml aliquots were removed after 0.5, 5, 15 and 30 min. Each aliquot was added to 1.2 ml of a 5:1 (v/v) ethanol:water solution containing 100 μg each of unlabelled E_2 and E_1 carriers. The solution was mixed and extracted immediately with 5 ml of ethyl acetate. After removing the organic phase, the samples were extracted again with 5 ml of ethyl acetate. The ethyl acetate extracts from each sample were pooled and brought to a final volume of 10 ml. For TLC, 2 ml portions of each extract were dried under a stream of nitrogen. The residues were redissolved in 50 μl of ethyl acetate and applied to silica gel plates. Chromatography was run in the solvent system chloroform/ethyl acetate, 4:1 (7). After chromatography, the E_1 and E_2 spots were scraped from the plates, added to 1 ml of ethanol in a scintillation vial, shaken for 15-30 min and counted in 7 ml of scintillation solution. Recovery of the steroids, calculated by adding ^{14}C-labelled E_2 or E_1 to some of the homogenates just prior to extraction, was 59-72%. Contamination of the E_1 spot with counts contributed by [6,7-^{3}H]E_2 was 0.39-0.45%. Enzyme activity was calculated from the slope of the plot of [^{3}H]E_1 produced versus time. Activity of 17β-HSD is expressed as pmol E_1 produced per hour per mg total homogenate protein. The final protein content of each diluted homogenate was measured by the method of Lowry et al. (19) using bovine serum albumin as standard.

Assay of ReRF Activity in vitro. Progesterone-dependent inactivation of nuclear Re in vitro was measured as described before (5,6). Briefly, duplicate aliquots of uterine nuclear extracts were diluted with an equal volume of buffer A_{30} plus 0.5 M KCl. One set of samples was incubated at 36°C for 30 min while the second set was kept at 0°C. The amounts of total Re and of unoccupied Re remaining after the 36°C incubation were measured by exchange assay with [2,4,6,7-$^3H_4$]E_2 as described before (3,4,16). Occupied Re was calculated by subtracting the amount of unoccupied Re from the quantity of total Re remaining in each sample. ReRF activity is measured as enhanced inactivation (or loss) of nuclear Re at 36°C in the progesterone-treated sample relative to the control.

General Methods. Scintillation solution was toluene-Triton X-100 (2:1, v/v) containing 5 g of diphenyloxazole and 50 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene per liter. Radioactive samples were counted in a Packard Tri-Carb scintillation spectrometer in the efficiency range 25-35% for tritium. Comparisons between means were done by two-tailed Student's t-test.

RESULTS

The data in Table I illustrate that there was no short-term effect of progesterone treatment on hamster uterine 17β-HSD activity measured as oxidation of [6,7-^{3}H]E_2, either 2 h (Exp. 1) or 4 h (Exp. 2) after hormone injection. Indeed, there were no differences between any of the 17β-HSD activity values measured in uteri from proestrous hamsters, comparing hormone-treated with control. Furthermore, similar control values were obtained whether injected with oil vehicle (Exp. 2) or left untreated (Exp. 1). However, progesterone action during early pregnancy appeared to have a small, but statistically insignificant, decremental

Table I. Effect of progesterone on 17β-hydroxysteroid dehydrogenase activity in hamster tissue homogenates.

Treatment Group	Activity	p[a]
	pmol/h/mg protein	
Experiment 1		
Control, untreated	840 ± 40 (3)[b]	
Progesterone, 2 h	880 ± 50 (3)	p>0.60
Pregnant ovary	6380 (1)	
Pregnant uterus	670 ± 70 (3)	0.05<p<0.10
Experiment 2		
Control, vehicle-injected	890 ± 50 (3)	
Progesterone, 4 h	900 ± 80 (3)	p>0.90

Tissue homogenates were prepared in 50 mM Tris-HCl, pH 8.0 (approx. 1 mg protein ml^{-1}) and incubated for 30 min at 35°C in the presence of 0.72 mM NAD^+ and 37 μM [6,7-^{3}H]E_2. The amount of [^{3}H]E_1 in each homogenate was measured at intervals during this incubation to calculate the enzyme activity, which is expressed as pmol [^{3}H]E_1 produced hr^{-1} mg protein^{-1}.

a) Compared to the appropriate control using Student's t-test.

b) Values are mean ± SEM. Parentheses contain n values for each group.

effect on hamster uterine 17β-HSD (Exp. 1). The enzyme assay could detect relatively high levels of activity, as shown by the values obtained for pooled ovaries from 2 1/2-day pregnant hamsters (Exp. 1). As expected from previous work (20), there was substantial 17β-HSD activity in the ovary of the pregnant hamster, the specific activity of which was ten-fold higher than that in the pregnant uterus.

Treatment of proestrous hamsters with progesterone for only 2 h results in a progesterone-dependent increase in the *in vitro* rate of nuclear Re inactivation (Table II). In agreement with our previous findings (2,3,5), the progesterone-dependent activity responsible for enhanced Re inactivation *in vitro* (defined as ReRF) acts preferentially on the occupied form of nuclear Re, with little or no effect of progesterone on the unoccupied receptor.

Table II. Effect of progesterone treatment *in vivo* on inactivation of estrogen receptor in uterine nuclear extracts *in vitro*.

Treatment	Estrogen Receptor, cpm/0.3 ml		Re Inactivated at 36°C	
	0°C	36°C	cpm	%
Total Re				
Control[a]	2310±40[b]	2350±40	-40	0
Progesterone	2360±60	1960±70	400	17
Unoccupied Re				
Control	790±80	920±70	-130	0
Progesterone	730±50	690±105	40	5
Occupied Re[c]				
Control	1520	1430	90	6
Progesterone	1630	1270	360	22

a) Control animals received corn oil vehicle.
b) Values are mean ± SEM of triplicate determinations made on nuclear extract from pooled tissue. This experiment is representative of 3 replicates.
c) Occupied Re = Total Re minus unoccupied Re.

DISCUSSION

These studies have shown that progesterone treatment of proestrous hamsters causes no change in the activity of uterine 17β-HSD during the relatively short time required for hormonal induction of a factor (ReRF) which selectively inactivates the nuclear estrogen-receptor complex *in vitro*. Previous studies by Okulicz *et al.* (3) showed that, 4 h after progesterone administration *in vivo*, there was no detectable occupied Re remaining in uterine nuclei, presumably due to ReRF action. After 4 h of progesterone treatment in the present study, we were unable to find any change in 17β-HSD activity in homogenates of whole uteri. Although these findings do not preclude the possibility that metabolism of estradiol by 17β-HSD plays some role in the progesterone-dependent inhibition of nuclear Re retention in the hamster uterus, our data indicate that the function of 17β-HSD in this process must be secondary to the primary effect of ReRF.

The observations which formed the hypothetical basis for the present study were those of Tseng and Gurpide (7-9), who showed that long-term progesterone domination of the human endometrium during pregnancy or in the secretory phase of the menstrual cycle leads to a marked stimulation of estradiol oxidation via increased 17β-HSD activity. That result has been confirmed by others in the human (11,21,22) and monkey (23), leading to the hypothesis that enhanced oxidation of E_2 in the progesterone-dominated human uterus constitutes a long-term mechanism for progesterone antagonism of estrogen action (7). Further evidence in favor of this hypothesis was provided recently by Gurpide and Marks (10), who showed that 17β-HSD-catalyzed conversion of E_2 to E_1 in human secretory endometrium *in vitro* inhibited [^{3}H]E_2

binding to nuclear estrogen receptor. Recently, Satyaswaroop et al. (11) showed that progesterone induction of 17β-HSD activity in vitro was restricted to glandular epithelial cells of the human endometrium.

In the rodent, estrogen exerts positive control over the amount of uterine 17β-HSD activity (12-15). Furthermore, progesterone domination of the uterus in the rat (12-15) and mouse (24) decreases the amount of 17β-HSD activity in this tissue. Although our studies do not address this question directly, it is likely that hormonal regulation of uterine 17β-HSD in the hamster is similar to that observed in the rat and mouse. In the hamster uterus, 17β-HSD activity declined somewhat during early pregnancy relative to the proestrous animal, as was observed in the rat by Kreitmann et al. (13). The specific activity of 17β-HSD expressed on a protein basis for the hamster uterus is in the same range as that measured in uterine homogenates from E_2-treated rats (13) and from cycling rats (14).

Previous work from our laboratory and by others lends further credence to the contention that ReRF is not 17β-HSD. Evans et al. (18) showed that progesterone treatment induces selective loss of nuclear Re in ovariectomized, E_2-implanted hamsters more rapidly than (and independently of) estrogen withdrawal. Thus, the effect of progesterone on nuclear Re retention takes place directly upon the hormone-receptor complex rather than on ligand availability. The ability to extract a factor from the nuclei of the progesterone-treated uterus which mimics progesterone-induced Re inactivation in vitro (Table II) is pertinent since the 17β-HSD activity of the uterine nucleus is believed to be negligible relative to the whole tissue (7). It is improbable, therefore, that 17β-HSD interacts directly with the estrogen-receptor complex

within the nucleus. Thus, the available evidence argues against the possibility that ReRF is 17β-HSD or a modifier of this enzyme which is detectable *in vitro* under the conditions of our studies. Our previous work (5,6) suggested that ReRF may be an acid phosphatase whose activity increases in the uterine nucleus following progesterone administration. Szego *et al.* (25,26) showed that acid phosphatase as well as other lysosomal hydrolases are rapidly recompartmentalized from lysosomes to the nucleus of the rat preputial gland and uterus after E_2 administration. The possibility remains that progesterone may enhance nuclear acid phosphatase and ReRF activities through a similar mechanism in the hamster uterus.

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27. ABBREVIATIONS: Re, estrogen receptor; ReRF, estrogen receptor-regulatory factor; E_2, estradiol-17β or estradiol; E_1, estrone; 17β-HSD, 17β-hydroxysteroid dehydrogenase. This activity could be contributed by one or more enzymes: 3(17)β-hydroxysteroid: NAD oxidoreductase (E.C. 1.1.1.52) or estradiol-17β: NAD oxidoreductase (E.C. 1.1.1.62).