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REGULATION OF 3-HYDROXYL-3-METHYLGLUTARYL-COENZYME A REDUCTASE ACTIVITY IN RAT UTERINE TISSUES

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Summary—Initiation of uterine DNA synthesis and mitosis in response to estrogen appears to depend upon the stimulation of protein synthesis. 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase could have a key function in controlling uterine mitosis through its control of mevalonic acid and cholesterol synthesis as the rate-limiting enzyme in their synthetic pathways. These studies were initiated to examine the kinetics of the uterine increases in HMG-CoA reductase activity in response to estradiol. In the uterus of the ovariectomized mature rat, estradiol increased levels of enzyme activity in both the luminal epithelium and stroma-myometrium up to 12 h after estradiol treatment. Levels of HMG-CoA reductase activity decreased after 12 h in the luminal epithelium and further increased in the stroma-myometrium. Previous studies have shown that estradiol does not increase DNA synthesis and mitosis in the stroma-myometrium of the uterus of the ovariectomized mature rat. Since estradiol increased HMG-CoA reductase activity in both the luminal epithelium and stroma-myometrium, we conclude that even though increased HMG-CoA reductase activity may be a prerequisite for increased DNA synthesis, increases in uterine HMG-CoA reductase activity are not necessarily followed by increased DNA synthesis.

INTRODUCTION

During the estrous cycle, estrogen and progestin secretion control the timing and histological location of endometrial DNA synthesis and mitosis [1, 2]. The sequential secretion of estrogen and progestin and the variability in tissue responses result in peaks of mitotic activity at different times in the three main endometrial tissues, luminal epithelium, glands, and stroma. Estradiol treatment of ovariectomized rodents initiates a marked increase in the mitotic index of luminal epithelial cells 18–24 h after a single treatment [3–5]. Prior to the increase in epithelial mitosis, there is enhanced DNA synthesis which can be demonstrated by the uptake of labeled thymidine.

The initiation of mitosis in the uterus in response to estrogen appears to depend upon prolonged stimulation of protein synthesis and one of the epithelial proteins induced by estradiol is HMG-CoA reductase [6]. Through its control of mevalonic acid and cholesterol synthesis as the rate-limiting enzyme in their synthetic pathways, HMG-CoA reductase could have a key function in controlling epithelial mitosis [7–10]. In addition to cholesterol, cell growth also requires a nonsterol metabolite of mevalonate [10]. Evidence suggests that mevalonate production, independent of its function in cholesterol synthesis, plays an essential and specific role in regulating S-phase DNA replication [11].

Previous studies in the mouse demonstrated that estradiol increased HMG-CoA reductase activity in uterine luminal epithelial cells in close synchrony with S phase DNA replication [6]. To examine this relationship before further studies of the hormonal control of enzyme synthesis, we determined the effects of estradiol on HMG-CoA reductase activity in the rat uterine tissues. Estradiol increased the activity of HMG-CoA reductase in the luminal epithelium prior to expected increases in epithelial DNA synthesis, but enzyme activity also increased in the stroma-myometrium where estrogen-induced increases in DNA synthesis are not observed.

EXPERIMENTAL

Mature Sprague–Dawley female rats (Zivic-Miller, Zelienople, Penn., 150–175 g) were maintained on standard rat chow and water *ad libitum* in animal facilities illuminated between 0500–1900 h. At 1–3 weeks after ovariectomy, rats were injected with either estradiol (500 ng/0.1 ml sesame oil, s.c., Sigma, St Louis, Mo.) or medroxyprogesterone acetate (MPA, 3.5 mg/0.1 ml saline, s.c., Upjohn, Kalamazoo, Mich.) and sacrificed at intervals thereafter. The Department of Laboratory Animal Medicine at the University of Cincinnati maintains AAALAC

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accreditation of its animal facilities, and all experiments were conducted in accord with NIH standard established by the *Guidelines for Care and Use of Experimental Animals.*

Uterine horns were excised, trimmed of extraneous connective tissue, slit longitudinally, and placed in cold phosphate-buffered saline (PBS). For the preparation of luminal cell extracts, six horns were placed in a round bottom tube containing 1 ml of isolation buffer (0.1 M sucrose, 50 mM KCl, 50 mM potassium phosphate, 0.3 mM dithioerythritol, DTE, pH 7.2) and freshly added antipeptidases (5 mM EGTA, 0.1 mM TLCK, 0.1 mM TPCK, 50 μg/ml leupeptin). The uterine horns were mixed with five 5-mm glass balls with a Vortex mixer for 2 min at 4°C [12]. Luminal epithelial extracts were removed and homogenized once (15 s) with a Polytron (Brinkman, Westbury, N.Y.) before preparation of a microsomal fraction. Stroma-myometrial tissue was frozen between two blocks of dry ice and stored at −70°C. For the preparation of microsomes, approximately 100 mg of stroma-myometrium was homogenized with 3 × 15 s Polytron bursts separated by 30 s in cold homogenization buffer (HB, 280 mM sucrose, 10 mM Tris–HCl, 100 μM EDTA, 3 mM dithioerythritol). Epithelial and stroma-myometrial homogenates were then centrifuged at 105,000 *g* for 60 min in a SW 50.1 rotor (Beckman, Fullerton, Calif.) and resulting microsomal pellets were frozen and stored at −70°C.

HMG-CoA reductase activity was measured by enzyme assay procedures with slight modifications [13, 14]. Microsomal pellets were resuspended in HB and the protein concentration determined by the bicinchoninic acid protein assay [15]. Approximately 150–250 μg protein in 40 μl were preincubated for 1 h at 37°C. Enzyme reactions were initiated by the addition of 25 μl of substrate mix containing lyophilized glucose-6-phosphate (5.2 mM), $NADP^+$ (1.53 mM), 150 mM EDTA, 160 mM potassium phosphate, 1.5 mM [^{14}C]HMG-CoA (30,000 dpm/nmol), 2.3 mM KCl, [^{3}H]mevalonic acid lactone (13.3 μCi/ml) glucose-6-phosphate dehydrogenase (Bakers yeast type VII, Sigma, St Louis, Mo.). Blank tubes received 20 μl, 6 N HCl. Reactions were terminated after 15, 30 and 45 min by the addition of 40 μl, 6 NHCl and 25 μl of substrate mix was added to blank tubes. Following additional incubation for 30 min at 37°C, the reaction tubes were centrifuged. An aliquot (25 μl) of each of the supernatants were spotted on an activated TLC sheet (Chromagram silica gel sheets, Kodak, Rochester, N.Y.) and the chromatogram was developed with benzene:acetone (1:1). Mevalonic acid product was scraped from the chromatograph (R_f 0.5–1.0) into neutralizing scintillation cocktail (RPI, Mt Prospect, Ill.) and counted to uniform statistical error of 0.2% error in the ^{3}H-channel and 2.0% in the ^{14}C-channel of a scintillation counter (460CD, Packard Instruments, Downers Grove, Ill.) programmed for dual label detection and DPM conversion. After correction for percentages of recovery and the subtraction of the reaction blank, enzyme activities were determined by linear regression from the available enzyme reaction time points. HMG-CoA reductase enzyme activity is expressed as nmoles product/min/mg protein. Figure 1 shows that the time-course of the enzyme reaction was linear during the enzyme assay.

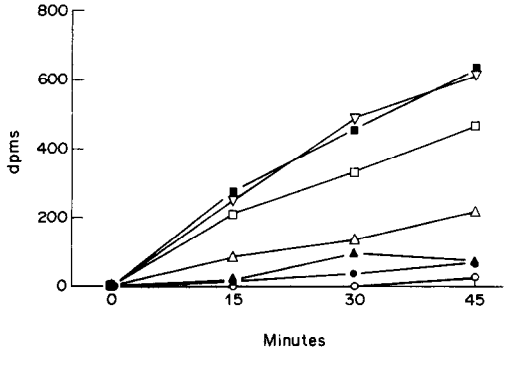

Fig. 1. Time-course of HMG-CoA reductase enzyme reaction. Microsomes from uterine stroma-myometrium were prepared at intervals after estradiol treatment (○) zero h, (●) 3 h, (△) 6 h, (▲) 9 h, (□) 12 h, (■) 15 h, and (▽) 18 h. Enzyme activity was determined by linear regression analysis of mevalonic acid produced (dpm) during 45 min as described in Materials and Methods.

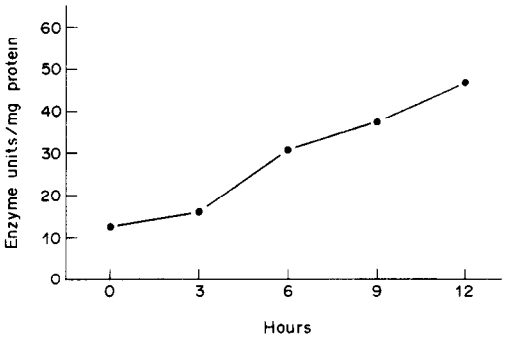

Fig. 2. Effect of estradiol on levels of uterine HMG-CoA reductase activity. Each point represents the average of data from 2 experiments, 2 assays of 5 uterine horns per experiment.

RESULTS

The effects of estradiol on HMG-CoA reductase activity in the rat uterus are shown in Fig. 2. Estradiol increased levels of the enzyme activity over the entire time period examined. In contrast to these results, MPA had no significant effect on uterine enzyme activity (data not shown). Increased enzyme activity and mRNA for HMG-CoA reductase were observed in the liver during the 12 h after estradiol injection. However, when the time of estradiol injection was varied with the time of sacrifice of all animals held constant, no significant effect of estradiol on liver HMG-CoA reductase activity was observed (data not shown).

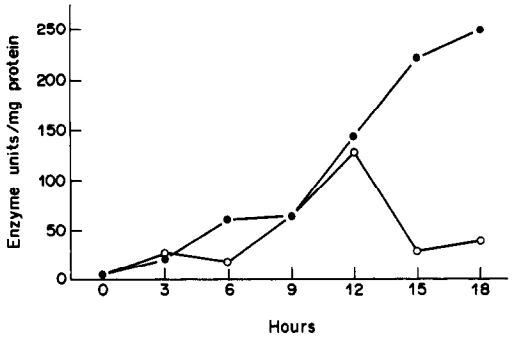

Fig. 3. Effect of estradiol on levels of uterine HMG-CoA reductase activity in luminal epithelium (○) and stroma-myometrium (●). Each point represents the average of data from 2 experiments, 2 assays of 5 uterine horns per experiment.

To identify the uterine tissue in which estradiol induced the HMG-CoA reductase activity, levels of enzyme activity were determined in the luminal epithelium and stroma-myometrium at intervals after estradiol injection. Figure 3 shows that estradiol increased the enzyme activity of both uterine tissues up to 12 h. Following 12 h, levels of enzyme activity increased further in the stroma-myometrium as they decreased in the luminal epithelium.

DISCUSSION

HMG-CoA reductase is the rate limiting enzyme in mevalonic acid and cholesterol synthesis and thus may have a key function in controlling mitosis [7–10]. Although the role of cholesterol in the cell cycle is not entirely clear, its continued synthesis in the G1 phase appears to be required for traverse of the cell cycle [16, 17]. Increased synthesis of cholesterol precedes synthesis of DNA, and inhibition of HMG-CoA reductase inhibits incorporation of thymidine into DNA. Cholesterol may be required for membrane formation or have a regulatory role in relation in some critical event in the cell cycle [16, 17].

In addition to cholesterol, cell growth also requires a nonsterol metabolite of mevalonate [10]. Evidence suggests that mevalonate production, independent of its function in cholesterol synthesis, plays an essential and specific role in regulating S-phase DNA replication [11, 18–20]. The effects of mevalonate in controlling S-phase DNA replication appear to be mediated by a metabolite(s) of mevalonate, specifically isopentenyladenine or a close related isoprene purine derivative [21]. Although cholesterol is necessary in early G1 for the passage of cells through G1, it has no direct effect on S phase replication. In contrast and independent of its function in cholesterogenesis, mevalonic acid through its metabolites is required in late G1 for cells to carry out S phase DNA replication [11].

Previous studies of the effect of estrogen treatment on uterine HMG-CoA reductase activity in the ovariectomized mature mouse showed two peaks of activity at 9 and 15 h after hormone treatment in luminal epithelial cells [6]. Epithelial DNA content doubled between 6 and 12 h and it was suggested that the peak of enzyme activity at 9 h was associated with an early S-phase in these cells and the second peak with a second S-phase or with mitosis. Further studies examined the modulation of the uterine enzyme through reversible phosphorylation, but it was concluded that this mechanism did not have a role in the uterine response to estrogen [22].

The time-course of the response of uterine HMG-CoA reductase that we observed in response to estradiol in the ovariectomized mature rat was significantly different from the observations of Wilce *et al.* [6]. In contrast to their observations, estradiol treatment resulted in a smooth increase in enzyme activity to a maximum in the luminal epithelium at 12 h with further increases also observed in the stroma-myometrium up to 18 h. Differences between our experimental design and those of Wilce *et al.* [6] include length of time after ovariectomy, pretreatment with estradiol in drinking water, enzyme assay procedures, and the rodent species used in the experiments.

Despite our success in detecting liver HMG-CoA reductase enzyme protein on Western blots and liver HMG-CoA reductase mRNA on Northern blots, we were unable to measure either uterine protein or mRNA by these procedures. In addition to Northern blot analysis, we used the more sensitive ribonuclease protection assay to examine levels of uterine HMG-CoA reductase mRNA after estradiol treatment. A protected fragment of the expected size was detected, but no induction after estradiol was observed (data not shown).

In the ovariectomized immature rat, administration of a single injection of estradiol causes a maximum increase in DNA synthesis in the luminal epithelium and stroma-myometrium approximately 24 h later which then decreases to control levels at 36 h [23]. In the luminal epithelium, this peak of DNA synthesis is preceded by an increase in nuclear DNA polymerase α activity at 20–24 h after a single estradiol injection [24]. Quite different effects of estradiol on uterine DNA synthesis and mitosis are observed in the ovariectomized mature rat. Estradiol stimulates DNA synthesis and mitosis in the luminal epithelium but has no effect on the underlying stroma or myometrium [1, 25]. We observed that estradiol increases the activity of HMG-CoA reductase in the luminal epithelium prior to expected increases in epithelial DNA synthesis, but that estradiol also increases the enzyme activity in the stroma-myometrium where estrogen-induced increases in DNA synthesis are not observed. Since estradiol increases HMG-CoA reductase activity in uterine tissues regardless of effects on DNA synthesis, we conclude that although increased HMG-CoA reductase activity may be a prerequisite for increased DNA synthesis, these two effects of estradiol are not tightly coupled.

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