Progestin and Estrogen Control of Cathepsin D Expression and Processing in Rat Uterine Luminal Epithelium and Stroma-Myometrium (43486)

BRUCE C. MOULTON*,[1] AND SOHAIB A. KHAN†

Departments of Obstetrics and Gynecology and Anatomy and Cell Biology,† University of Cincinnati, College of Medicine, Cincinnati, Ohio 45267*

Abstract. **Progestins increase the activity and rate of synthesis of cathepsin D, a lysosomal aspartyl protease, in the uterine luminal epithelium in ovariectomized rats. Western blot analysis of luminal epithelial proteins determined that the progestin, medroxyprogesterone acetate (MPA) increased the 43-kDa form of cathepsin D by 7-fold in 24 hr, whereas estradiol increased the amount of the same form by only 2-fold. To examine the precursor-product relationship between cathepsin D proteins in the luminal epithelium and stroma-myometrium after progestin or estradiol treatment, uterine proteins were prelabeled by incubation with [^{35}S]methionine *in vitro*, cathepsin D was isolated by immunoprecipitation, and equal amounts of labeled cathepsin D were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After each hormonal treatment in each uterine tissue, a 48-kDa precursor was processed into a 44-kDa cathepsin D product. Endoglycosidase H digestion of [^{35}S]methionine-labeled cathepsin D from the luminal epithelium and stroma-myometrium of medroxyprogesterone-treated rats shifted the molecular masses of the cathepsin D proteins by approximately 5.7 kDa. To examine the contribution of increased mRNA to increased rates of cathepsin D synthesis, we measured levels of cathepsin D mRNA in uterine tissues after progestin and estrogen treatment. Total RNA was isolated from the uterine luminal epithelium and from the stroma-myometrium. Northern blot analysis identified a single 2.2-kb RNA band corresponding to the size expected for cathepsin D mRNA. Medroxyprogesterone increased levels of cathepsin D mRNA in the luminal epithelium (>17-fold) and in the stroma myometrium (3-fold), with maximum increases at 9 hr after treatment. Estradiol also increased cathepsin D mRNA levels in both uterine tissues, but by only 2-fold. No hormonal effects on liver cathepsin D mRNA were observed. Increases in cathepsin D synthesis and activity in uterine tissues in response to progestin and estrogen appear to depend in part upon increased levels of mRNA.** [P.S.E.B.M. 1992, Vol 201]

Cathepsin D (EC 3.4.23.5) is a lysosomal aspartyl endopeptidase involved in intracellular protein degradation (1). The enzyme is a major endopeptidase in lysosomes and appears to have a function in a wide variety of tissues during tissue remodeling or regression and apoptotic cell death in particular. Cathepsin D levels increase in cells prior to cell death during blastocyst implantation (2–5), postpartum uterine involution (6), follicular atresia (7), and mammary gland involution (8). In the prostate, removal of testosterone initiates apoptotic cell death and increases cathepsin D activity (9).

In human breast cancer cells (MCF_7), cathepsin D mRNA is induced by estrogens and growth factors, but not by progestins (10, 11). Of the 52-kDa cathepsin D protein synthesized in response to estradiol, 40% is secreted, whereas the remainder is processed into 48-kDa and 34-kDa proteins (10). In the uterus, however, cathepsin D synthesis is specifically increased by progestins rather than estradiol (4, 5, 12, 13). Immunohis-

[1] To whom requests for reprints should be addressed at Department of Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0526.

Received February 10, 1992. [P.S.E.B.M. 1992, Vol 201]
Accepted April 24, 1992.

0037-9727/92/2011-0098$3.00/0

tochemistry and measurements of enzyme activity demonstrated that cathepsin D is concentrated in luminal and glandular epithelia (4, 5, 12). Progestin treatment of ovariectomized rats increased cathepsin D activity in luminal epithelial cells and specifically increased the rate of cathepsin D synthesis (5). Although estradiol also increased rates of cathepsin D synthesis in the uterus, the increases paralleled the general increase in protein synthesis (4, 5). In the human uterus, endometrial epithelial levels of cathepsin D are higher during the luteal phase, and the progestin R5020, rather than estradiol, induces the enzyme in primary cultures of endometrium (13).

Steroids regulate not only rates of mRNA transcription, but also the posttranscriptional processing and compartmentalization of proteins (14, 15). Since progestins and estradiol have contrasting effects on rates of synthesis of cathepsin D in specific uterine tissues, we decided to examine the processing of cathepsin D in the luminal epithelium and stroma-myometrium after hormonal treatments. Furthermore, estradiol preferentially stimulates the glycosylation apparatus in the uterus (16) so that changes in the molecular weights of cathepsin D could have resulted from altered rates of glycosylation. We also examined the effects of progestin and estrogen on levels of cathepsin D mRNA in specific uterine tissues to assess their contribution to increased rates of cathepsin D synthesis.

Materials and Methods

Animals. Mature Sprague-Dawley female rats (150–175 g; Zivic-Miller, Zelienople, PA) were maintained on standard rat chow and water *ad libitum* in animal facilities illuminated between 0500 and 1900 hr. At 2–3 weeks after ovariectomy, rats were injected with either estradiol (500 ng/0.1 ml of sesame oil sc; Sigma Chemical Co., St. Louis, MO) or medroxyprogesterone acetate ([MPA] 3.5 mg/0.1 ml of saline sc; Upjohn, Kalamazoo, MI) and sacrificed at intervals thereafter. The Department of Laboratory Animal Medicine at the University of Cincinnati maintains AAALAC accreditation of its animal facilities, and all experiments were conducted in accord with NIH standards established by the Guidelines for Care and Use of Experimental Animals.

Tissue Preparation. Uterine horns were excised, trimmed of extraneous connective tissue, slit longitudinally, and placed in cold phosphate-buffered saline (PBS) containing 0.05% Nonidet P-40, 1 μM leupeptin, 200 μM L-1-chloro-3-(4-tosylamido)-4-phenyl-2-butanone (TPCK), 100 μM L-1-chloro-3-(4-tosylamido)-7-amino-2-heptanone-HCl (TLCK). For the preparation of the luminal epithelial cell fraction, five horns were placed in a round-bottom tube containing 1 ml of PBS and five 5-mm glass balls and mixed with a vortex mixer for 2 min at 4°C (17). The epithelial fraction was removed, freeze-thawed three times, and centrifuged at 800*g* for 15 min. Equal amounts of the supernatant protein were subjected to sodium dodecyl sulfate (SDS)-gel electrophoresis and to Western blot analysis using a polyclonal antibody to rat spleen cathepsin D, characterized previously (4, 5).

In pulse-chase experiments, uterine horns were removed, slit longitudinally, and placed in minimum essential medium (MEM) containing 0.1% of normal methionine concentration (MEM-met) at 37°C. After incubation in this medium for 1 hr (37°C, 95% O_2 and 5% CO_2), the horns were incubated in MEM-met containing 100 μCi/ml of Trans ^{35}S-Label (ICN Biomedical, Irvine, CA) for 1 hr. The uterine horns were rinsed with MEM containing 10 times the concentration of methionine (MEM-10 × met) and incubated in this chase medium for 0, 1, or 3 hr. At the end of the incubations, luminal epithelial fractions were prepared as described above and the remaining stroma-myometrium was frozen on dry ice.

Western Blot Analysis and Immunoprecipitation of Cathepsin D. After transblotting of proteins to nitrocellulose membranes, membranes were blocked by incubation for 1 hr in Tris-saline (10 mM Tris and 154 mM NaCl, pH 7.5) containing 5% nonfat dry milk and then incubated with cathepsin D antibody (1:2000 in Tris-saline) for 4 hr at 4°C. Membranes were rinsed in Tris-saline and then incubated with secondary antibody conjugated to alkaline phosphatase (Promega, Madison, WI) for 1 hr. After washing, antibody antigen complexes were detected using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate substrates for alkaline phosphatase. Membranes were scanned with a GS300 scanning densitometer (Hoefer Scientific Instruments, San Francisco, CA) to determine the relative quantity of enzyme product.

Labeled cathepsin D proteins were isolated from uterine homogenates by immunoprecipitation (13). Protein A cell suspension (35 μl/ml; Sigma) was added to each homogenization supernatant (27,000*g*, 20 min), incubated for 15 min on ice, and then removed by centrifugation. Cathepsin D antibody (125%) was added in solubilization buffer (SB) (250 mM NaCl, 25 mM Tris-HCl, 5 mM EDTA, 1% Triton X-100, and 0.05% Na deoxycholate) containing bovine serum albumin (50 mg/ml) and incubated overnight at 4°C. Protein A cell suspension was added, incubated 5 min at room temperature, and layered over 1 M sucrose before centrifugation. The pellet was washed with SB and 10 mM Tris-5 mM EDTA (pH 7.5) buffer. Peptides were eluted from the pellet by incubation in 1% SDS-PBS for 10 min at room temperature and centrifugation. Cathepsin D antibody was added to the supernatant in 1.5 ml of SB + bovine serum albumin (10 mg/ml) and incubated for 4 hr at room temperature. Protein A cell suspension (35 μl) was added, incubated for

5 min at room temperature, collected by centrifugation, washed with SB and Tris-EDTA, and frozen. Immunoprecipitated peptides were eluted by boiling with SDS, and equal amounts of radioactivity in each lane were electrophoresed by SDS-polyacrylamide gel electrophoresis [PAGE] and transferred to nitrocellulose by electroblotting. Blots were dried and exposed to x-ray film at −70°C, and scanned with a GS300 scanning densitometer (Hoefer Scientific Instruments) to determine the density and molecular weight of the labeled proteins relative to coelectrophoresed labeled standards.

To assess the effect of endoglycosidase H digestion on cathepsin D proteins, immunoprecipitates were dissolved in 50 m*M* sodium phosphate buffer (pH 5.4) and 1% SDS, and heated for 1 min at 100°C. After dilution 10 times with water, samples were frozen or incubated with and without endoglycosidase H (25 mU/ml; Sigma) for 18 hr at 37°C. At the end of the incubation, proteins were precipitated with 10% trichloroacetic acid, redissolved in SDS, and subjected to SDS-PAGE.

Cathepsin D mRNA and Northern Analysis. For the preparation of luminal cell fractions for RNA extraction, five horns were placed in a round-bottom tube in 1 ml of PBS containing heparin (1 mg/ml; Sigma) and ribonucleoside vanadyl complexes (10 m*M*; Sigma) and five 5-mm glass balls and mixed with a vortex mixer for 2 min at 4°C (17). Immediately after preparation, the epithelial extract was transfered to 6 *M* guanidinium thiocyanate (Sigma) for isolation of total RNA. The remaining stroma-myometrial tissue was frozen on dry ice.

Tissue samples of stroma-myometrium (100–300 mg) were homogenized and extracted with guanidinium thiocyanate-phenol-chloroform for the isolation of total RNA (18). Total RNA was analyzed by Northern blot procedures (19). Equal amounts of uterine RNA, as determined by uv absorbance from each experimental group, were denatured in formamide, electrophoresed on 1% agarose gel containing 3.5% formaldehyde. The RNA was transferred to nitrocellulose filters, immobilized, prehybridized, and hybridized in 50% formamide at 42°C with a ^{32}P-labeled cathepsin D cDNA probe. Hybridized nitrocellulose filters were washed to 0.1 × standard saline citrate stringency, exposed to x-ray film at −70°C, and scanned with a GS300 scanning densitometer (Hoefer Scientific Instruments) to determine the density of the label.

A plasmid, pHCpDEco1.1, encoding human cathepsin D was kindly provided by Dr. John Chirgwin (University of Texas Health Science Center, San Antonio, TX).

Results

Synthesis and Processing of Cathepsin D. To determine the effects of estrogen and progestin treatment on levels of cathepsin D protein in luminal epithelial cells, ovariectomized rats were sacrificed at intervals after hormone treatments and epithelial fractions were prepared and subjected to SDS-PAGE and Western blot analysis. Figure 1 shows that MPA treatment increased the relative amount of the 43-kDa cathepsin D peptide by approximately 7-fold. The level of a 39-kDa cathepsin D peptide was slightly increased by 12 hr of MPA treatment; the 31-kDa peptide did not increase. Estradiol treatment increased the 43-kDa cathepsin D peptide by approximately 2-fold by 12 hr, with no further increase at 24 hr.

In our previous studies of the hormonal control of cathepsin D synthesis, cathepsin D was purified before immunoprecipitation using overnight dialysis against citrate buffer (pH 3.5) and ammonium sulfate fractionation. After this purification procedure, our polyclonal antibody against rat spleen cathepsin D precipitated a single protein with a molecular mass of 43 kDa, although minor bands were occasionally observed. The more rapid double immunoprecipitation procedure used in this study revealed multiple forms of cathepsin D. Pulse-chase experiments were performed to demonstrate a precursor-product relationship among these proteins.

Figure 2 shows the forms of cathepsin D synthesized in uterine luminal epithelial cells after MPA and estradiol treatment. Uterine horns of ovariectomized

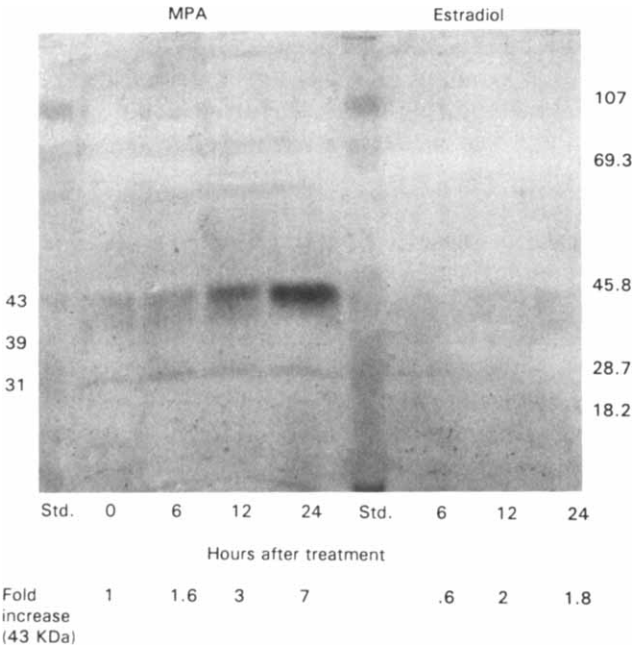

Figure 1. Effect of MPA and estradiol on levels of cathepsin D in uterine luminal epithelial cells detected by Western blot. At intervals after hormonal treatment of ovariectomized rats, luminal epithelial supernatants were prepared, subjected to SDS-PAGE and Western blot analysis, and scanned densitometrically to assess relative amounts of immunoreactive cathepsin D proteins. Fold increases are the average of two Western blots.

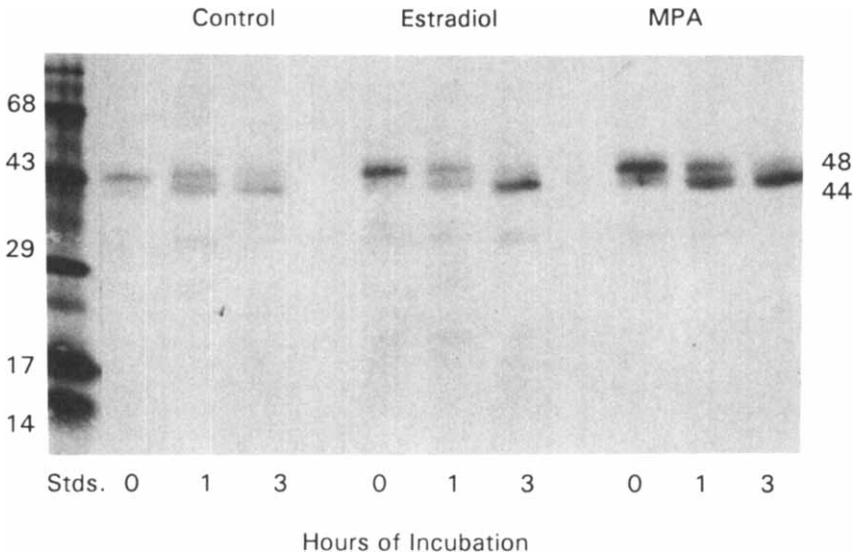

Figure 2. Forms of cathepsin D synthesized in uterine luminal epithelial cells after MPA and estradiol treatment and pulse-chase incubation. Uterine horns of ovariectomized rats treated with MPA, estradiol, or vehicle for 24 hr were incubated with [^{35}S]methionine for 1 hr before incubation in chase medium for 0, 1, or 3 hr. Luminal epithelial extracts were prepared, cathepsin D protein was isolated by immunoprecipitation, and immunoprecipitates were subjected to SDS-PAGE and exposed to x-ray film.

rats treated with MPA, estradiol, or vehicle for 24 hr were incubated with [^{35}S]methionine for 1 hr before incubation in chase medium for 0, 1, or 3 hr. After each hormonal treatment, a 48-kDa protein was the major species synthesized during the 1-hr-labeling period. During the subsequent chase periods of 1 and 3 hr, the relative amount of the 48-kDa protein decreased as the amount of a 44-kDa protein increased. The difference in molecular mass between these two proteins was 3.5 kDa. Additional proteins were detected at 36 kDa, 33 kDa, and 26 kDa, but their amounts did not increase during the chase period and the 36-kDa and 26-kDa proteins were detected only after estradiol treatment. SDS-PAGE revealed a single 51-kDa protein band in the incubation media in greatest abundance after MPA treatment (data not shown).

The forms of cathepsin D synthesized in uterine stroma-myometrium after MPA and estradiol treatment were also examined. Figure 3 shows that after each hormonal treatment, both 48-kDa and 44-kDa forms of cathepsin D were synthesized. During the chase incubation, the relative amount of the 48-kDa form decreased as the 44-kDa form increased. Lower molecular mass species were observed in some experiments, but their relative amounts did not change during the chase incubation.

To examine the content of high mannose carbohydrate in cathepsin D, ovariectomized rats were pretreated with MPA before incubation of uterine horns with [^{35}S]methionine. Cathepsin D immunoprecipitates were prepared from the luminal epithelium and stroma-myometrium and then incubated with or without endoglycosidase H (Endo-H), which cleaves internal *N*-acetylglucosamine residues of high mannose carbohydrates. As shown in Figure 4, Endo-H digestion shifted the apparent molecular mass of cathepsin D proteins isolated from both the luminal epithelium and stroma-myometrium. Before Endo-H digestion, cathepsin D proteins were observed at 48 kDa and 43 kDa and afterward at 43 kDa and 37 kDa in both uterine tissues. The changes in molecular masses were 5.7 kDa. Other lower molecular mass species between 31 kDa and 16 kDa were observed in both tissues after Endo-H digestion.

Hormonal Regulation of Cathepsin D mRNA. To determine the effects of estrogen and progestin on levels of mRNA encoding uterine cathepsin D, total RNA was isolated from treated ovariectomized rats, and equal amounts at each time point from luminal epithelium and stroma-myometrium were electrophoresed. Ethidium bromide staining showed discrete bands at 18 and 28 S, indicating undegraded RNA. Northern blot analysis using human cathepsin D cDNA as a probe revealed the expected 2.2-kb cathepsin D mRNA species. Figure 5 shows the effect of estradiol and MPA on luminal epithelial cathepsin D mRNA. Levels of cathepsin D mRNA in control epithelia were not detectable; estradiol doubled levels at 9 hr. MPA treatment, however, increased levels of cathepsin D mRNA by greater than 9-fold at 6 hr, with a maximum 17-fold increase observed at 9 hr.

The effects of estradiol and MPA on levels of cathepsin D mRNA in the stroma-myometrium were also examined. Figure 6 shows that estradiol treatment increased levels slightly. MPA treatment increased levels at 6 hr by approximately 3-fold, with no further increase thereafter.

To determine the tissue specificity of the response

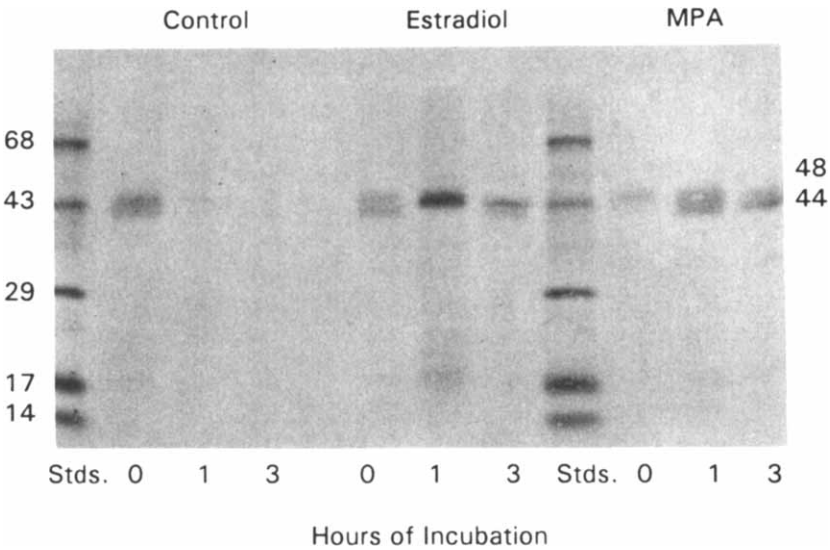

Figure 3. Forms of cathepsin D synthesized in uterine stroma-myometrium after MPA and estradiol treatment and pulse-chase incubation. Uterine horns of ovariectomized rats treated with MPA, estradiol, or vehicle for 24 hr were incubated with [^{35}S]methionine for 1 hr before incubation in chase medium for 0, 1, or 3 hr. After removal of luminal epithelial cells, cathepsin D protein was isolated by immunoprecipitation from homogenates of stroma-myometrium, and immunoprecipitates were subjected to SDS-PAGE and exposed to x-ray film.

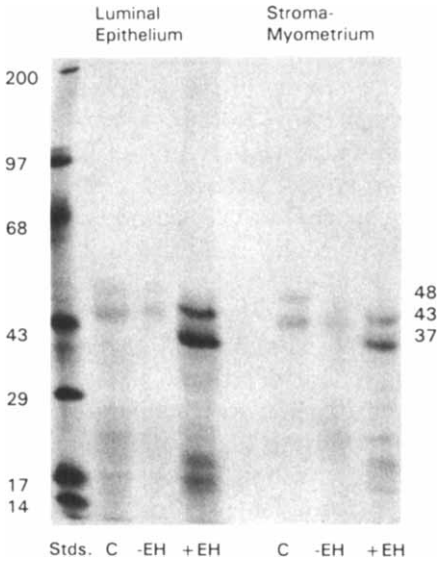

Figure 4. Effect of endoglycosidase H (EH) digestion on forms of cathepsin D in luminal epithelium and stroma-myometrium of MPA-treated ovariectomized rats. Uterine horns of ovariectomized rats treated with MPA for 20 hr were incubated with [^{35}S]methionine for 4 hr. After removal of luminal epithelial cells, cathepsin D protein was isolated by immunoprecipitation from homogenates of the luminal epithelium and stroma-myometrium. The immunoprecipitate was resolubilized and divided into fractions that were refrozen or incubated with or without endoglycosidase H for 24 hr. Proteins were precipitated with trichloroacetic acid and subjected to SDS-PAGE.

to estrogen and progestin, polyA$^+$ RNA was prepared from livers of estradiol- and MPA-treated rats and probed with the cathepsin D cDNA. Again, a single 2.2-kb species was detected. A constitutive level of expression of liver cathepsin D mRNA was detected, with no increase in levels of cathepsin D mRNA after hormone treatment.

Discussion

In MCF$_7$ breast cancer cells, [^{35}S]methionine incorporation into a 52-kDa protein is enhanced 4-fold by estradiol treatment (10). Before the 52-kDa protein was identified as cathepsin D, the processing and regulation of synthesis of the protein in MCF$_7$ cells were well described (10, 20). Following its synthesis, the 52-kDa protein is successively processed into 48-kDa and 34-kDa proteins, with the 34-kDa protein having a long half-life. Digestion of monoclonal immunoprecipitated proteins by endoglycosidase H decreased the molecular masses of these three species by 4 kDa. Although estradiol increased cathepsin D synthesis in these cells, no effect on the glycosylation of this protein was detected, and estradiol appeared to decrease the rate of cathepsin D processing (10).

In contrast to the regulation of cathepsin D synthesis in MCF$_7$ cells, progestins increase levels of cathepsin D in the luminal epithelium of the rat (4, 5) and human endometrium (13) and in epithelial endometrial cells in primary culture (13, 21). Increased synthesis of the 52-kDa precursor and 34-kDa large chain of cathepsin D was detected in the cultured epithelial cells in response to R5020, with a possible increase in a 48-kDa species also (13). Our Western blot analysis of luminal epithelial proteins showed that MPA treatment of ovariectomized rats increased the level of 43-kDa cathepsin D protein by 7-fold. This increase is somewhat greater than the 4-fold increase in enzyme activity observed previously, but reflects the 13-fold increase in the rate of synthesis of epithelial cathepsin D (5). Estradiol increased epithelial cathepsin D protein by approximately 2-fold, but had no effect on enzyme-specific activity or the specific rate of synthesis (4, 5).

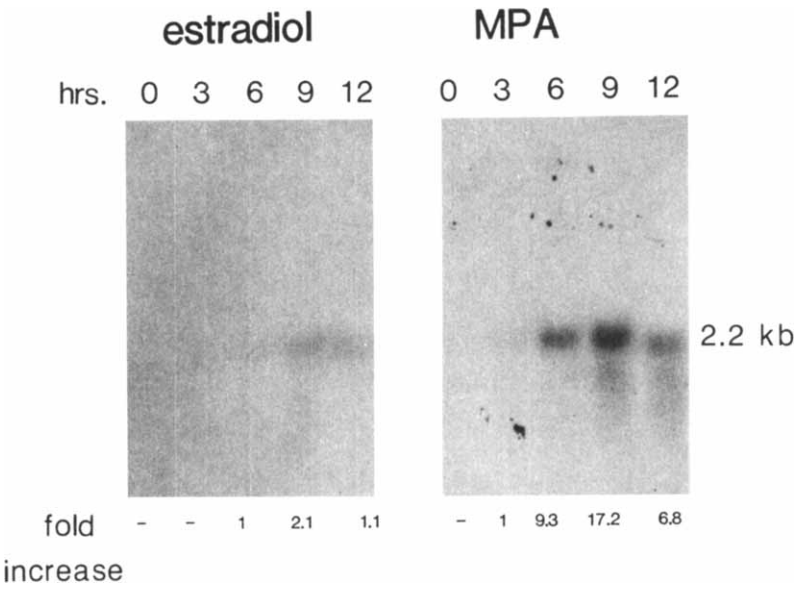

Figure 5. Effects of estrogen and progestin treatment on levels of cathepsin D mRNA in the luminal epithelium. Total cellular RNA (35 μg) was isolated from rat uterine luminal epithelium after estrogen or progestin treatment and electrophoresed on formaldehyde agarose gel together with RNA size markers. The RNA was transferred to nitrocellulose membrane and hybridized with ^{32}P-labeled cathepsin D cDNA. The fold increase indicated at each time point is the average of two experiments.

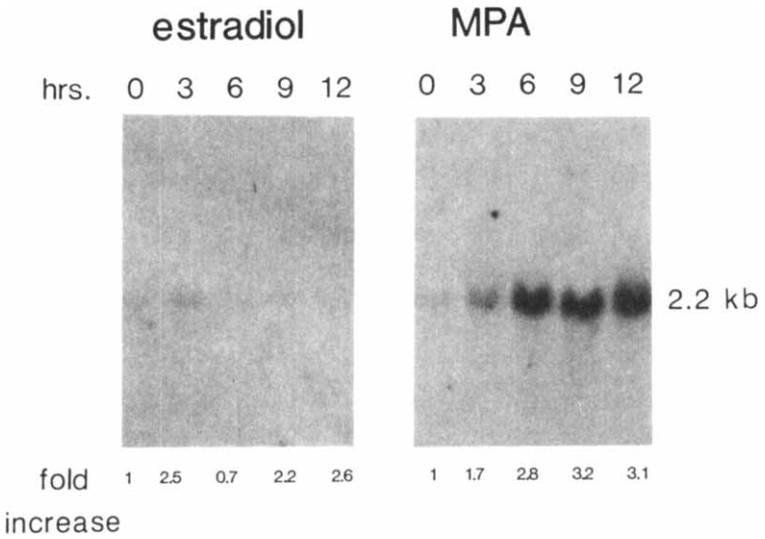

Figure 6. Effects of estrogen and progestin treatment on levels of cathepsin D mRNA in the stroma-myometrium. Total cellular RNA was isolated from rat uterine luminal epithelium after estrogen (50 μg) or progestin treatment (30 μg) and electrophoresed on formaldehyde agarose gel together with RNA size markers. The RNA was transferred to nitrocellulose membrane and hybridized with ^{32}P-labeled cathepsin D cDNA. The fold increase indicated at each time point is the average of two experiments.

With SDS-PAGE of equal amounts of radiolabeled immunoprecipitated cathepsin D protein, we were able to examine the processing of the enzyme protein after progestin, estradiol, or control treatments of ovariecomized rats in the luminal epithelium and stroma-myometrium. In both tissues and after all three hormonal treatments, a 48-kDa cathepsin D precursor was processed to a 44-kDa product during the 3-hr chase period. These forms were somewhat smaller than the cathepsin D proteins observed in the endometrial primary culture (13). However, a consistent difference of approximately 4 kDa between precursor and product was observed in our experiments and in the cultured endometrial cells. A single 51-kDa protein was detected in the incubation medium at the same size as an uncharacterized protein secreted by endometrial cells in response to progesterone (21). We were unable to detect differences in rates of processing of cathepsin D proteins in the luminal epithelium and stroma-myometrium after the hormonal treatments.

Endoglycosidase H digestion of immunoprecipitated cathepsin D was used to determine the extent of

cathepsin D glycosylation after MPA treatment. Similar decreases in molecular weight of epithelial and stroma-myometrial cathepsin D proteins were observed, which suggests comparable levels of glycosylation, but the 5.7-kDa decreases were significantly greater than the 4-kDa decrease observed after digestion of MCF_7 cathepsin D (10).

Progesterone, rather than estradiol, induces cathepsin D mRNA 3-fold in the immature rat uterus after 24 hr of treatment (12). Our results demonstrate that the major effect of progestin on uterine cathepsin D mRNA is in the luminal epithelium. In ovariectomized rats, MPA increased levels of cathepsin D mRNA in the luminal epithelium by greater than 17-fold, compared with a 3-fold effect on the stroma-myometrium and the approximately 2-fold effects of estradiol on levels in both uterine tissues. These results indicate that effects of MPA and estradiol on rates of synthesis of uterine cathepsin D observed in previous studies (4, 5) depend in part upon increased levels of mRNA.

Both progesterone and MPA increase cathepsin D activity and rates of synthesis in the luminal epithelium without significant effect on the stroma-myometrium (5). Estradiol does not specifically increase rates of uterine cathepsin D synthesis, but it increases cathepsin D synthesis only as part of the general increase of protein synthesis induced by estradiol (4). Estradiol increased cathepsin D mRNA levels by 2-fold, an increase comparable to the increase in cathepsin D protein detected by Western blot analysis. The 17-fold induction of cathepsin D mRNA by MPA compared with the 2-fold induction by estradiol would explain the differences between the two hormones in their effects on cathepsin D activity and synthesis.

Uterine cathepsin D concentrations have been identified immunohistochemically in both the luminal and glandular epithelium (2–4, 13). Although glandular epithelium constitutes only a small fraction of the rat endometrium, these cells may respond to MPA, and their inclusion with the stroma-myometrium we prepared in these experiments could explain the 3-fold increase observed in the cathepsin D mRNA. Rates of stroma-myometrial cathepsin D synthesis increased by 2.7-fold in response to MPA, but the effect was not significant because of experimental variability (5). The increase in epithelial cathepsin D mRNA in response to MPA (greater than 17-fold) appears to be significantly greater than increases in cathepsin D synthesis (13-fold) (4, 5) or in enzyme protein (7-fold), which suggests that MPA controls cathepsin D by mechanisms other than just transcription.

Estradiol treatment initiates a sequence of metabolic and morphological changes that result in overall growth and development of the uterus. The increase in the levels and diversity of uterine mRNA after estrogen treatment reflects these effects (22, 23). Although progestins initiate a wide range of modifications of the uterine endometrium, their primary effects appear more upon differentiation than growth. Relatively fewer newly synthesized proteins appear to be synthesized during the differentiation induced by progesterone compared with the uterine response to estrogens (21, 24). The increased degradative capacity of lysosomal cathepsin D resulting from progestin treatment might contribute to the remodeling of endometrial structure and function implicit in progestin-induced differentiation.

This research was supported by Grants HD 10721 and HD 22918 from the National Institute of Child Health and Human Development.

We thank Dr. John Chirgwin for providing the clone of human cathepsin D cDNA. We thank Heidi Scrable, Gretchen Langdon, Martha McKee, and Janice Anderson for excellent technical assistance and Karen Carr for the preparation of the manuscript.

1. Tang J, Wong RNS. Evolution in the structure and function of aspartic protease. J Cell Biochem **33**:53–63, 1987.
2. Elangovan S, Moulton BC. Blastocyst implantation in the rat and the immunohistochemical distribution and rate of synthesis of uterine lysosomal cathepsin D. Biol Reprod **23**:663–676, 1980.
3. Moulton B, Ingle CB. Uterine lysosomal cathepsin D activity, rate of synthesis, and immunohistochemical localization following initiation of decidualization in pseudo pregnant rats. Biol Reprod **25**:393–398, 1981.
4. Elangovan S, Moulton BC. Progesterone and estrogen control of rates of synthesis of uterine cathepsin D. J Biol Chem **155**:7474–7479, 1980.
5. Moulton BC, Koenig BB. Progestin increases cathepsin D synthesis in uterine luminal epithelial cells. Am J Physiol **244**:E442–E446, 1983.
6. Woessner JF. Acid hydrolases of the rat uterus in relation to pregnancy, post-partum involution and collagen breakdown. Biochem J **97**:855–866, 1965.
7. Dhanasekaran N, Sheelarani CS, Moudgal NR. Studies on follicular atresia: Lysosomal enzyme activity and gonadotropin receptors of granulosa cells following administration or withdrawal of gonadotropins in the rat. Mol Cell Endocrinol **33**:97–112, 1983.
8. Helminen HJ, Ericsson JLE. Quantitation of lysosomal enzyme changes during enforced mammary gland involution. Exp Cell Res **60**:419–426, 1970.
9. Sensibar JA, Liu X, Patai B, Alger B, Lee C. Characterization of castration-induced cell death in the rat prostate by immunohistochemical localization of cathepsin D. The Prostate **16**:263–276, 1990.
10. Morisset M, Capony F, Rochefort H. Processing and estrogen regulation of the 52-kilodalton protein inside MCF_7 breast cancer cells. Endocrinology **119**:2773–2782, 1986.
11. Westley BR, May FEB. Oestrogen regulates cathepsin D mRNA levels in oestrogen responsive human breast cancer cells. Nucleic Acids Res **15**:3773–3783, 1987.
12. Touitou I, Cavailles V, Garcia M, Defrenne A, Rochefort H. Differential regulation of cathepsin D by sex steroids in mammary cancer and uterine cells. Mol Cell Endocrinol **66**:231–238, 1989.
13. Maudelonde T, Martinez P, Brouillet J-P, Laffargue F, Pages A, Rochefort H. Cathepsin-D in human endometrium: Induction by progesterone and potential value as a tumor marker. Endocrinol Metab **70**:115–121, 1990.

14. Firestone GL, Payvar F, Yamamoto KR. Glucocorticoid regulation of protein processing and compartmentalization. Nature **300**:221–225, 1982.
15. Norvitch ME, Harvey S, Smith S, Hagstrom JE, Wieben ED. Androgens affect the processing of secretory protein precursors in the guinea pig seminal vesicle. I. Evidence for androgen-regulated proteolytic processing. Mol Endocrinol **3**:1788–1796, 1989.
16. Carson DD, Farrar JD, Laidlaw J, Wright DA. Selective activation of the N-glycosylation apparatus in uteri by estrogen. J Biochem Mol Biol **265**:2947–2955, 1990.
17. Fagg B, Martin L, Rogers L, Clark B, Quarmby VE. A simple method for removing the luminal epithelium of the mouse uterus for biochemical studies. J Reprod Fertil **57**:335–339, 1979.
18. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid quanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem **162**:156–159, 1987.
19. Thomas PS. Hybridization of denatured RNA and smaller DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA **77**:5201–5205, 1980.
20. Capony F, Morisset M, Barrett AJ, Capony JP, Broquet P, Vignon F, Chambon M, Louisot P, Rochefort H. Phosphorylation, glycosylation, and proteolytic activity of the 52-kD estrogen-induced protein secreted by MCF_7 cells. J Cell Biol **104**:253–262, 1987.
21. Aziz S, Balmain A, Knowler JT. Diversity and complexity of uterine mRNA from rats of differing hormonal states. Eur J Biochem **100**:95–100, 1979.
22. Hsu C-YJ, Komm BS, Lyttle CR, Frankel F. Cloning of estrogen-regulated messenger ribonucleic acids from rat uterus. Endocrinology **122**:631–639, 1988.
23. Maudelonde T, Rochefort H. A 51K progestin-regulated protein secreted by human endometrial cells in primary culture. J Clin Endocrinol Metab **64**:1294–1301, 1987.
24. Misrahi M, Atger M, Milgrom E. A novel progesterone-induced messenger RNA in rabbit and human endometria. Cloning and sequence analysis of the complementary DNA. Biochemistry **26**:3975–3982, 1987.