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Estrogenic Regulation of Murine Uterine 90-Kilodalton Heat Shock Protein Gene Expression

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Murine uterine steady-state protein levels of the 90-kilodalton heat shock protein (HSP90) have been demonstrated recently to be increased by estrogen in a target tissue- and steroid-specific manner (C. Ramachandran, M. G. Catelli, W. Schneider, and G. Shyamala, Endocrinology 123:956–961, 1988). We now report that this regulation occurred with both the HSP86 and HSP84 forms of HSP90 as well as with the 94-kilodalton glucose-regulated protein. At the mRNA level, this response was greatest for HSP86 (15-fold). In contrast, estradiol had no significant effect on HSP70.

All eucaryotes examined express stress or heat shock proteins of ~28, 70, and 90 kilodaltons (kDa) (5, 11). In steroid-responsive tissues, the 90-kDa heat shock protein (HSP90) has been found in association with steroid-receptor complexes, and this association has been shown to prevent the receptor from interacting with DNA in vitro (3, 6, 11, 16, 22). On the basis of these findings and others concerning the structure-function relationship of steroid receptors, it has been suggested that in vivo HSP90 may be responsible for maintaining the receptor in an inactive state in the absence of steroid (19).

For the last 2 decades, the rodent uterus has been widely used as a model for elucidating the mechanism of estrogen and estrogen receptor action in mammalian cells. In recent studies it was demonstrated that the level of murine uterine HSP90 was increased by estradiol in a target tissue- and steroid-specific manner (20), thereby raising the possibility that in steroid target tissues, steroid hormones and HSP90 may mutually regulate their cellular functions. In murine tissues, HSP90 has been shown to consist of two forms which have molecular masses of 84 and 86 kDa (2, 18, 25) (designated HSP84 and HSP86, respectively [2, 25]) and which exhibit distinct developmental regulation (2). Consistent with these findings, the two forms of murine HSP90 have been shown to be synthesized from distinct mRNAs, and two non-cross-hybridizing cDNA clones specific for HSP84 and HSP86 have been isolated from both human and murine tissues (4, 9, 10, 17, 18, 21). Therefore, to further explore the estrogenic regulation of murine uterine HSP84 and HSP86 and its overall physiological significance in mammalian cells, we have examined the effect of estradiol on the steady-state protein and mRNA levels of HSP84 and HSP86.

Uteri were obtained from ovariectomized BALB/c mice injected with saline or estradiol-17β, as described previously (20). The HSP84 probe was a 0.7-kilobase *Eco*RI-*Bam*HI segment derived from the 3′ coding region of a cDNA clone isolated from a mouse meth A library (17). The HSP86 probe was a 1.2-kilobase *Eco*RI segment derived from a cDNA clone isolated from the same library (18). The latter probe contained 800 nucleotides of the 3′ portion of the HSP86 coding region and 400 nucleotides of the 3′ untranslated region (18). Murine GRP94 (94-kDa glucose-regulated protein) cDNA clone (14) was obtained from Michael Green at the Saint Louis University School of Medicine. Murine HSP70 cDNA clone (pM 1.8) was a gift from R. Morimoto at Northwestern University.

Total RNA was prepared by the phenol-chloroform method and subjected to slot blot and Northern (RNA) blot analyses by using standard procedures (13, 15). The blots were hybridized at 65°C with random primer-labeled (2×10^9 to 3×10^9 cpm/μg of DNA) or nick-translated (2×10^8 to 4×10^8 cpm/μg of DNA) probes and sequentially washed at 65°C with 6, 1, and 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) solutions containing 0.1% sodium dodecyl sulfate. The dried filters were autoradiographed, and the relative intensities of the autoradiographic bands were determined by scanning laser densitometry to quantify the relative mRNA concentration in each sample (hybridized to the ^{32}P-labeled probe) in the linear response range of film. Immunoblot analyses were performed exactly as described previously (20), except for the use of a discontinuous polyacrylamide gel containing 5.0% acrylamide–0.133% bis in the resolving gel and 3% acrylamide–0.08% bis in the stacking gel in order to resolve HSP84 and HSP86. HSP84 and HSP86 were detected by incubating the blot with a rabbit antiserum prepared against a mixture of murine HSP84 and HSP86 (25). For estimation of HSP70, a rabbit antiserum prepared against its C-terminal peptide (8) was used. GRP94 was detected with a rat monoclonal antibody prepared against avian HSP108 (originally believed to be the B subunit of avian progesterone receptor [7] and later shown to correspond to GRP94 [1]) provided by Dean Edwards at the University of Colorado. The blots were incubated with ^{125}I-labeled protein A, autoradiographed, and scanned with a computerized laser scanning densitometer (LKB 2200) to estimate the relative steady-state levels of the various antigens.

Effect of estradiol on uterine HSP90. Initial time course experiments (Fig. 1) revealed time-dependent increases in HSP84 and HSP86 mRNA due to estradiol detectable as early as 30 min after steroid administration, reaching a maximal accumulation in 2 to 4 h, and declining to control baseline values around 24 h after steroid administration. The effect of estradiol was much more pronounced on HSP86 mRNA than on HSP84 mRNA. Northern blot analyses

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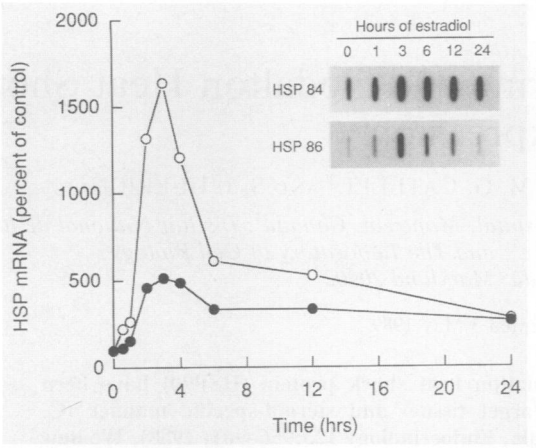

FIG. 1. Time course of the effect of estradiol on murine uterine HSP84 and HSP86 mRNAs. Ovariectomized mice were treated with either saline (control) or estradiol for indicated times prior to the processing of the uteri for slot blot analyses of HSP86 (○) and HSP84 (●) mRNAs as described in the text. The data represent the densitometric scan of autoradiographs presented as percentages of control (zero time, saline group) and is the average of two or three separate experiments. Inset, Autoradiograph of a typical experiment.

confirmed these results, showing that estradiol caused a much greater increase in the 3.1-kilobase transcript corresponding to HSP86 than in the 2.8-kilobase transcript corresponding to HSP84 (Fig. 2; compare lanes 1 and 2 in each panel). In addition, while estradiol has a greater effect on HSP86 mRNA than on HSP84 mRNA, HSP84 mRNA was present at a higher basal level in the control untreated tissues (Fig. 1 and 2). A combined analysis of data from several experiments revealed that, in control tissues, the basal level of HSP86 mRNA was roughly between 30 and 50% of the basal level of HSP84; these mRNA levels became approximately equivalent between 2 and 4 h after estradiol treatment.

In our previous studies reporting the estrogen-dependent augmentation of HSP84 and HSP86 protein levels in murine

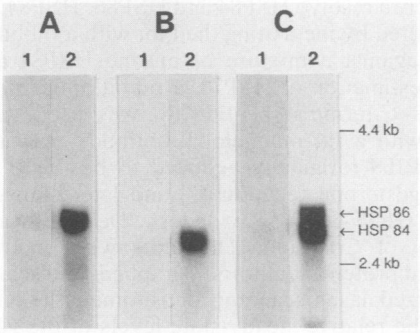

FIG. 2. Northern blot analyses of murine uterine HSP90 mRNAs. Total cellular RNA (15 μg) isolated from uteri of ovariectomized mice treated with saline (lanes 1) or estradiol (lanes 2) for 3 h was loaded in each lane and hybridized with ^{32}P-labeled cDNA probes of similar specific activity. The blots shown in panels A and B were hybridized with HSP86 and HSP84, respectively, while that in panel C was hybridized with both probes. The blots shown in panels B and C were autoradiographed for 7 h, while that in panel A was autoradiographed for 17 h for better visualization of the basal level of HSP86.

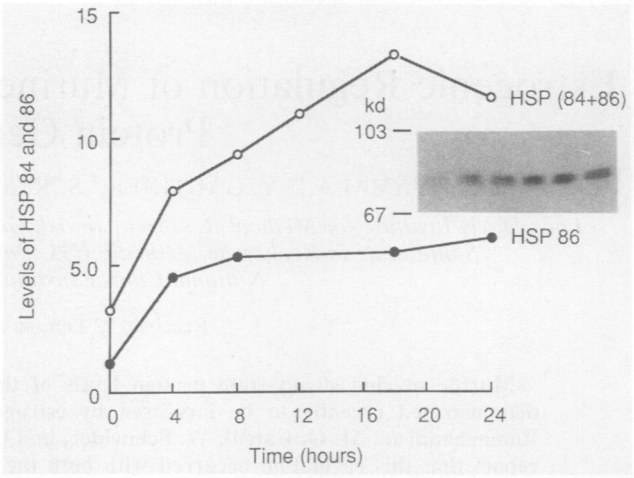

FIG. 3. Immunoblot analyses of the two forms of HSP90 in uterine cytoplasmic extracts. Ovariectomized mice were treated with saline or estradiol for indicated times prior to processing the uteri for immunoblot analyses as described in the text. The data represent the relative densitometric scanning units corresponding to ^{125}I-labeled protein A bound to the antibody-antigen complex.

uterus (20), our sodium dodecyl sulfate-polyacrylamide gel electrophoresis conditions were unable to resolve HSP84 and HSP86. In order to better define the relationship between estrogen-dependent increase in the HSP84 and HSP86 steady-state mRNA and protein levels, we reanalyzed the effect of estradiol on uterine HSP90 by using the antiserum exhibiting reactivity with HSP84 and HSP86. As previously observed (20), overall there was approximately a fivefold increase in HSP84 plus HSP86 due to estradiol, which resulted in the production of approximately equal levels of both proteins following induction (Fig. 3).

Effect of estradiol on GRP94 and HSP70. GRPs are proteins which are synthesized in response to glucose starvation but are often classified as stress proteins (5, 12). Previous studies have shown that GRP94 is regulated by steroid hormones in avian oviducts (1). On the basis of nucleotide and predicted amino acid sequence homology, it has been proposed (23) that GRP94 and HSP90 may have similar functional properties in vivo. Similarly, in a wide range of organisms and tissues examined so far, it appears that the two most abundant and highly conserved HSPs are HSP90 and HSP70 (5, 12, 18). Therefore, to gain a better understanding of the significance of estrogen-dependent increase in HSP84 and HSP86, we also examined the effect of estradiol on uterine GRP94 and HSP70 by performing a detailed time course study similar to that illustrated by Fig. 1. Estradiol caused an approximate sixfold maximal accumulation of GRP94 mRNA (Fig. 4), and the detailed time course for accumulation was similar to that observed with HSP84 and HSP86 (data not shown). Parallel assays for GRP94 by immunoblot analyses also revealed an increase in the protein level, and, as in the cases of HSP84 and HSP86, this was observed between 18 and 24 h after steroid administration. In contrast to GRP94, estradiol had no significant effect on the accumulation of HSP70 mRNA (Fig. 4) or protein (data not shown).

We have demonstrated that in murine uteri, HSP84 and HSP86 are regulated by estrogen at the mRNA and protein levels. At the mRNA level, the effect of estradiol was approximately three times greater on HSP86 than on HSP84. This may be a reflection of the two- to threefold higher basal level of HSP84 mRNA relative to HSP86. After estrogen

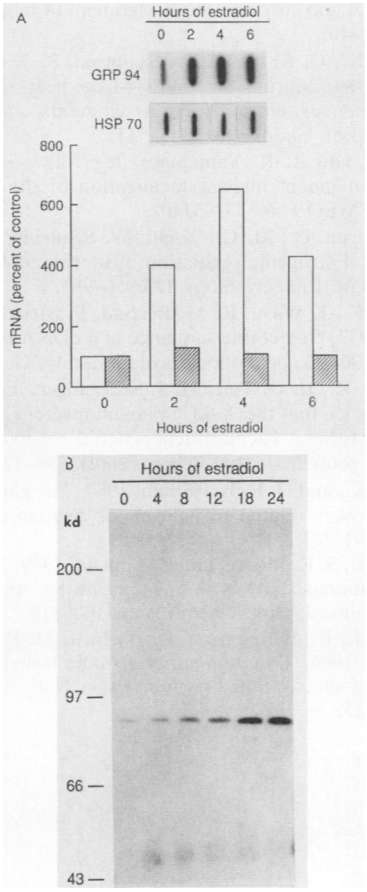

FIG. 4. Effect of estradiol on uterine GRP94 and HSP70. Ovariectomized mice were injected with saline or estradiol for indicated times prior to removal of the uteri. (A) Total RNA (5 μg) from each group was immobilized on nitrocellulose membrane and hybridized with ^{32}P-labeled probes specific for either GRP94 (□) or HSP70 (▨). The bar graph represents the relative densitometric scanning units presented as percentages of control (zero time, saline group); data are averages of two experiments. The top of the panel shows the autoradiograph from one of these experiments. (B) Autoradiograph of cytoplasmic extracts analyzed by Western blot (immunoblot) for GRP94. Molecular sizes are shown in kilodaltons (kd).

treatment, the levels of mRNA for both HSP84 and HSP86 became roughly equivalent. This was subsequently reflected at the steady-state level of the proteins: HSP84 and HSP86 were present in estrogen-treated tissue in approximately equal concentrations. The data from time course studies showed that the maximal accumulation of mRNA was observed approximately 4 h after steroid administration, with the protein steady-state level reaching a maximum at about 18 h. These findings suggest that the estrogenic regulation of uterine HSP84 and HSP86 may occur primarily at the level of transcription.

The differential responses by HSP84 and HSP86 to estradiol were analogous to those seen with heat shock in murine fibroblasts (2, 24). In these cells, HSP84 is predominantly synthesized in the absence of stress, whereas HSP86 is predominantly synthesized in response to stress. However, in murine fibroblasts, the preferential synthesis of HSP86 triggered by stress is also accompanied by elevated synthesis of HSP70 (2), whereas estradiol has no apparent effect on the synthesis of HSP70. In this regard, the estrogenic regulation of HSP84 and HSP86 is different from that of heat shock induction and is more characteristic of developmental regulation. Studies on the developmental induction of HSPs in *Drosophila* spp. have shown that this type of induction is distinguished from that of heat shock by the absence of HSP70 synthesis, interestingly, the trigger for the developmental induction in *Drosophila* spp. is believed to be the steroid hormone ecdysone (12). Since the principal and most dramatic effect of estradiol was that on growth in the rodent uterus, it is conceivable that at the cellular level, HSP84 and HSP86 synthesized in response to estrogen have an important role in mediating estrogen-dependent growth.

In addition to affecting HSP84 and HSP86, estradiol also caused a small increase in uterine GRP94 at both the protein and mRNA levels. GRP94 has been shown previously to be induced primarily by estrogens and progestins in avian oviducts (1). A principal difference between estrogenic stimulation of GRP94 mRNA in avian oviducts and that in murine uteri appears to be one of magnitude: the increase in avian oviducts is about 20-fold (1) compared with a 3-fold increase in murine uteri. However, as in the murine uterus, the estrogen-dependent increase in GRP94 mRNA in the avian oviduct is also target tissue specific and is not accompanied by an increase in HSP70 mRNA. These data, together with the results presented in this paper, suggest that in target tissues for estrogen, the family of proteins consisting of HSP84, HSP86, and GRP94 may be regulated by this steroid, with the magnitude of response by any individual member of this family being modulated by tissue-specific factors.

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