

Changes in Heat Shock Protein-90 and -70 Messenger Ribonucleic Acid in Uterine Tissues of the Ewe in Relation to Parturition and Regulation by Estradiol and Progesterone*

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ABSTRACT

Steroid receptors, including estrogen receptor (ER) and progesterone receptors (PR), form associations with heat shock proteins (Hsps). Dissociation of Hsps activates PR, whereas retention of Hsp90 *in vitro* stimulates ER. Progesterone and estrogen, interacting with their receptors, regulate myometrial contractility throughout pregnancy and during parturition. We hypothesize that uterine ER and PR changes concurrent with changes in Hsp90 and -70 abundance could alter uterine function. We quantified changes in Hsp90 and -70 messenger RNA (mRNA) abundance in pregnant sheep myometrium, endometrium, and fetal placenta during glucocorticoid-induced preterm and spontaneous labor. The effects of estradiol and progesterone on Hsp90 and -70 mRNA in myometrium and endometrium were examined in ovariectomized nonpregnant ewes. Hsp90 and -70 mRNA

distribution was evaluated by *in situ* hybridization in myometrium and endometrium. Dramatic tissue-specific increases in Hsp90 and -70 mRNA were observed in myometrium and endometrium ($P < 0.05$) during spontaneous and glucocorticoid induced labor. Hsp90 and -70 mRNA localized in myometrial, arterial smooth muscle, and endometrial gland epithelial cells. Estradiol increased Hsp90 and -70 mRNA in myometrium and endometrium of nonpregnant ewes. Progesterone did not affect Hsp90 and -70 mRNA abundance, but inhibited the estradiol-stimulated increase. These data support our hypothesis that at term, increased abundance of Hsp90 and -70 may inhibit uterine PR and stimulate ER function in uterine tissues. Similar changes, if present, would be of importance in species showing no progesterone withdrawal before labor, such as primates, including pregnant women. (*Endocrinology* 137: 5685–5693, 1996)

THERE IS NOW considerable evidence that several different steroid receptors can associate with heat shock proteins (Hsps) (1–6) with variable and sometimes opposing effects on the functional activity of each steroid. The association of Hsp90 and -70 with the progesterone receptor (PR) has been shown in cell-free systems. PR associated with Hsp is maintained in a nonactivated state (3, 7, 8). These studies suggest that progesterone-dependent activation of the PR is accompanied by dissociation of Hsps from the Hsp-receptor complex *in vitro* (3, 7), as nuclear progesterone receptor is mainly Hsp90 free *in vivo* (9). Dissociation of Hsps from the PR has been proposed as the mechanism of PR activation. Hsp90 represses PR DNA-binding activity by blocking receptor dimerization *in vitro* in cell-free systems (3). In contrast to their effect on the PR, Hsps assist and stimulate the binding of estrogen receptor (ER) to its cognate response element *in vitro* (10), which, in turn, activates the transcriptional function of the ER.

Several different Hsps have been described. The two major groups of Hsps (Hsp90 and -70) are the ones best charac-

terized in relation to steroid receptor function. It is apparent that multiple forms of Hsp90 and -70 exist in mammalian cells, as different genes coding for each individual subtype of Hsp90 and -70 have been cloned. Two distinct forms of Hsp90 complementary DNA (cDNA), designated Hsp90- α and - β , have been identified (11–13). There are also multiple subtypes of the Hsp70 family (14–16). Although the association and dissociation of Hsps with steroid receptors has been extensively studied at molecular and cellular levels, the physiological significance of changes in Hsps in relation to the steroid receptors during pregnancy and labor has not been determined in experimental animal models in which the changes in other major regulators of parturition have been clearly defined. In addition, no studies have been performed to differentiate the expressions of the different subtypes of Hsp90 or -70 in any physiological conditions.

In pregnant sheep, the fetal adrenal glucocorticoid-induced increase in the activity of placental 17 α -hydroxylase and 17–20 desmolase expression leads to withdrawal of the action of progesterone (17) and increased action of estrogen (18) on uterine tissues. These changes promote a switch of myometrial contractures to labor-type contractions. Together with rupture of the membranes and dilation of the cervix, these changes in the pattern of myometrial contractility result in delivery. In this species, altered steroid receptor function contributes to the mechanisms that regulate these physiological changes. At the time of parturition, ER messenger RNA (mRNA) content increases in the pregnant sheep myometrium and endometrium accompanied by a rise in estro-

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gen receptor protein (19, 20). A rise in maternal plasma estrogen appears to be a feature common to many species in late gestation, including pregnant women (21–23), monkeys (24), and baboons (25). However, the fall in the maternal plasma progesterone concentration that occurs in the days immediately before birth in sheep has not been observed in any primate species to date.

In contrast to these well documented changes in plasma hormone concentrations and ERs, there is as yet no direct evidence for withdrawal of progesterone action at the receptor level on target tissues in any species. In the present study we wished to determine whether increases in Hsp90 and -70 expression in myometrium and endometrium occur in pregnant sheep in a direction that would be able to facilitate ER and inactivate PR function in the uterus during labor. We, therefore, quantified changes in Hsp90 and -70 mRNA abundance in the pregnant sheep myometrium, endometrium, and fetal placenta during the last third of gestation and during both glucocorticoid-induced and spontaneous labor.

We also determined whether the changes in Hsps are specifically associated with labor or due to the direct effect of glucocorticoid. We compared Hsp90 mRNA concentrations in myometrium from pregnant ewes in which we successfully induced premature labor with glucocorticoid infusion to the fetus with the Hsp90 mRNA concentrations in myometrium obtained from sheep similarly infused with glucocorticoids that had not yet begun labor as well as those in control ewes at the same stage of gestation and not in labor. To investigate potential regulatory mechanisms we also examined the effect of estradiol and progesterone replacement in ovariectomized nonpregnant sheep on Hsp90 and -70 mRNA abundance in myometrium and endometrium. Finally, histological distribution of Hsp90 and -70 mRNA was evaluated by *in situ* hybridization in myometrium and endometrium to correlate cellular localization of Hsps with available evidence on the distribution of the various steroid receptors.

Materials and Methods

Animals and tissue collection

Twenty-nine pregnant Rambouillet-Dorset ewes bred only on a single occasion and carrying fetuses of known gestational age were studied. Experimental procedures were approved by the Cornell University institutional animal care and use committee. The Cornell facilities are approved by the American Association for the Accreditation of Laboratory Animal Care. At 120 days gestational age (dGA) ewes from which tissues were obtained were instrumented with electromyogram (EMG) leads sewn into the myometrium and fetal and maternal carotid arterial and jugular venous catheters (26). Labor was defined as having occurred when the myometrial EMG record showed a clear switch from contractions to contractions followed by contraction activity for at least 5 h (27). To examine the effect of labor on Hsp90 and -70 mRNA, tissues were obtained from ewes during labor in a comparative study to determine whether there were any differences in labor induced by the infusion of betamethasone (Celestone phosphate, Schering, Bloomfield, NJ; n = 5) or dexamethasone (Azium, Schering; n = 5; beginning at 127 dGA). Dexamethasone or betamethasone was administered iv into the fetal jugular vein continuously over a period of 48 h in a total dosage of 0.5 mg. The animals underwent elective necropsy 3 days after initiation of the infusion, or earlier if they went into labor due to glucocorticoid administration. Labor occurred in all five ewes carrying betamethasone-infused fetuses, but in only two of the ewes carrying dexamethasone-infused fetuses. Betamethasone has been shown to be 1.4 times as ef-

fective a glucocorticoid as dexamethasone (28). This group of 7 of the 10 glucocorticoid-infused fetuses was designated glucocorticoid infused in labor (GL). The remaining 3 glucocorticoid-infused fetuses were removed before labor started and were designated glucocorticoid infused, not in labor (GI:NL). Labor was determined according to the presence or absence of myometrial contraction activity. Tissues were also obtained from contemporary control ewes (n = 5) at the same stage of gestation (131 dGA). These ewes were designated as early controls, not in labor (ECNL), in which the fetus had received equivalent volumes of physiological saline vehicle. These ewes were not in labor as judged from the myometrial EMG, which showed only contractions. Tissues were

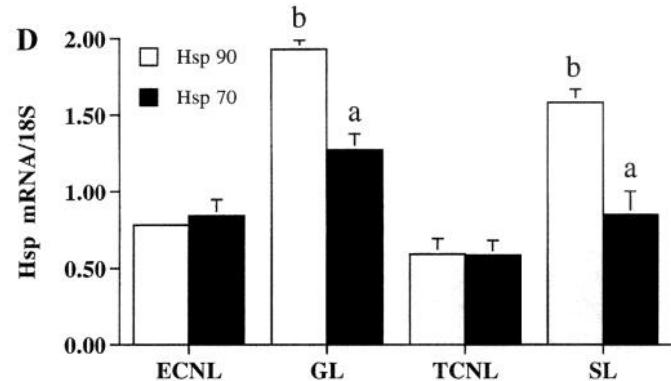
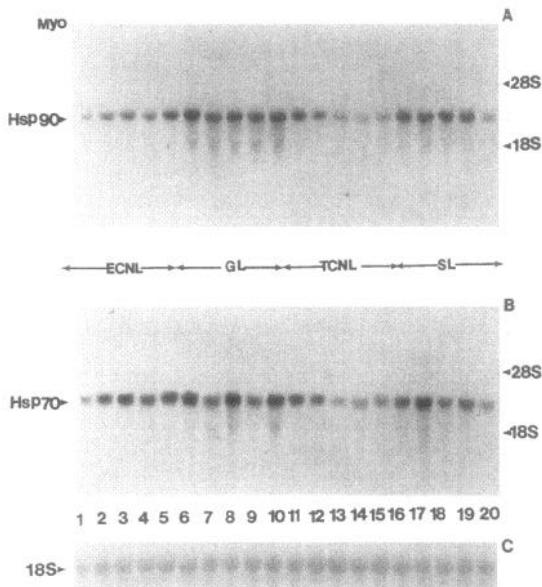


FIG. 1. Comparison of levels of Hsp90 and -70 mRNA in the myometrium (Myo) of pregnant sheep. A, Presentation of Northern blot analysis of Hsp90 mRNA in five separate samples in each group in ECNL (lanes 1–5), GL (lanes 6–10), TCNL (lanes 11–15), and SL (lanes 16–20) groups. B, Presentation of Northern blot analysis of Hsp70 mRNA in five separate samples in each group. C, Hybridization of the same blot with 18S cDNA probe to demonstrate relative amounts of total RNA in each lane. D, Northern blot signals for Hsp90 and -70 mRNA and 18S in the myometrium of pregnant sheep were quantified by densitometry and expressed as the ratio of Hsp90 or -70 mRNA to 18S (mean \pm SEM) in the ECNL (n = 5), GL (n = 5), TCNL (n = 7), and SL (n = 7) groups. Hsp90 and -70 mRNAs were significantly increased (a, $P < 0.05$; b, $P < 0.01$) in each group in which labor was present compared with levels in their gestational age-matched controls. Northern blotting was performed as described in *Materials and Methods*.

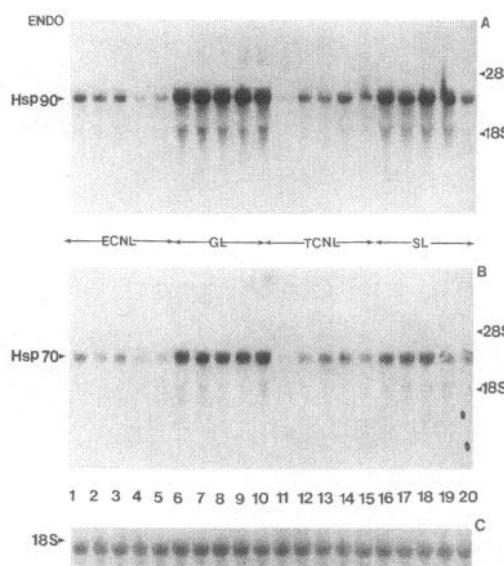


FIG. 2. Comparison of level of Hsp90 and -70 mRNA in the endometria (ENDO) of pregnant sheep. A, Presentation of Northern blot analysis of Hsp90 mRNA in five separate samples in each group in ECNL (lanes 1–5), GL (lanes 6–10), TCNL (lanes 11–15), and SL (lanes 16–20) groups. B, Presentation of Northern blot analysis of Hsp70 mRNA in five separate samples in each group. C, Hybridization of the same blot with 18S cDNA probe to demonstrate the relative amount of total RNA in each lane. D, Northern blot signals for Hsp90 and -70 mRNA and 18S in the endometria of pregnant sheep were quantified by densitometry and expressed as ratio of Hsp90 or -70 mRNA to 18S (mean \pm SEM) in the ECNL ($n = 5$), GL ($n = 5$), TCNL ($n = 7$), and SL ($n = 7$) groups. Hsp90 and -70 mRNA were significantly increased (b, $P < 0.01$) in each group associated with labor compared with levels in their gestational age-matched controls. Northern blotting was performed as described in Materials and Methods.

also collected from ewes in spontaneous term labor (SL; $n = 7$) and term control ewes ($n = 7$) not in labor (TCNL) at the same gestational age (140–145 dGA). To maintain catheter patency, all pregnant ewes were infused throughout the study with physiological saline at 2 ml/h into each maternal and fetal catheter.

To reduce endogenous ovarian steroid levels to a minimum, 32 non-pregnant ewes were ovariectomized on the day of ovulation, which was induced by placement of an intravaginal progesterone sponge followed by its removal 14 days later. A jugular venous and carotid arterial catheters were placed at the time of ovariectomy. Forty days after ovariectomy were allowed to elapse to ensure total clearance of the hormones. Nonpregnant ewes were divided into four groups ($n = 8$ in each): the control group (C), which was infused only with physiological saline (2

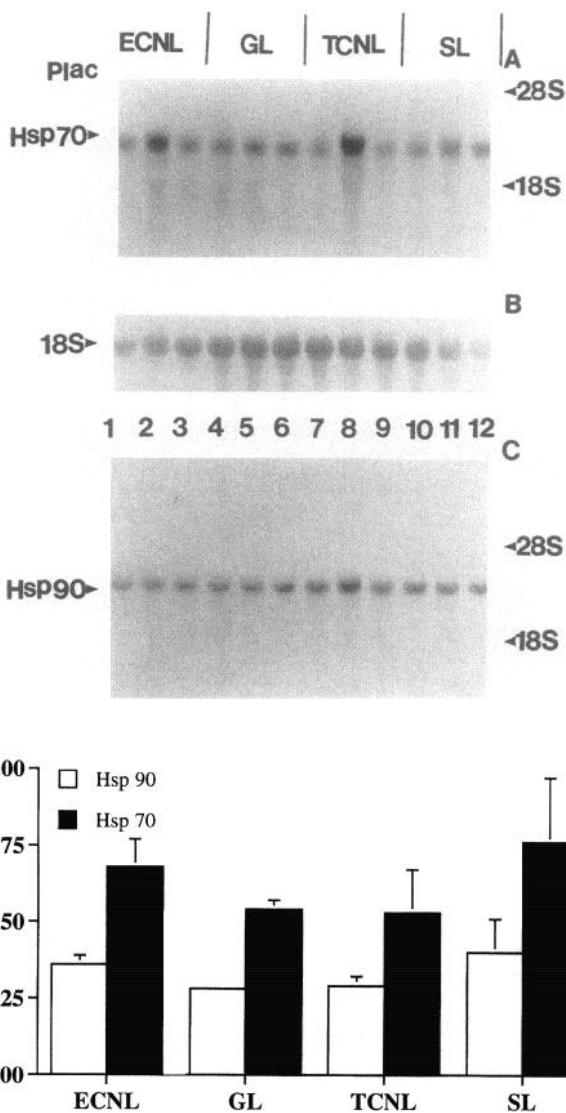


FIG. 3. Comparison of levels of Hsp90 and -70 mRNA in the fetal placenta (Plac) of pregnant sheep. A, Presentation of Northern blot analysis of Hsp70 mRNA in three separate samples in each group in ECNL (lanes 1–3), GL (lanes 4–6), TCNL (lanes 7–9), and SL (lanes 10–12) groups. B, Hybridization of the same blot with 18S cDNA probe to demonstrate relative amounts of total RNA in each lane. C, Presentation of Northern blot analysis of Hsp90 mRNA in three separate samples in each group. D, Northern blot signals for Hsp70 mRNA, Hsp90 mRNA, and 18S in the fetal placenta were quantified by densitometry and expressed as the ratio of Hsp70 and Hsp90 mRNA to 18S (mean \pm SEM) in the ECNL ($n = 5$), GL ($n = 5$), TCNL ($n = 7$), and SL ($n = 7$) groups. There were no significant changes in Hsp70 and -90 mRNAs in the groups associated with labor. Northern blotting was performed as described in Materials and Methods.

ml/h) throughout the study; the estradiol group (E), which received 50 μ g/day estradiol for 2 days (infused iv); the progesterone group (P), which received a 0.3-g progesterone sponge for 10 days (intravaginally); and the estradiol plus progesterone group (EP), in which a progesterone sponge was placed for 8 days and left *in situ* while the ewes were further treated as in group E for an additional 2 days. Progesterone sponges were purchased from Carter Holt Harvey Plastic Products (Hamilton, New Zealand) and produce plasma concentrations ranging from 1.5–2 ng/ml (provided by the manufacturer). All animals were infused with the same rate of physiological saline as the control group.

Myometrium and endometrium were always removed from the ven-

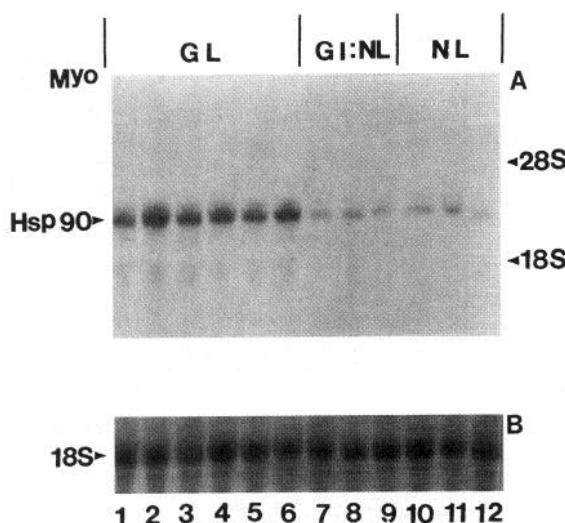


FIG. 4. Comparison of Hsp90 mRNA levels measured by Northern blot analysis in the myometrium (Myo) in GL, GI:NL, and NL groups. A, Northern analysis of Hsp90 in myometrium of pregnant ewes. Lanes 1–6, GL; lanes 7–9, GI:NL; lanes 10–12, NL. B, Hybridization of the same blot with an 18S cDNA probe to demonstrate the relative amounts of RNA in each lane. C, Northern blot signals for Hsp90 mRNA and 18S in the myometrium were quantified by densitometry and expressed as the ratio of Hsp90 mRNA to 18S (mean \pm SEM) in the NL ($n = 3$), GI:NL ($n = 3$), and GL ($n = 6$) groups. Hsp90 mRNA was only significantly increased (a, $P < 0.05$) in the group associated with labor. Northern blotting was performed as described in Materials and Methods.

tral aspect of the midportion of the body of the uterus. Uterine wall strips were rapidly dissected into their component layers. Fetal cotyledons, referred to as fetal placenta, were dissected out of the whole placenta. Endometrium, myometrium, and fetal placenta were frozen separately in liquid nitrogen for later RNA extraction. Another similar portion of the myometrium and endometrium was fixed in 4% paraformaldehyde for 24 h and embedded in paraffin for later *in situ* hybridization analysis. Frozen tissues were stored at -80°C until extracted for RNA.

Total RNA preparation and blot analysis

Total RNA was prepared from individual tissues as previously described (19). Briefly, total RNA was isolated from frozen tissues by homogenization in 4.2 M guanidinium thiocyanate solution. RNA was pelleted through a 5.7-M cesium chloride cushion. The RNA purity and recovery of each tissue was determined by UV spectrophotometry (260 and 280 nm). There were no differences in the yield of RNA per mg tissue over increasing gestational age, between ewes in labor and ewes not in

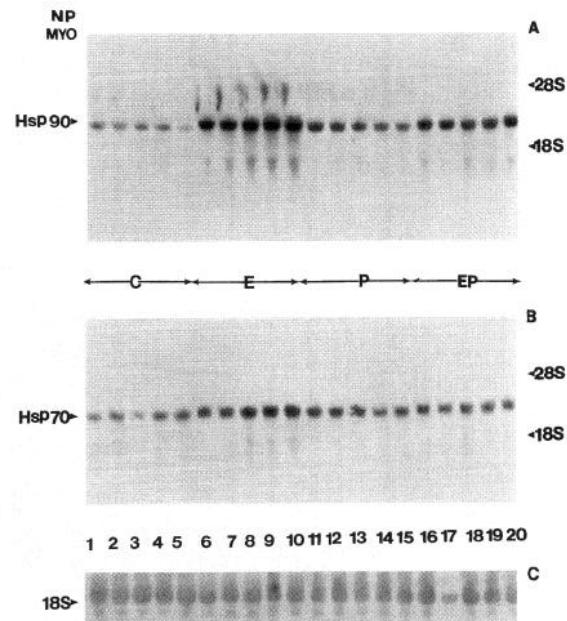


FIG. 5. Comparison of the levels of Hsp90 and -70 mRNA in myometrium (Myo) of ovariectomized nonpregnant (NP) ewes. A, Presentation of Northern blot analysis of Hsp90 mRNA in five separate samples in each group: C, control, saline infused (lanes 1–5); E, estradiol treated (lanes 6–10); P, progesterone treated (lanes 11–15); and EP, estradiol and progesterone treated (lanes 16–20). B, Presentation of Northern blot analysis of Hsp70 mRNA in five separate samples in each group. C, Hybridization of the same blot with 18S cDNA probe to demonstrate relative amounts of total RNA in each lane. D, Northern blot signals for Hsp90 and -70 mRNAs in the myometrium of nonpregnant ewes were quantified by densitometry and expressed as the ratio of Hsp90 or -70 mRNA to 18S (mean \pm SEM; $n = 5$ for each group). Hsp90 and -70 mRNAs were significantly increased (b, $P < 0.01$) in the myometrium after estradiol treatment. Progesterone antagonized the stimulatory effect of estradiol on Hsp90 and -70 mRNA expression (a, $P < 0.05$). Northern blotting was performed as described in Materials and Methods.

labor, or between different steroid treatments of nonpregnant ewes. Purified RNA was resuspended in 1 mM EDTA and stored at -80°C .

Samples of total RNA (30 $\mu\text{g}/\text{lane}$) from each tissue were denatured in 17.4% (vol/vol) formaldehyde, 50% (vol/vol) formamide, 20 mM MOPS [3-(N-morpholino)propanesulfonic acid], 5 mM sodium acetate, and 1 mM EDTA, pH 7.0, for 5 min at 65°C and separated on a 1% (wt/vol) agarose-0.66 M formaldehyde gel. Ethidium bromide-stained ribosomal RNA (ribosomal RNA) bands were visualized (UV) to insure that RNA degradation had not occurred and an equal amount of RNA had been loaded into each lane. After electrophoresis, RNA was trans-

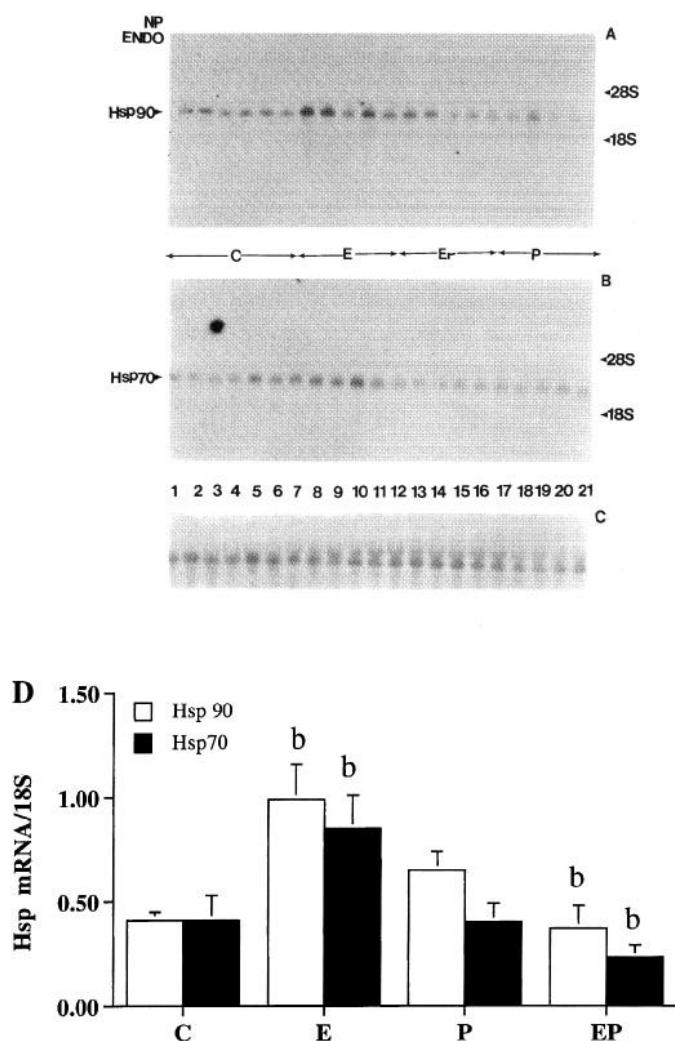


FIG. 6. Comparison of the levels of Hsp90 and -70 mRNA in endometria (ENDO) of ovariectomized nonpregnant (NP) ewes. A, Presentation of Northern blot analysis of Hsp90 mRNA in five or six separate samples in each group: C, control, saline infused (lanes 1–6); E, estradiol treated (lanes 7–11); P, progesterone treated (lanes 12–16); and EP, estradiol and progesterone treated (lanes 17–21). B, Presentation of Northern blot analysis of Hsp70 mRNA in five or six separate samples in each group, as described in A. C, Hybridization of the same blot with 18S cDNA probe to demonstrate relative amounts of total RNA in each lane. D, Northern blot signals for Hsp90 and -70 mRNAs in the endometria of nonpregnant ewes were quantified by densitometry and expressed as the ratio of Hsp90 or -70 mRNA to 18S (mean \pm SEM; n = 8 for each group). Hsp90 and -70 mRNAs were significantly increased (b, $P < 0.01$) in the endometrium after estradiol treatment. Progesterone antagonized the stimulatory effect of estradiol on Hsp90 and -70 mRNA expression (b, $P < 0.01$). Northern blotting was performed as described in Materials and Methods.

fected to a nylon membrane (Gene Screen Plus, New England Nuclear, Dupont, Wilmington, DE) by capillary blotting for 24 h in 10 \times SSC (1 \times SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0). Completion and uniformity of transfer were assessed by determining transfer of 28S and 18S ribosomal RNA from the gel. Membranes were prehybridized at 42 °C for 5 h in hybridization solution [50% (vol/vol) deionized formamide, 50 mM sodium phosphate, 0.8 M NaCl, 2% (wt/vol) SDS, 100 μ g salmon sperm DNA/ml, 20 μ g transfer RNA/ml, and 1 \times Denhardt's (1 \times 1% solution of BSA, Ficoll, and polyvinylpyrrolidone)].

Recombinant human Hsp90- α (catalogue no. 78313) and Hsp70-1

(catalogue no. 78318) cDNA were purchased from American Type Culture Collection (Rockville, MD) and labeled with [α -³²P]deoxy-CTP (3000 Ci/mmol) for Northern blot analysis and with [α -³⁵S]deoxy-CTP (1000–1500 Ci/mmol) for *in situ* hybridization using the random priming method (New England Nuclear-DuPont, Boston, MA) to specific activities of approximately 1 \times 10⁹ cpm/ μ g. Labeled cDNA was used at a final concentration of 1 \times 10⁶ cpm specific probe/ml hybridization solution.

Hybridization was carried out at 42 °C for 20 h. Membranes were washed sequentially in 2 \times SSC at room temperature for 10 min and in 0.5 \times SSC with 0.1% SDS at 65 °C for 30 min. Kodak X-Omat AR film was exposed to the membrane with intensifying screens at –80 °C. Exposure duration was varied to achieve hybridization signals within the limited linear range for densitometry.

Membranes were stripped of Hsp probes by boiling in 0.1 \times SSC with 0.1% (wt/vol) SDS for 30 min and rehybridized with α -³²P-labeled 18S cDNA probe to normalize Hsp90 and -70 mRNA levels. Autoradiographed signals were quantified by scan densitometry.

In situ hybridization

In situ hybridization was performed as described previously (29) with the following modifications: paraffin sections (4 μ m) of pregnant sheep myometrium and endometrium were cut and mounted on poly-L-lysine-coated slides. Tissue sections were treated with 0.2 N HCl for 20 min at room temperature, followed by incubation in 2 \times SSC for 10 min at 70 °C. The sections were then digested with proteinase K (10 μ g/ml) at 37 °C for 15 min in 100 mM Tris-Cl at pH 7.5. Prehybridization was performed at 42 °C for 1 h in prehybridization buffer containing 50% formamide, 5 \times SSPE (1 \times SSPE = 0.18 M NaCl, 10 mM NaH₂PO₄, and 1 mM EDTA), 0.1% (wt/vol) SDS, 0.1% (vol/vol) Denhardt's solution, 200 μ g denatured salmon testis DNA/ml, and 200 μ g transfer RNA/ml. Hybridization was carried out for 18 h at 42 °C in hybridization buffer [prehybridization buffer plus 4% (wt/vol) dextran sulfate] containing 1.5 \times 10⁶ cpm ³⁵S-labeled Hsp70 or -90 cDNA probe/section. After hybridization, the sections were rinsed at room temperature for 2 h in 2 \times SSC, for 2 h in 1 \times SSC, for 1 h in 0.5 \times SSC, and finally for 1 h in 0.5 \times SSC at 37 °C. The sections were then dehydrated by passing them through an alcohol series containing 300 mM ammonium acetate and coated with liquid photographic emulsion (NTB2, Eastman Kodak, Rochester, NY). After 14 days of exposure, the sections were developed and stained with hematoxylin and eosin.

Controls

Serial sections were treated with pancreatic ribonuclease A (RNase A; 20 μ g/ml) for 30 min at room temperature before hybridization. After enzyme treatment, the sections were rinsed in three changes of 2 \times SSC (5 min each) and hybridized with the labeled probe as described above.

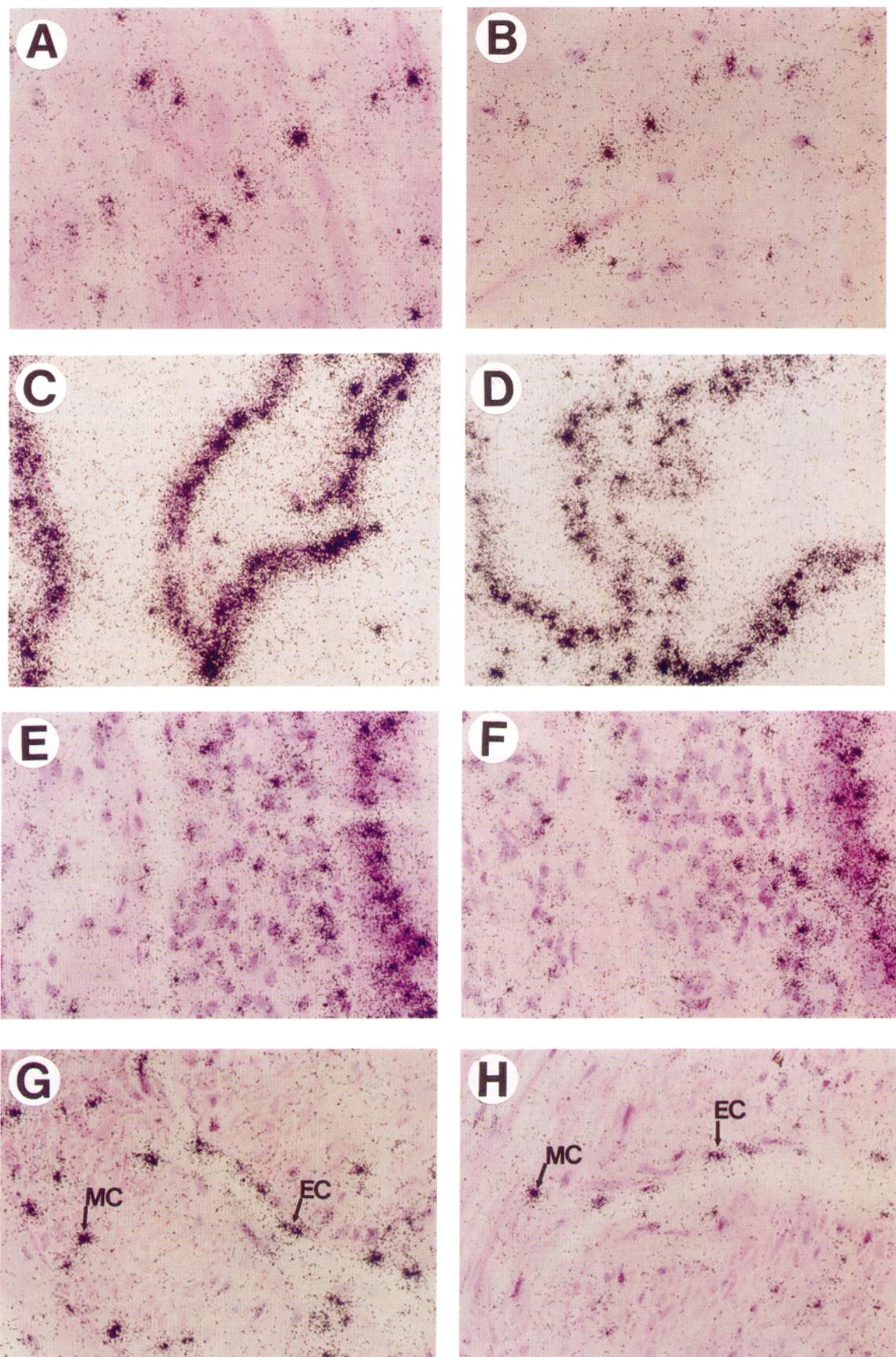
Statistical analysis

The Hsp90 and -70 mRNA concentrations in each Northern blot were normalized to the content of 18S ribosomal RNA in individual samples and expressed as a ratio of Hsp90 and -70 mRNA to 18S. Differences between different groups for Northern blot analysis were examined by one-way ANOVA. Data throughout are presented as the mean \pm SEM. There was no difference in either Hsp90 or Hsp70 mRNA level between the animals whose fetus received betamethasone or dexamethasone if the ewe was in labor; therefore, we pooled data from those two groups into a single group, defined as glucocorticoid-induced labor (GL).

Results

Northern blot analysis

Effect of labor on expression of Hsp90 and -70 mRNA. The effects of labor on Hsp90 and -70 mRNA contents in the pregnant sheep myometrium and endometrium were examined by Northern blot analysis. For this analysis, we probed tissues from all five ECNL, all seven TCNL, all seven SL, and five of the seven GL. Dramatic increases in Hsp90 and -70 mRNA



were observed in the pregnant sheep myometrium and endometrium (Figs. 1 and 2: a, $P < 0.05$; b, $P < 0.01$) during spontaneous labor. Glucocorticoid-induced labor was accompanied by the same biochemical changes in Hsp90 and -70 mRNA levels associated with spontaneous labor (Figs. 1 and 2). There was no significant difference between GL and SL for either Hsp90 or -70 mRNA.

To determine whether the effect of labor on Hsp mRNA abundance was target tissue specific, fetal placenta from the same animals was also analyzed for Hsp90 and -70 mRNA contents. As shown in Fig. 3, there were no changes in Hsp90 and -70 mRNA levels in the fetal placenta from sheep in labor compared with those in age-matched control animals.

In glucocorticoid-treated animals, Hsp90 mRNA in the myometrium increased only in association with labor (Fig. 4). The mRNA level of Hsp90 in tissues obtained from ewes that had been carrying glucocorticoid-treated fetus but had not yet developed clearly defined myometrial contractions were not different from that in gestational age-matched controls. For this analysis, we probed tissues from six of the seven GL, three GI:NL, and three of the five ECNL.

Effects of estradiol and progesterone on Hsp90 and -70 mRNA expression. To determine whether estradiol and progesterone play a role in regulating Hsp90 and -70 mRNA abundance in uterine tissues, we examined the effect of estradiol and progesterone administration on uterine Hsp90 and -70 mRNA abundance in ovariectomized nonpregnant sheep. The results from these *in vivo* experiments are shown in Figs. 5 and 6. Sufficient myometrial tissue was available from 5 of the 8 ewes in each group. Enough endometrium was available from all 32 ewes, 8 in each group. After estradiol administration, there were significant increases in both Hsp90 and -70 mRNAs in the myometrium (Fig. 5) and endometrium (Fig. 6). Progesterone alone had no significant effect on Hsp90 and -70 mRNA expression. However, progesterone inhibited the estradiol-stimulated increase in Hsp90 and -70 mRNA. As a result of this suppression, Hsp90 mRNA in endometrium and Hsp70 mRNA in myometrium and endometrium were no longer significantly elevated above control levels. Progesterone combined with estrogen significantly inhibited Hsp90 mRNA in myometrium, but at the doses used, this combination was still significantly greater than the control value (Figs. 5 and 6).

In situ hybridization

In situ hybridization demonstrated that both Hsp90 and -70 mRNAs were associated with myometrial cells in myometrium (Fig. 7, A and B), epithelial cells of endometrial glands (Fig. 7, C and D), and epithelial cells of the lumen (Fig. 7, E and F). Hsp90 and -70 mRNAs were also localized around the blood vessels (Fig. 7, G and H), and endometrial

stromal cells stained positively for Hsp90 and -70 mRNAs (Fig. 7, E and F). Control sections treated with RNase showed no specific signals.

Discussion

Although the interaction of Hsp with steroid receptors has been extensively studied at the molecular and cellular levels, changes in Hsp have not been determined under different physiological conditions. It is of particular interest that no data are available for changes in Hsp in critical uterine tissues in relation to parturition in any species. The procedures we used produce physiological changes in plasma estradiol concentrations as well as EMG activity (30). We have demonstrated increases in ER mRNA and its protein in sheep uterine tissues during labor (19, 20). Increased ER will enhance the effect of increased plasma estradiol concentration on the myometrium, thereby contributing to the mechanism by which estrogen can exert its influence on the process of parturition. To date, there is no direct evidence for a withdrawal of progesterone's action at the receptor level on critical target tissues in any species. Our study is the first to examine parturition-related changes in Hsp90 and -70 mRNA in pregnant sheep uterine tissues obtained from animals in whom myometrial activity and the onset of labor have been precisely monitored by recordings of myometrial activity patterns. Increased expression of Hsp90 and -70 may act as potent modulators of PR and ER functions in uterine tissues immediately before and during parturition. In sheep, these changes in Hsp may be considered back-up redundant mechanisms, but similar changes, if they occur, would be of considerable importance in species in which there is no evidence for progesterone withdrawal before labor.

The demonstration that similar changes in Hsp90 and -70 gene expression are associated with both spontaneous and glucocorticoid-induced labor supports the view that increased uterine Hsp90 and -70 production plays an important role in the process of labor, regardless of the manner in which labor is initiated. The rise in Hsp gene expression has been shown to be labor specific in the myometrium, as Hsp90 mRNA had not risen above control values in tissues collected from the animals that received the same amount of glucocorticoids but had not yet developed the typical myometrial contractions characteristic of labor. In addition, the changes in Hsp90 and -70 mRNAs are tissue specific, as Hsp did not increase in the fetal placenta. These findings also suggest that glucocorticoids are unlikely to have a direct effect on Hsp70 and -90 mRNA regulation. The tissue- and labor-specific increases in Hsp90 and -70 mRNAs in late pregnancy in the present studies indicate the potential for these Hsp to exert specific and critical functions in the process of labor. Further studies need to examine specific interactions of steroid re-

FIG. 7. *In situ* hybridization of Hsp90 and -70 mRNAs in myometrium and endometrium from a pregnant sheep in labor. Silver grains are formed by Hsp90 and -70 cDNA probes hybridized with Hsp90 and -70 mRNAs. A and B, *In situ* localization of Hsp90 (A) and Hsp70 (B) mRNAs in pregnant sheep myometrium; silver grains are associated with myometrial cells. C and D, *In situ* localization of Hsp90 (C) and Hsp70 (D) mRNAs in pregnant sheep endometrium; the silver grains are associated with the epithelial cells of the glands. E and F, *In situ* localization of Hsp90 (E) and Hsp70 (F) mRNAs in pregnant sheep endometrium; silver grains are associated with stromal cells as well as epithelial cells of the lumen. G and H, *In situ* hybridization of Hsp90 and -70 mRNAs in pregnant sheep myometrium; silver grains are associated with the endothelial cells (EC) and smooth muscle cells (MC) around the blood vessels.

ceptors with Hsps in this and other physiological conditions related to pregnancy and parturition, such as implantation and preterm labor.

The changes we observed in pregnant sheep uterine tissues during labor do not clearly indicate which hormonal agents regulate Hsp90 and -70 mRNA abundance. Parturition in sheep is accompanied by both a rise in estradiol and a fall in progesterone in maternal plasma together with changes in a large number of other potential regulators. As we consider estradiol and progesterone to be two of the major potential regulators involved in the control of Hsp90 and -70 mRNA in pregnant sheep uterine tissues, additional studies were performed in ovariectomized nonpregnant sheep treated with estradiol or/and progesterone to examine the regulatory effects of these two important hormones on Hsp90 and -70 mRNA abundance. Estradiol-dependent induction of both Hsp90 and -70 mRNAs was observed in both the myometrium and endometrium. This finding is consistent with previous observations of estradiol induction of Hsp90 protein in full thickness uterine wall of the mouse (31). Our studies extend this previous study by demonstrating both tissue specificity and that progesterone inhibited the estradiol-stimulated increase in Hsp90 and -70 mRNAs. Direct induction of Hsp90 and -70 mRNA by estradiol in uterine tissues of ovariectomized animals further supports the view that the changes in Hsp mRNA in uterine tissues of pregnant sheep in labor resulted from a combination of effects caused by the changes in plasma estradiol and progesterone concentrations that preceded labor in sheep.

The cellular locations of Hsp90 and -70 mRNA corresponded with the sites of PR and ER reported previously in uterine tissues (20, 32–35). This finding provides the histological and cellular evidence that interactions of Hsp90 and -70 with steroid receptors occur in uterine tissues. The marked appearance of Hsp in the endothelium of the blood vessels is in keeping with the distribution of Hsp25 in the endothelial cells of the endometrial vessels in the rat in early gestation (36). Hsp would, therefore, be in a position to play a role in steroid regulation of uterine blood flow. It is extremely difficult to physically resolve the individual subtype Hsps at the protein level, and there are currently no specific antibodies available to differentiate each individual subtype of Hsp90 and -70. As a result, none of currently available studies has made a distinction among the different subtypes of Hsp in relation to steroid receptors. The present study, using the specific probes encoding for Hsp90- α and Hsp70 (the protein 1 subtype), is the first attempt to evaluate Hsp90 and -70 subfamily function at the mRNA level under changing physiological conditions.

In conclusion, Hsp90 and -70 mRNA increase in pregnant sheep myometrium and endometrium in association with labor. We hypothesize that at term, increased expression of Hsp90 and -70 may act to inhibit PR function and stimulate ER function in uterine tissues during labor. In addition, our data support a role for both estradiol and progesterone in the control of Hsp90 and -70 mRNA abundance. Similar changes, if they occur, would be of considerable importance in species that do not demonstrate a withdrawal of progesterone at the plasma level before labor.

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