Characterization of two labor-induced genes, DSCR1 and TCTE1L, in the pregnant ovine myometrium

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Abstract

In the present study we characterized two labor-induced genes, DSCR1 (Down syndrome candidate region 1) and TCTE1L (murine t-complex like), which were identified by suppression subtractive hybridization in the pregnant ovine myometrium. DSCR1 and TCTE1L cDNA sequences were retrieved from a custom-made labor-myometrial cDNA library by hybridization screening. The characterized cDNA sequences include 5'-untranslated region (UTR), coding region and 3'-UTR, which are 12 bp, 351 bp and 1716 bp for TCTE1L, and 64 bp, 594 bp and 1539 bp for DSCR1 respectively. The two cDNA sequences encode proteins of 116 and 197 amino acids for TCTE1L and DSCR1 respectively. Northern analysis further confirmed the significant increases of myometrial DSCR1 and TCTE1L mRNA associated with spontaneous term labor ($n=6$) compared with gestation-matched controls not in labor ($n=6$). The abundance of DSCR1 and TCTE1L mRNA was attenuated when myometrial contraction was inhibited by Nimesulide ($n=6$), a specific prostaglandin H synthase 2

inhibitor. Fetal occupancy greatly upregulated DSCR1 and TCTE1L mRNA in the gravid horn during betamethasone-induced premature labor ($n=6$) compared with the non-gravid horn not in labor ($n=3$). Estradiol upregulated TCTE1L mRNA, but had no effect on DSCR1 mRNA expression in the non-pregnant sheep myometrium. Progesterone alone had no effect on both DSCR1 and TCTE1L mRNA expression, however progesterone antagonized estradiol's stimulating effect on myometrial TCTE1L mRNA expression in ovariectomized non-pregnant sheep.

Upregulation of DSCR1 and TCTE1L in both betamethasone-induced premature labor and spontaneous term labor and inhibition of their expression by Nimesulide suggest a functional role of these two genes in myometrial activation associated with onset of labor. Mechanical stretch, labor and steroids differentially regulated DSCR1 and TCTE1L mRNA in the pregnant and non-pregnant sheep myometrium.

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Introduction

The onset of labor in sheep is associated with a clear switch in myometrial contractility patterns from contractures to contractions (Nathanielsz *et al.* 1980, Harding *et al.* 1982). This switch is accompanied by myometrial activation (Lye 1994) involving changes in expression of a collection of contraction-associated proteins, such as estrogen receptor (Wu *et al.* 1995), gap junctions (Garfield 1988, Lye *et al.* 1993) and oxytocin receptor (Fuchs *et al.* 1983, Wu & Nathanielsz 1994, Zingg *et al.* 1995, Chen *et al.* 1996). Each member of the cassette of genes contributes to some extent in the switch in myometrial contractility patterns from contractures to contractions as well as the progression

of established labor. Altered abundance of members of this cassette of genes in the myometrium is a prerequisite for labor and delivery. Myometrial activation before and during labor appears to play a critical role in connecting fetal signals that indicate fetal readiness for birth to maternal factors that carry labor forward to expel the fetus.

Our understanding of the molecular basis of myometrial activation is incomplete. More information is needed on the genes involved during labor and the factors regulating their expression. To understand the molecular basis of myometrial activation, the relevant subsets of differentially expressed genes associated with the various stages of parturition must be identified and studied in detail. By using suppression subtractive hybridization, we identified

marked transcript increases associated with parturition of two labor-induced genes in the pregnant ovine myometrium (thrombospondin 1 and decorin), which had not been reported in any previous studies of myometrium in association with parturition (Wu *et al.* 1999a, 2000). The elucidation of the labor-related changes in thrombospondin 1 and decorin demonstrated that suppression subtractive hybridization is a powerful technique for identifying changes in abundance of mRNAs associated with the onset of labor.

In the current study, we proceed to characterize two other labor-induced genes identified by suppression subtractive hybridization, murine t-complex like (TCTE1L) and Down syndrome candidate region 1 (DSCR1) in the ovine myometrium. We cloned and sequenced DSCR1 and TCTE1L cDNAs from our custom-made ovine labor-myometrial cDNA library. The cDNA sequences of DSCR1 and TCTE1L have not been characterized in sheep and their expression in the pregnant myometrium in relation to labor has not been examined in any species.

Premature labor is not predictable in most clinical circumstances and an appropriate treatment to arrest the progress of labor that has already commenced is a very important clinical goal. In addition, the majority of the previous studies did not dissect mechanisms that occur prior to labor and after establishment of labor. Thus our current study also determined the effect of inhibition of myometrial contraction by prostaglandin H synthase 2 inhibitor (Nimesulide) on the regulation of the expression of uterine DSCR1 and TCTE1L during the progression of labor.

We also determined regulatory mechanisms on the expression of myometrial TCTE1L and DSCR1 by stretch and steroids. The sheep is a unique animal model that can be used for studying the regulatory role of stretch *in vivo*. In this species, uterine occupancy of the gravid horn can be compared with the non-gravid horn to examine the effect of stretch on the activation of myometrial contraction associated protein both before and during labor in the same animals. We have demonstrated recently that mechanical stretch differentially regulated myometrial contraction associated protein mRNA expression, such as oxytocin receptor and prostaglandin H synthase 2 (Wu *et al.* 1999b) in the ovine gravid horn and non-gravid horn, providing firm evidence for the regulatory role of mechanical stretch on myometrial contraction associated protein expression *in vivo* in this species. Therefore in the present study we compared DSCR1 and TCTE1L mRNA expression in the gravid horn and non-gravid horn of the pregnant sheep bearing a single fetus during betamethasone-induced labor.

The effects of endocrine factors have been studied extensively in regulation of myometrial contraction associated proteins. A major focus has been on the effects of the two steroid hormones, estrogen and progesterone, on the expression of myometrial contraction associated proteins

(Lye 1994, Wu *et al.* 1996a,b). In this study we examined the regulatory control of estrogen and progesterone on expression of myometrial DSCR1 and TCTE1L mRNA in the ovariectomized non-pregnant sheep.

Materials and Methods

Animals and tissue collection

Pregnant Rambouillet–Dorset ewes bred 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 120–130 days of gestational age (dGA) ewes from which tissues were obtained were instrumented with electromyogram leads sewn into the myometrium and fetal and maternal carotid arterial and jugular venous catheters (Nathanielsz *et al.* 1980, Harding *et al.* 1982). Labor was defined as having occurred when the myometrial electromyogram record showed a clear switch from contractures to contractions followed by contraction activity for at least 5 h (Nathanielsz *et al.* 1980). Myometrium from the ventral aspect of the mid-portion of the body of the gravid horn was collected from ewes in spontaneous term labor as judged from well-established labor type myometrial contraction at 145–147 dGA ($n=6$) and term control ewes ($n=6$) not in labor at the same gestational age (143–147 dGA).

Nimesulide infusion

Nimesulide (D-5648, Sigma Chemical Co., St Louis, MO, USA) infusion to the ewe ($n=6$) i.v. (30 mg bolus, followed by 6 h infusion, 30 mg/h) commenced 9 h after onset of labor at 147–148 dGA.

Betamethasone (celestone phosphate, Schering, 10 µg/h) was administered intravenously into the fetal jugular vein continuously over a period of 48 h to precipitate betamethasone-induced premature labor ($n=6$). Myometrium from the ventral aspect of the mid-portion of the body of the gravid horn and the non-gravid horn of the uterus was obtained from ewes in betamethasone-induced premature labor at 130–140 dGA as well as contemporary control ewes ($n=3$) whose fetuses were infused with saline at the same stage of gestation (130–140 dGA). These ewes were designated as controls, not in labor since myometrial electromyogram showed only contractures and no contractions.

Twenty non-pregnant ewes were ovariectomized on the day of ovulation. Forty days later ewes were treated with saline (controls, $n=5$) or estradiol infused intravenously for 2 days (50 µg/day, $n=5$) in 2 ml saline per hour or an intravaginal progesterone sponge (Carter Holt

Harvey Plastic Products, Hamilton, New Zealand) for 10 days (containing 0.3 g progesterone, $n=5$) or an estradiol plus progesterone group in which the intravaginal progesterone sponge was placed for 10 days and estradiol (50 µg/day) was infused intravenously on days 9 and 10 with the progesterone sponges still in place. The doses for estradiol and progesterone used in the present study were established in our previous studies (Wu *et al.* 1996a,b, 2000). At the end of each treatment period myometrium was removed under halothane general anesthesia prior to necropsy. Myometrium was frozen in liquid nitrogen for later RNA extraction. Frozen tissues were stored at -80°C .

Total RNA and Poly-A+ RNA isolation

Total RNA was isolated from myometrium of the sheep in spontaneous term labor and term control not in labor as described previously (Wu *et al.* 1999a). Poly-A+ RNA was extracted from total RNA using the Micro-FastTrack kit as suggested by the manufacturer (Invitrogen, San Diego, CA, USA). The Poly-A+ RNA from the two groups (spontaneous term labor and term control not in labor) was purified in parallel using the same reagents and protocol.

cDNA subtraction library

The construction of the subtracted cDNA library has been described in detail previously (Wu *et al.* 1996a). The library was made using PCR-Select cDNA Subtraction Kit according to the protocol provided by the manufacturer (CLONTECH Laboratories, Inc., Palo Alto, CA, USA).

Cloning and sequencing of DSCR1 and TCTE1L cDNAs from ovine myometrial cDNA library

The ovine TCTE1L and DSCR1 clones were retrieved from a custom-made ovine labor-myometrial cDNA library (CLONTECH Laboratories, Inc., Palo Alto, CA, USA), with probes from positive clones of subtraction, by differentiation screening (Wu *et al.* 2000).

5'- and 3'-RACE

Rapid amplification of cDNA ends (RACE) was used to isolate the 5' and 3' sequences. The marathon RACE kit was purchased from CLONTECH Laboratories, Inc. Poly-A+ RNA used for making the RACE library was from term control not in labor myometrium with a similar gestational age to spontaneous term labor myometrium for the subtraction library. Based on the sequences retrieved from the subtraction and cDNA libraries, two primers, NVR2 (reverse, 5'-GGGGGAATAGTCAGTTGAGATCAT-3') and NVF2 (forward, 5'-GGTGACAGACTT

CACAGATGCCTT-3'), from cloned ovine DSCR1 sequence were synthesized and used with AP1 primer from the kit for 5'- and 3'-RACE respectively. For TCTE1L, RP3 (reverse, 5'-CCTATGAGACTGCTTGCTTGAC-3') and RP2 (forward, 5'-CTGTGCCGTGGTCCAGAGGAGTCCA-3') primers were used with AP1 primer for 5'- and 3'-RACE.

The purified plasmid DNA was sequenced from at least two different clones and from both strands at the core facility of Cornell University. The acquired sequence data (nucleotides and amino acids) were aligned against the GenBank databases (nucleotides and protein) at the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA), using BLAST to search sequence matches. The sequence alignments were done using the Clustal W program (<http://www2.ebi.ac.uk/clustalW/>). The potential functional sites for the proteins were predicted using the ScanProsite website software (<http://expasy.hcuge.ch/tools/scnpsite.html>).

Identification of DSCR1 and TCTE1L as upregulated genes in parturition

At the end of suppression subtractive hybridization, DSCR1 and TCTE1L were isolated from the tester cDNA population (spontaneous term labor myometrium); they represented the upregulated genes from the myometrium of the pregnant sheep during labor. Northern blot analysis was performed to confirm the finding by suppression subtractive hybridization. All the samples for comparison were run on the same gel. Northern blot analysis was carried out as described previously (Wu *et al.* 1999a, 2000). The cloned ovine DSCR1 and TCTE1L cDNAs were used as probes and labeled with α - ^{32}P dCTP using the random priming method (NEN-Dupont, Boston, MA, USA) to specific activities of approximately 1×10^9 c.p.m./µg and utilized at a final concentration of 1×10^6 c.p.m. specific probe per milliliter of hybridization solution.

Statistical analysis

Following normalization of the content of DSCR1 and TCTE1L mRNA to 18S rRNA in individual samples, the DSCR1 and TCTE1L mRNA concentration in each Northern blot was expressed as a ratio of DSCR1 or TCTE1L mRNA to 18S rRNA. There appeared to be two bands for DSCR1 mRNA. Since both bands changed in the same pattern following labor onset or steroid treatment, the quantified data from both bands were combined and averaged for each animal. Differences between different groups for Northern blot analysis were analyzed by ANOVA followed by multiple comparison using a Tukey-Kramer procedure. Data throughout are presented as means $\pm$ S.E.M. The significant level was set at $P \leq 0.05$.

Results

Cloning and characterization of ovine DSCR1 and TCTE1L cDNAs

The Blastn search revealed that two upregulated gene clones were ovine orthologous genes of TCTE1L and DSCR1, which have not been previously reported in sheep. The acquired cDNA nucleotide sequences are 2079 bp for TCTE1L and 2197 bp for DSCR1. The characterized regions of the cDNAs include 5'-untranslated region (UTR), coding sequence and 3'-UTR, which are 12 bp, 351 bp and 1716 bp for TCTE1L, and 64 bp, 594 bp and 1539 bp for DSCR1 respectively. There are two polyadenylation signal sites (AATAAA) for TCTE1L (1612–1617, 2042–2047, accession no. AY205338) and one for DSCR1 (1706–1711, accession no. AY205232). The deduced amino acids are 116 and 197 for TCTE1L and DSCR1 respectively. The amino acid residues of human and ovine DSCR1 are almost entirely identical except for the only five conserved substitutions (97% identity), reflecting the functional constraint during evolution. A similar conservation of amino acids is also found for human and ovine TCTE1L, with one semi-conserved and five conserved substitutions (94% identity, Fig. 1). The putative functional sites of phosphorylation and glycosylation are illustrated in Fig. 1.

The effect of labor on expression of DSCR1 and TCTE1L mRNA

DSCR1 and TCTE1L were identified as two of the upregulated genes from the subtraction cDNA library representing transcripts expressed at higher levels in the myometrium during spontaneous term labor compared with gestation-matched controls. Northern blot was performed to further confirm the upregulation of DSCR1 and TCTE1L mRNA in the pregnant sheep myometrium during labor. DSCR1 mRNA significantly increased in ovine myometrial tissue in spontaneous term labor indicating strong induction of two approximately 2.3 and 2.1 kb transcripts in the myometrium (Fig. 2A,D, $P < 0.01$). TCTE1L mRNA also significantly increased during labor, indicating strong induction of an approximately 2.1 kb transcript in the myometrium (Fig. 2B,D, $P < 0.01$). Following Nimesulide infusion, DSCR1 and TCTE1L mRNAs significantly decreased below levels seen in spontaneous term labor in the myometrium (Fig. 2, $P < 0.05$), but remained significantly higher than term control ewes not in labor ($P < 0.01$).

The effect of stretch on expression of DSCR1 and TCTE1L mRNA

DSCR1 mRNA The DSCR1 mRNA level was similar in the gravid horn and non-gravid horn before onset of

labor. During betamethasone-induced labor, the increase of DSCR1 mRNA in non-gravid horn did not reach the significant level (Fig. 3, $P > 0.05$), however there was a significant increase in the gravid horn for DSCR1 mRNA during betamethasone-induced labor (Fig. 3, $P < 0.05$).

TCTE1L mRNA Gravid horn myometrial TCTE1L mRNA was significantly higher than the non-gravid horn before labor. During betamethasone-induced labor, there was a further increase of myometrial TCTE1L mRNA in the gravid horn (Fig. 4, $P < 0.05$). In addition, TCTE1L mRNA in the non-gravid horn during labor is higher than before labor (Fig. 4, $P < 0.05$).

The effects of estradiol and progesterone on expression of DSCR1 and TCTE1L mRNA

After estradiol treatment of non-pregnant ovariectomized ewes, TCTE1L mRNA concentration analyzed by Northern blot increased significantly ($P < 0.05$, Fig. 5) in the myometrium, whereas progesterone treatment alone had no effect on TCTE1L mRNA abundance. Progesterone antagonized estradiol's stimulatory effect on TCTE1L mRNA expression when used in combination with estradiol ($P < 0.05$, Fig. 5). Estradiol or progesterone alone or in combination had no effect on myometrial DSCR1 mRNA expression in non-pregnant sheep (Fig. 5).

Discussion

In this study we characterized two labor-induced genes in pregnant and non-pregnant sheep myometrium, which have not been described previously in uterine myometrium in any species. In addition, the present study is the first to characterize the complete DSCR1 and TCTE1L cDNA sequences in this important experimental species.

The human TCTE1L was first isolated from Xp21 during a positional cloning of retinitis pigmentosa 3 gene but excluded as a candidate gene (Roux *et al.* 1994). Its function is still unknown while its homologue Tctex-1 (mouse T complex-associated-testis expressed) has been suggested as one of three light chains of cytoplasmic dynein, a microtubule-based molecular motor exhibiting ATPase activities (King *et al.* 1996). TCTE1L has been indicated as a variant cytoplasmic dynein light chain that is differentially expressed in certain tissues (King *et al.* 1996). Therefore, the upregulated TCTE1L transcript might be related to the increased activity of uterine myometrial cells during labor.

DSCR1 was isolated as a candidate gene from Down's syndrome critical region on chromosome 21 (Fuentes *et al.* 2000) and identified as an inhibitor of calcineurin, a

(A)		Myristyl	CK2	
human	MEEYHRHCDEVGFNAEEAHNIVKECVDGVLGGEDYNHNNINQWTASIVEQSLTHLVKLGK	60		
ovine	MEEYHRPCDEVGFNADEAHNIVKECIDGVLGGEDYNQNNINQWTASIVEQSLAHLVKLGK	60		
***** :*****:*****:*****:*****				
	CK2	PKC	N	
human	AYKIYIVTCAVVQKSAYGFHTASSCFWDTTSDGTCTVRWENRTMNCIVNVFAIAIVL	116		
ovine	AYKIYIVTCAVVQKSPYGFHTASSCFWDTTSDGTCTVRWENRTMNCIVNVFAIAIVL	116		
*****:*****				
(B)				
	N	CK2	PKC	N
human	MHFRNFNYSESSLIACVANSIDIFSESETRAKFESLFRTYDKDITFQYFKSFKRVIRNFSN	60		
ovine	MHFRNFNYSESSLIACVANSEIFSESETRAKFESLFRTYDKDITFQYFKSFKRVIRNFSN	60		
*****:*****				
	CK2			
human	PFSAADARLQLHKTEFLGKEMKLYFAQTLHIGSSHLAPPNPDQFLISPPASPPVGWKQV	120		
ovine	PLSAADARLQLHKTEFLGKEMKLYFAQTLHIGSSHLAPPNPDQFLISPPASPPVGWKQV	120		
*:*****				
	CK2			
human	EDATPVINYDLLYAISKLGPGKEYELHAATDTTPSVVVHVCESDQEEEEEEMERMRRPK	180		
ovine	EDATPVINYDLLYAISKLGPGKEYELHAATDTTPSVVVHVCESDQENEEEDEMERMKRPK	180		
*****:***:***:***				
	PKC			
human	PKIIQTRRPEYTPIHLS	197		
ovine	PKIIQTRRPEYTPIHLS	197		

* identical amino acids; : conserved substitution; . semi-conserved substitution

Figure 1 Comparison of the deduced amino acid sequences of ovine TCTE1L (A) and DSCR1 (B) with human orthologs. The amino acid sequences of ovine TCTE1L and DSCR1 and of human TCTE1L and DSCR1 were deduced from respective cDNA sequences (GenBank accession nos: ovine TCTE1L, AY205338; ovine DSCR1, AY205232; human TCTE1L, AL121578; human DSCR1, NM-004414). The putative functional sites for N-glycosylation (N), protein kinase C phosphorylation (PKC), casein kinase II phosphorylation (CK2) and N-myristoylation (Myristyl) are shaded and marked accordingly.

Figure 2 Northern blot analysis of DSCR1 (A) and TCTE1L (B) mRNA in the pregnant sheep myometrium in term control not in labor (lanes 1–6), spontaneous term labor (lanes 7–12) and Nimesulide-infused ewes (lanes 13–18). (C) The same blot was probed for 18S. (D) Densitometric analysis of the ratio of DSCR1 (■) and TCTE1L (□) mRNA and 18S rRNA in myometrium from term control not in labor (TCNL; $n=6$), spontaneous term labor (STL; $n=6$) and Nimesulide-infused ewes (NIM; $n=6$). There was a significant increase in DSCR1 and TCTE1L mRNA during spontaneous term labor ($*P<0.01$). Following Nimesulide infusion, DSCR1 and TCTE1L mRNA decreased significantly in myometrium ($+P<0.05$). Means $\pm$ S.E.M. are shown.

serine/threonine protein phosphatase regulated by Ca^{2+} /calmodulin (Fuentes *et al.* 2000, Rothermel *et al.* 2000). It has been suggested that the cells may transiently increase DSCR1 expression to provide short-term protection against acute chemical or mechanical stress (Ermak *et al.* 2002, Wang *et al.* 2002).

Both DSCR1 and TCTE1L mRNA levels increase in pregnant sheep myometrium during betamethasone-induced premature labor and spontaneous term labor, indicating that the increases in DSCR1 and TCTE1L mRNA levels are a common feature associated with the switch of myometrial activity from contractures to

Figure 3 Northern blot analysis of DSCR1 mRNA (A) expression in the gravid horn (GH, lanes 1–9) and non-gravid horn (NGH, lanes 10–18) from the pregnant sheep not in labor (GHNL: lanes 1–3 and NGHNL: lanes 10–12) and during betamethasone-induced labor (GHBL: lanes 4–9 and NGHBL: lanes 13–18). (B) Hybridization of the same blot with an 18S cDNA probe to demonstrate relative amounts of total RNA in each lane. (C) Northern blot signals for DSCR1 mRNA and 18S in the myometrium from GHNL, GHBL, NGHNL and NGHBL were quantified by densitometry and expressed as a ratio of DSCR1–18S (data are means $\pm$ S.E.M.). There were significant increases in myometrial DSCR1 mRNA during betamethasone-induced labor (* $P < 0.05$) in the gravid horn.

contraction. The mRNA levels of DSCR1 and TCTE1L expressed in the myometrium were differentially regulated by stretch and steroid hormones, indicating that different myometrial contraction associated proteins are activated by different pathways in association with the onset of labor.

Nimesulide infusion inhibited myometrial contractility (Unno *et al.* 1997) and prostaglandin E_2 (PGE_2) and cortisol production in the fetal circulation (Unno *et al.* 1998). Both the attenuated fetal plasma cortisol and PGE_2 that accompany the administration of Nimesulide decreased placental P450–17 α hydroxylase mRNA expression observed in our previous study (Ma *et al.* 1999), which may result in a decreased placental conversion of progesterone to estrogen.

To further test the hypothesis that estradiol and/or progesterone are involved in the regulation of DSCR1 and

TCTE1L mRNA expression, we conducted studies in ovariectomized non-pregnant sheep treated with estradiol or/and progesterone. Estradiol-dependent induction of TCTE1L mRNA was observed in the myometrium. Progesterone alone had no effect on myometrial TCTE1L mRNA expression. In combination with estradiol, progesterone did antagonize estradiol's positive effect on TCTE1L expression. These results support the view that changes in TCTE1L mRNA in uterine tissues of pregnant sheep in labor are regulated by plasma estradiol and progesterone changes that precede labor in sheep. However, estradiol or progesterone alone or in combination did not have any effect on myometrial DSCR1 mRNA expression, indicating that myometrial DSCR1 mRNA may not be induced by these two steroid hormones during labor.

Figure 4 Northern blot analysis of TCTE1L mRNA (A) expression in the gravid horn (GH, lanes 1–9) and non-gravid horn (NGH, lanes 10–18) from the pregnant sheep not in labor (GHNL: lanes 1–3 and NGHNL: lanes 10–12) and during betamethasone-induced labor (GHBL: lanes 4–9 and NGHBL: lanes 13–18). (B) Hybridization of the same blot with an 18S cDNA probe to demonstrate relative amounts of total RNA in each lane. (C) Northern blot signals for TCTE1L mRNA and 18S in the myometrium from GHNL, GHBL, NGHNL and NGHBL were quantified by densitometry and expressed as a ratio of TCTE1L to 18S (data are means $\pm$ S.E.M.). TCTE1L mRNA was significantly higher in gravid than in non-gravid horn before labor onset (* $P < 0.05$). There was a significant increase in myometrial TCTE1L mRNA during betamethasone-induced labor in the gravid horn (** $P < 0.05$) and non-gravid horn (+ $P < 0.05$) compared with gravid and non-gravid horn not in labor.

As discussed above, overexpression of DSCR1 can protect cells against multiple stresses mediated by calcium and mechanical stretch (Ermak *et al.* 2002); our next experiment was designed to identify the effect of myometrial stretch on induction of DSCR1 in the pregnant sheep uterus. The bicornuate sheep uterus provides a powerful natural experimental system in which the degree of stretch is much greater in the gravid horn of sheep carrying a single fetus than the non-gravid horn. The following information can be obtained by using this *in vivo* stretch model: (1) changes due to stretch from the differences between gravid and non-gravid horn before labor; (2) changes due to labor-related endocrine effects from differences in the non-gravid horn before and after labor;

and (3) changes due to the combination of endocrine effects and stretch from the difference between non-gravid horn not in labor and gravid horn in labor.

Before labor onset, TCTE1L mRNA was higher in the gravid horn than the non-gravid horn. In addition, TCTE1L mRNA was higher during labor than before labor in non-gravid horn. Furthermore TCTE1L mRNA was maximal in the gravid horn during labor. These data suggested that myometrial distention induced by fetal occupancy and endocrine factor such as increased estradiol associated with onset of labor are both involved in the regulation of myometrial TCTE1L mRNA expression. Furthermore, the effect induced by both stretch and endocrine change was additive, which resulted in the

Figure 5 Northern blot analysis of the effect of estradiol (E) and progesterone (P) alone or in combination (EP) on regulation of DSCR1 and TCTE1L mRNA expression in ovariectomized non-pregnant sheep myometrium. Top panel: estradiol or progesterone or the estradiol and progesterone combination had no significant effect on DSCR1 mRNA expression compared with controls (C). Bottom panel: estradiol stimulated myometrial TCTE1L mRNA expression (* $P < 0.05$). Progesterone alone had no effect on myometrial TCTE1L, however progesterone antagonized estradiol's stimulating effect (+ $P < 0.05$ when the estradiol and progesterone combination is compared with estradiol).

maximal induction of TCTE1L mRNA in gravid horn during labor.

In contrast to TCTE1L, DSCR1 mRNA was not induced by stretch alone. In addition, endocrine changes associated with onset of labor were not able to induce DSCR1 mRNA expression either. DSCR1 mRNA was only upregulated in the gravid horn during labor, indicating (1) that the synergic effect of endocrine changes and stretch may be required for induction of myometrial DSCR1 mRNA; or (2) that the increase of DSCR1 may be due to mechanical stress such as myometrial contraction and/or high cellular calcium concentrations during labor to protect cells from these labor-related changes. Further studies are required to differentiate the potential mechanisms that regulate myometrial DSCR1 mRNA expression during labor.

In conclusion, the upregulation of DSCR1 and TCTE1L in both betamethasone-induced premature labor and spontaneous term labor and inhibition of their expression by Nimesulide suggest a functional role of

these two genes in myometrial activation associated with onset of labor. Mechanical stretch, labor and steroids differentially regulated DSCR1 and TCTE1L mRNA in the pregnant and non-pregnant sheep myometrium.

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