A decline in the levels of progesterone receptor coactivators in the pregnant uterus at term may antagonize progesterone receptor function and contribute to the initiation of parturition

Jennifer C. Condon*, Pancharatnam Jeyasuria†, Julie M. Faust*, James W. Wilson*, and Carole R. Mendelson*‡§

Departments of *Biochemistry, †Internal Medicine, and ‡Obstetrics and Gynecology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9038

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The molecular events that lead to the onset of labor in humans and in other mammalian species remain unclear. We propose that a decline in coactivators containing histone acetylase activity in myometrium may contribute to the onset of labor by impairing the function of the progesterone–progesterone receptor (PR) complex. As assessed by semiquantitative and real-time RT-PCR, immunohistochemistry, and immunoblotting, expression of the PR coactivators cAMP-response element-binding protein (CREB)-binding protein and steroid receptor coactivators 2 and 3 was decreased in fundal uterine tissue of women in labor. Using the mouse as an animal model, we also found decreased coactivator levels in uterine tissues at term. In both human and mouse, the levels of acetylated histone H3 were also decreased in uterine tissues at term. Administration of trichostatin A, a specific and potent histone deacetylase inhibitor, to pregnant mice late in gestation increased histone acetylation and delayed the initiation of parturition by 24–48 h, suggesting the functional importance of the decline in histone acetylation in the initiation of labor. These findings suggest that the decline in PR coactivator expression and in histone acetylation in the uterus near term may impair PR function by causing a functional progesterone withdrawal. The resulting decrease in expression of PR-responsive genes should increase sensitivity of the uterus to contractile stimuli.

pregnancy | labor | histone acetylation | human | mouse

In many species, progesterone withdrawal appears to be a critical event in the initiation of parturition, which is evidenced by the fact that a decline in progesterone levels precedes the onset of parturition and that progesterone administration delays the process (1). However, a role for progesterone withdrawal in the control of human labor has not been identified, because plasma progesterone levels do not decline before or during labor (2). Nevertheless, the fact that the progesterone receptor (PR) antagonist mifepristone can enhance cervical ripening and initiate labor (3) suggests some form of functional progesterone deprivation might be involved in the initiation of labor in women. Functional progesterone withdrawal could be mediated by a variety of mechanisms, including accelerated inactivation/metabolism of progesterone within target cells, formation of metabolites that antagonize binding of progesterone to its receptor, reduction in the amount of PR, and/or impairment of the transcriptional activity of the progesterone–PR complex by alterations in the levels of essential coregulators. Because levels of uterine PR do not decline prior to or during labor (4), in the present study we analyzed the potential role of changes in coregulator levels in the initiation of labor.

Nuclear receptors such as the PR interact with coregulators, coactivators, and corepressors, which increase and decrease their transcriptional activities, respectively. Coactivators likely function by bridging sequence-specific activators, such as ligand-bound receptors, to response elements in promoters and to the basal transcription machinery, resulting in stabilization of the preinitiation complex and activation of transcription initiation (5). Some coactivators contain histone acetyltransferase (HAT) activity, which catalyzes acetylation of histones, resulting in a remodeling of chromatin structure and increased access of transcription factors and polymerase to the promoter (6). Conversely, some corepressors can recruit histone deacetylases, resulting in a closing of chromatin structure and transcriptional silencing (7). In light of the importance of coregulators in the control of transcriptional activity of nuclear receptors, we measured expression levels of key coactivators and corepressors of the PR in myometrium from the uterine fundus of pregnant women in labor and not in labor toward the end of gestation. We hypothesized that a decline in PR coactivators may compromise PR function by decreasing uterine histone acetylation, resulting in the closing of the chromatin structure with decreased expression of PR-responsive genes and increased myometrial sensitivity to contractile stimuli. In parallel, we analyzed expression of key coactivators and levels of histone acetylation in mouse uterus from late gestation to term. The coactivator, cAMP-response element-binding protein (CREB)-binding protein (CBP), and members of the steroid receptor coactivator (SRC) family were markedly reduced in fundal myometrium of women in labor and in mouse uterus at term. This decline in coactivators was associated with a marked decrease in histone acetylation within the uterus. Importantly, we also found that treatment of pregnant mice with trichostatin A (TSA), a histone deacetylase inhibitor, delayed the onset of labor by 24–48 h. On the basis of these findings, we suggest that decreased expression of coactivators in uterine tissue leading to a reduction in histone acetylation at term results in a functional progesterone withdrawal that may initiate the onset of labor in a variety of species.

Materials and Methods

Subjects and Tissue Acquisition. Fundal myometrial tissues were biopsied at term from pregnant women undergoing cesarean section or at the time of cesarean hysterectomy. Informed consent was obtained in accordance with the Institutional Review Board of the University of Texas Southwestern Medical Center. Fundal biopsies were obtained by dissecting a strip of myometrium from the inner aspect of the posterior uterine wall. Myometrial smooth muscle was dissected from each of the biopsy samples, cut into appropriate-sized pieces for subsequent

Abbreviations: PR, progesterone receptor; HAT, histone acetyltransferase; CBP, cAMP-response element-binding protein; SRC, steroid receptor coactivator; TSA, trichostatin A; $PGF_{2\alpha}R$, prostaglandin $F_{2\alpha}$ receptor; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; CnX43, connexin-43; OTR, oxytocin receptor.

§To whom correspondence should be addressed. E-mail: cmende@biochem.swmed.edu.

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Table 1. Primers used in the RT-PCR analysis of myometrial biopsies from near-term women in labor and not in labor

Gene	Forward primer	Reverse primer	Ref. or accession no.
SRC-1	5′-CATGCTTATGAGGCAGCAAA-3′	5′-ATTCCAGTGCCAAACTGTCC-3′	XM_03978.5
SRC-2	5′-CCGTTTTCCCACAGCAGTAT-3′	5′-TCCAGGCCTCAGAGTCAAGT-3′	XM_011633
SRC-3	5′-TAATCCCTAGGCCAAGCAG-3′	5′-CGCATCTGTGTTGCTGAGAA-3′	XM_030032.4
CBP	5′-ATAGCTTAATTAATCAGGCTTCA-3′	5′-CTTATGAGCATGAAGCAGTAGAA-3′	36
NCoR	5′-ACAGGCAGAGGGAATGGGGCAA-3′	5′-GGGTGGGGAGATGGGCTCGTAG-3′	XM_037176
SMRT	5′-TACACCCGGCACCACCCACAG-3′	5′-TACCGTCCTCACCACCCAAGA-3′	NM006312.1
SRA	5′-TAAAGAAGAGAATGGCTCTA-3′	5′-GTATGGTATGGTTCTTCTCA-3′	XR_000089
NRIP-1	5′-ACGAATCTTCCTGATGTGAC-3′	5′-TACCCATTGGATTTTGAAAC-3′	XM_009699
Alien	5′-ATGTGGATTTGGAAAATCAG-3′	5′-CTGCACTCCGAATATAGGTC-3′	37
HMG-1	5′-AATAACACTGCTGCAGAT-3′	5′-TTTTCAGCCTTGACAAT-3′	BC030981
HMG-2	5′-TCTGAGCAGTCAGCCAAA-3′	5′-CCTGTTGGCCTGCCAGGG-3′	38
$PGF_{2\alpha}R$	5′-ACCTGCCAGACGGAAAACC-3′	5′-GCCTTCATGAGAATGGCGAT-5′	AF004021
CnX43	5′-TGGCCTTCTTGCTGATCCAG-3′	5′-GGTCGCATGGTAAGGGTCG-3′	39
OTR	5′-ACATCACCTTCCGCTTCTAC-3′	5′-TCTGCCAGATCTTGAAGCTG-3′	XM_003177
G3PDH	5′-CCACCCATGGCAAATTCCATGG-3′	5′-TCTAGACGGCAGGTCAGGTCCAC-3′	BL026907

Forward and reverse primers for analysis by RT-PCR of coregulators and other gene products are shown with their GenBank accession numbers or reference numbers.

immunohistochemical analyses, flash frozen in liquid nitrogen, and stored at −80°C.

Uterine tissues ($n = 3$ at each time point) were obtained from outbred Institute for Cancer Research (ICR) pregnant mice at 16–19 days gestation. The uteri collected on day 19 were from mice in labor. The tissues were washed in PBS, and all fetal membranes were removed. One-half of the uterus was flash frozen and stored at −80°C; the remaining tissue was fixed in 10% buffered formalin and embedded in paraffin. To analyze the importance of histone acetylation in the initiation of labor, another group of ICR pregnant mice ($n = 9$) received daily i.p. injections of 15 μg of TSA in 200 μl of DMSO (Sigma) at 15, 16, and 17 days postcoitum (dpc) and with 30 μg of TSA in 200 μl of DMSO at 18 and 19 dpc. Control mice ($n = 6$) were injected i.p. with 200 μl of DMSO alone. Three of the TSA-treated mice and three vehicle controls were killed at 19 dpc, and the uteri were removed for analysis of levels of acetylated histone H3. The uteri were dissected free of fetal membranes, washed in PBS, flash frozen, and stored at –80°C. Serum progesterone levels were analyzed at 19 dpc in the untreated, vehicle control, and TSA-treated animals.

Semiquantitative RT-PCR. Total RNA from fundal myometrial tissues of 12 women in labor and 12 women not in labor was extracted by the one-step method of Chomczynski and Sacchi (8) (TRIzol, Invitrogen). The first-strand cDNA synthesis reaction was catalyzed by Superscript II RNase H-reverse transcriptase (Invitrogen) and primed by random hexamers. Amplification of target cDNAs from the first-strand synthesis reaction was performed by PCR. A semiquantitative measurement of relative levels of gene expression in the in-labor samples, as compared with the not-in-labor samples, was performed for SRC-1, -2, and -3; CBP; steroid receptor RNA activator; nuclear receptor corepressor; silencing mediator for retinoid and thyroid hormone receptor; Alien; RIP-140; high-mobility group proteins 1 and 2; prostaglandin $F_{2\alpha}$ receptor ($PGF_{2\alpha}R$); glyceraldehyde-3-phosphate dehydrogenase (G3PDH); connexin-43 (CnX43); and oxytocin receptor (OTR). These target genes were amplified using gene-specific primers and reaction conditions recommended by the Primer Express oligo program (Perkin–Elmer, Applied Biosystems); primers are presented in Table 1. The constitutively expressed G3PDH transcript was used as a reference to normalize mRNA levels and to evaluate data from the exponential phase of PCR amplification.

Real-Time RT-PCR. Reactions were performed by using an ABI PRISM 7700 apparatus (Perkin–Elmer, Applied Biosystems). Thermocycling was done in a final volume of 10 μl containing 1.5 μl of cDNA sample (diluted 1:10) or calibrator; 3 mM $MgCl_2$; 0.5 μM each of the SRC-1, -2, and –3; and CBP primers or 0.3 μM each of the G3PDH primers; 1 μl of LC-Fast Start Reaction Mix SYBR green I; and 1 μl of LC-Fast Start DNA Master SYBR green, under conditions recommended by Applied Biosystems. To correct for differences in RNA quantity among samples, data were normalized by using the ratio of the target cDNA concentration to that of G3PDH.

Immunohistochemistry. Sections (8 μm thick) were cut from paraffin-embedded tissues and mounted on silane-coated slides, deparaffinized, and rehydrated in a graded alcohol series. Sections were preincubated with 1.5% horse serum in PBS (pH 7.5) and then incubated with primary antibodies diluted in PBS (1:100). Immunoreactivity was detected by using the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) and a Vector Nova red detection kit (Vector); the immunoreactive proteins appeared as a red end product.

Immunoblot Analysis. Tissue lysates and nuclear extracts prepared as described (9) were electrophoresed with a precast Novex gel electrophoresis system with 3–8% Tris acetate gels or 4–12% Bis-Tris gels (Invitrogen). Proteins were electrophoretically transferred onto polyvinylidene fluoride membranes, which were incubated for 1 h at room temperature with antibodies (1:500) directed against CBP (Santa Cruz Biotechnology), SRC-1 and -3, acetyl histone H3 (Lys-14), and histone H3 (Affinity BioReagents, Golden, CO). Membranes were incubated with horseradish peroxidase-conjugated rabbit secondary antibodies, and immunoreactive bands were visualized [SRC-1 (160 kDa), SRC-3 (155 kDa), and CBP (275 kDa)].

Results

SRC-2 and -3 and CBP mRNA Levels Are Significantly Reduced in Fundal Myometrium of Women in Labor. Semiquantitative RT-PCR was used to compare expression of genes encoding a variety of coregulators and other proteins known to modulate PR transcriptional activity in human fundal myometrial tissues obtained from six women in labor and six not in labor. After normalization of input for GP3DH transcripts, mRNA levels for the coactivators SRC-2 and -3 and CBP were found to be reduced in the

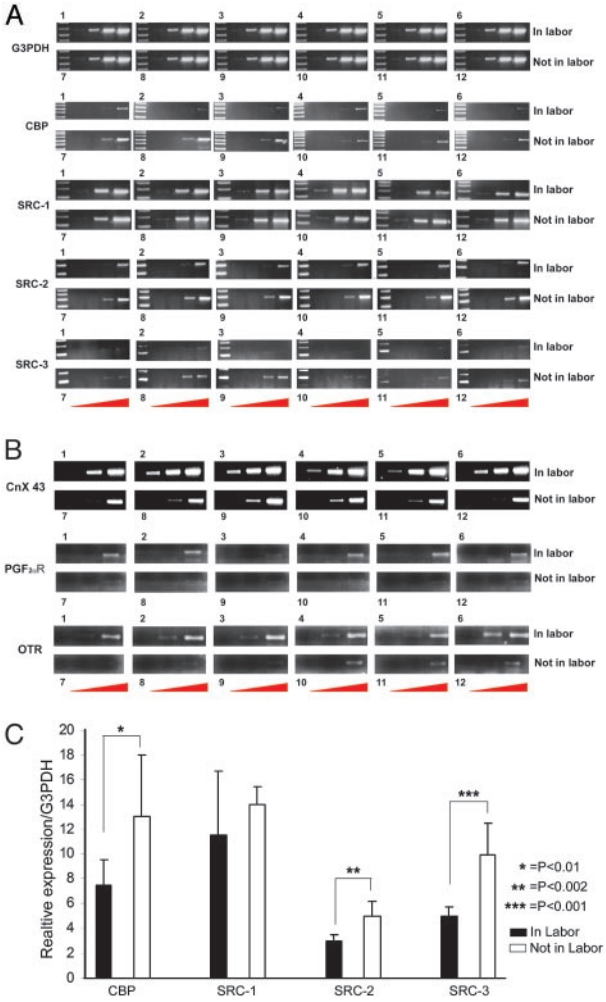

Fig. 1. mRNA levels for CBP and SRC-2 and -3 are decreased at term in fundal myometrium from women in labor, as compared with tissues from women not in labor. (*A*) Semiquantitative RT-PCR was used to analyze mRNA expression levels for CBP and SRC-1, -2, and -3 in myometrial biopsies obtained from the uterine fundus of women in labor as compared with those not in labor. G3PDH mRNA levels were analyzed to ensure that equal amounts of cDNA were added to each reaction. The wedges signify increasing numbers of PCR cycles. (*B*) Semiquantitative RT-PCR analysis of CnX43, $PGF_{2\alpha}R$, and OTR mRNA levels as an index of ''labor status.'' The wedges indicate increasing numbers of PCR cycles. (*C*) Real-time RT-PCR analysis was used to compare mRNA expression levels of CBP and SRC-1, -2, and -3 in fundal myometrium biopsies from 12 women in labor (solid bars) and 12 not in labor (open bars). Data are the mean ± SEM. ($n = 12$). *, $P < 0.01$; **, $P < 0.002$; ***, $P < 0.001$.

in-labor samples as compared with the not-in-labor samples (Fig. 1*A*). No consistent differences in mRNA levels between the in-labor and not-in-labor samples were observed for SRC-1 (Fig. 1*A*). mRNA levels for other coregulators (steroid receptor RNA activator, nuclear receptor corepressor, silencing mediator for retinoid and thyroid hormone receptor, Alien, and RIP-140) (10–12) and proteins that facilitate PR DNA binding (high-mobility group proteins 1 and 2) (13) were variable, with no obvious trend between the in-labor and not-in-labor samples (data not shown).

It has previously been reported that CnX43, OTR, and $PGF_{2\alpha}R$ are expressed in pregnant myometrium and increase with the onset of labor (14–16). To confirm at the molecular level that the myometrial tissues were classified correctly with regard to ''labor'' status, mRNA levels for the gap junction protein, CnX43, the OTR, and $PGF_{2\alpha}R$ were analyzed by using

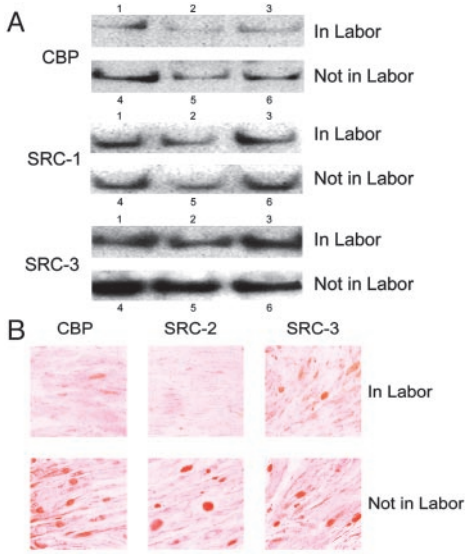

Fig. 2. Nuclear protein levels of CBP and SRC-2 are markedly decreased in myometrial tissues from women in labor, as compared with tissues from women not in labor. (*A*) Levels of expression of CBP and SRC-1 and -3 proteins were analyzed in nuclear fractions isolated from fundal myometrial biopsies from six in-labor and six not-in-labor subjects by immunoblotting (three in-labor and three not-in-labor subjects shown). (*B*) Immunohistochemistry was used to analyze expression and subcellular localization of CBP and SRC-2 and -3 (red reaction product) in fundal myometrial samples obtained at term from six women in labor and six women not in labor. Shown are representative sections from one subject in labor and one not in labor.

semiquantitative RT-PCR. In the 12 samples analyzed and normalized for G3PDH mRNA, CnX43, OTR, and $PGF_{2\alpha}R$ mRNA levels were higher in the in-labor samples (Fig. 1*B*). These findings indicate that the ''labor status'' of the myometrial samples analyzed was assigned correctly, and that a decline in coactivators CBP and SRC-2 and -3 occurs in the human myometrium in labor.

In addition, levels of SRC-1, -2, and -3 and CBP mRNA in fundal myometrium from the 12 subjects studied above and from six additional women in labor and six not in labor were analyzed by real-time RT-PCR (Fig. 1*C*). SRC-1 mRNA levels were similar in the in-labor and not-in-labor samples, but levels of SRC-2 and -3 and CBP mRNA were significantly reduced in fundal myometrium of women in labor (Fig. 1*C*).

Nuclear Levels of SRC-2 and -3 and CBP Proteins Are Decreased in Fundal Myometrium of Women in Labor as Compared with Tissues from Women Not in Labor. Levels of immunoreactive CBP and SRC-1 and -3 were analyzed by immunoblotting of nuclear extracts of fundal myometrium from six in-labor and six not-in-labor subjects. SRC-2 nuclear protein levels were not studied because of the lack of a commercially available antibody suitable for immunoblotting analysis. Nuclear levels of immunoreactive CBP were markedly reduced in all in-labor samples, and a modest but consistent decline in SRC-3 expression was also evident in the in-labor as compared with the not-in-labor samples (Fig. 2*A*). By contrast, SRC-1 protein expression was no different between the in-labor and not-in-labor samples (Fig. 2*A*). The samples shown are representative of the total six in-labor and six not-in-labor samples analyzed.

Immunohistochemistry was used to analyze the cellular localization and expression levels of SRC-2 and -3 and CBP proteins in myometrial tissues obtained from the uterine fundus of six women in labor and six not in labor. As can be seen by the red immunostaining in representative micrographs of fundal myo-

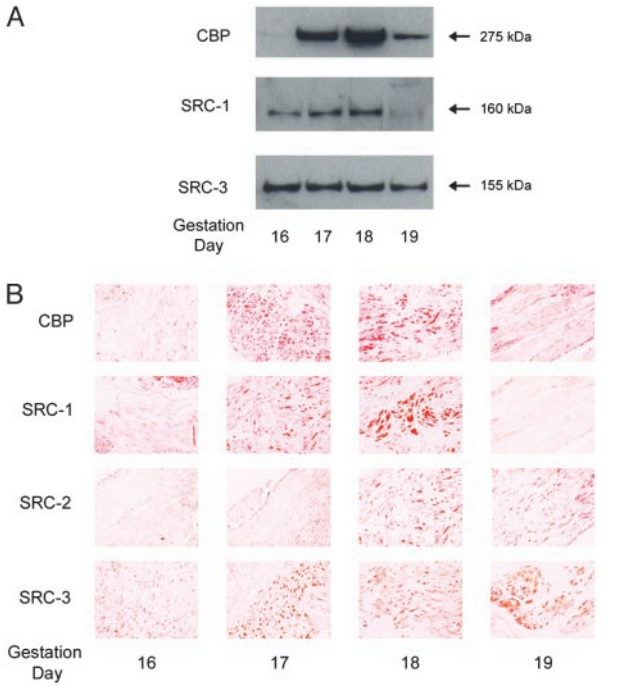

Fig. 3. CBP and SRC-1 and -2 protein levels decline markedly in smooth muscle cell nuclei of the pregnant mouse uterus at term. (*A*) Levels of immunoreactive CBP and SRC-1 and -3 proteins were analyzed by immunoblotting in nuclear fractions from pregnant mouse uteri obtained on days 16–19 (in labor) of gestation. (*B*) Immunohistochemical analysis of CBP and SRC-1 and -2 proteins (red reaction product) in pregnant mouse uteri on days 16–19 (in labor) of gestation.

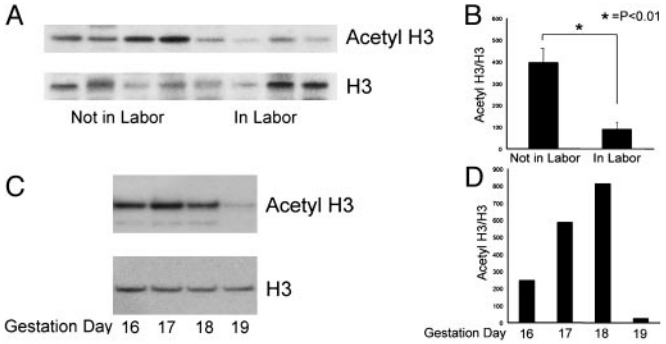

Fig. 4. Levels of acetyl H3 are greatly reduced in fundal myometrium from at-term pregnant women in labor, as compared with tissues from women at term who are not in labor and in pregnant mouse uterus at term. (*A*) Nuclear protein levels of acetyl H3 and total histone H3 were analyzed in nuclear extracts of fundal myometrium from four women in labor and four women not in labor by immunoblotting. (*B*) Densitometric analysis of acetyl H3 relative to total histone H3 levels (mean ± SEM) in four in-labor and four not-in-labor human myometrium samples. (*C*) Nuclear levels of acetylated and total histone H3 in uteri of pregnant mice at days 16–19 (in labor) of gestation were analyzed by immunoblotting. (*D*) Densitometric analysis of acetyl H3 relative to total histone H3 levels (mean ± SEM) in pregnant mouse uteri at days 16–19 (in labor) of gestation.

metrium from one subject in labor and one not in labor, CBP and SRC-2 and -3 were localized primarily to the nuclei of uterine smooth muscle cells. Immunoreactive levels of CBP and SRC-2 were markedly reduced in the in-labor samples. SRC-3 immunoreactivity was modestly decreased in nuclei of myometrial tissues from women in labor. The marked decline in nuclear levels of CBP and SRC-2 proteins in myometrium of women in labor is in accord with the observed changes in mRNA levels (Fig. 1).

SRC-1 and -3 and CBP Nuclear Protein Levels Decline in Mouse Uterus During Labor. In light of these findings in human myometrium at term, it was of interest to analyze temporal changes during the latter part of gestation, and we chose the mouse as a model for these studies. Nuclear protein levels of CBP and SRC-1 and -3 were analyzed in pregnant mouse uterus from gestation days 16–19 (in labor) by immunoblotting. Interestingly, CBP nuclear protein, which was essentially undetectable on gestation day 16, increased dramatically on day 17, increased further on day 18, and then dropped precipitously at term (Fig. 3*A*). SRC-1 nuclear protein levels increased steadily from gestation days 16–18; however, they were barely detectable on day 19 (Fig. 3*A*). SRC-3 nuclear protein levels remained constant from gestation days 16–18 and declined modestly on day 19.

Immunohistochemistry was used to analyze the cellular localization and expression levels of SRC-1, -2, and -3 and CBP proteins in pregnant mouse uterine tissues obtained on gestation days 16, 17, 18, and 19. All coactivators analyzed were localized primarily within nuclei of the smooth muscle cells. Immunoreactive levels of CBP and SRC-1, which were relatively low on gestation day 16, increased dramatically on day 17, increased further on day 18, and then dropped precipitously at term (Fig. 3*B*). Immunoreactive levels of SRC-2, which were undetectable on gestation days 16 and 17, increased dramatically on gestation day 18 and decreased at term. SRC-3 immunoreactivity was clearly detectable on gestation day 16 and remained elevated to term.

Levels of Acetyl Histone H3 Decrease in Human and Mouse Uterus at Term. To assess the functional consequences of the marked decline in expression of coactivators with HAT activity in human myometrium during labor, we analyzed nuclear levels of acetylated and total histone H3 in fundal myometrial samples from four term subjects in labor and four term subjects not in labor by immunoblotting. As can be seen in Fig. 4*A*, the levels of acetyl H3 were reduced in nuclei of myometrial tissues from women in labor, whereas levels of total histone H3 in the same samples were variable between the two groups. When expressed relative to levels of total histone H3 in the same samples, a marked and significant ($P < 0.01$) decrease in the relative levels of acetylated to total histone H3 was observed in the in-labor group (Fig. 4*B*).

In consideration of the gestational changes observed in expression of coactivators with HAT activity in mouse uterine tissues, nuclear protein levels of acetylated and total histone H3 also were analyzed in mouse uterus from gestation days 16–19 by immunoblotting. Acetyl H3 levels were relatively constant from gestation days 16–18. However, on day 19, acetyl H3 levels dropped precipitously (Fig. 4*C*). On the other hand, the levels of total histone H3 remained relatively constant during this period. Similar findings were obtained in uteri from two other series of pregnant mice. When the data from the three series of mice were collectively analyzed for acetyl H3 relative to total histone H3, a dramatic decline in acetyl H3 was evident on day 19 of gestation (Fig. 4*D*).

TSA Administration Increased Uterine Levels of Histone H3 Acetylation and Delayed the Initiation of Parturition in Pregnant Mice. To determine the functional importance of the state of uterine histone acetylation in the initiation of parturition, we investigated the effect of daily TSA injections into pregnant mice, on the timing of parturition, and on the level of histone H3 acetylation in the uterus. Nine pregnant mice were injected daily on days 15–19 of gestation with TSA, and six pregnant mice were injected with DMSO vehicle. Three mice in each group were

Table 2. Administration of the histone deacetylase inhibitor TSA to pregnant mice delays parturition by 24–48 h

	Term delivery	Post-term delivery, 24- to 48-h delay
TSA	–	6/6
DMSO	6/6	–

Pregnant mice were injected i.p. with the histone deacetylase inhibitor TSA or with DMSO vehicle, as control, on days 15–19 of gestation. All six of the TSA-treated mice delivered live pups 24–48 h late, whereas all six of the DMSO-injected mice delivered at term (day 19).

killed on gestation day 19 for analysis of uterine levels of acetylated and total histone H3. Daily administration of TSA to pregnant mice did not result in any apparent toxicity to the mother and did not alter maternal serum progesterone levels as compared with control mice [10.5 ng/ml noninjected ($n = 3$) vs. 7.8 ng/ml TSA treated ($n = 3$)]. Intriguingly, in all TSA-treated mice allowed to deliver spontaneously, parturition was delayed by 24–48 h. All of the vehicle-injected controls delivered normally on gestation day 19 (Table 2). All TSA-treated mice allowed to deliver had viable pups of normal mean litter size (9 ± 3 SEM) as compared with the vehicle injected controls (10 ± 4 SEM).

Effects of TSA administration on steady-state levels of histone H3 acetylation in the maternal uterus at gestation day 19 were analyzed by immunoblotting by using antibodies that recognize total and acetylated forms of histone H3. Levels of acetyl H3 relative to total histone H3 were increased in the uterine nuclear fraction of a TSA-injected mouse as compared with DMSO vehicle-injected and noninjected controls (Fig. 5*A*). Similar results were obtained in uterine homogenates of two additional TSA-injected mice and two additional vehicle and noninjected controls. Densitometric analysis revealed that levels of acetylated relative to total histone H3 were increased 200-fold over DMSO-injected and noninjected controls ($P < 0.003$) (Fig. 5*B*).

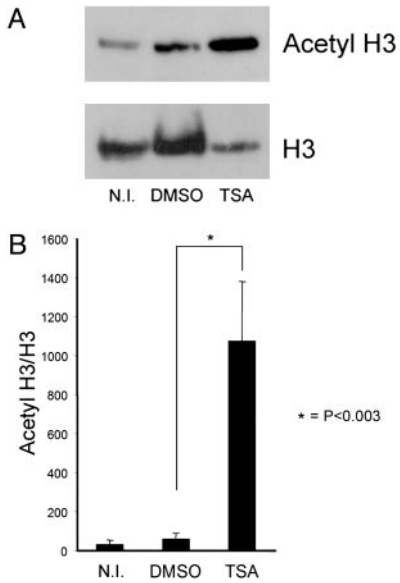

Fig. 5. Histone acetylation is greatly increased in uteri of at-term pregnant mice injected with TSA. (*A*) Levels of acetylated and total histone H3 proteins were analyzed in uterine nuclear fractions prepared from noninjected (N.I.), DMSO-injected controls, and TSA-injected mice on gestation day 19 by immunoblotting. (*B*) Densitometric measurements of acetylated relative to total histone H3 levels (mean ± SEM) for N.I., DMSO-injected controls, and TSA injected animals ($n = 3$).

Discussion

The transition of the pregnant myometrium from a state of relative quiescence to an activated state of contraction is associated with an increase in myometrial OTRs, increased numbers of gap junctions between myometrial cells and cervical softening and ripening (2). In mice, rats, rabbits, and sheep, the onset of labor may be a consequence of a decline in progesterone levels in uterine tissues caused by decreased circulating levels of progesterone and/or increased progesterone catabolism in progesterone target tissues (17). In these species, progesterone supplementation delays the onset of parturition. By contrast, circulating levels of progesterone in pregnant women remain elevated throughout gestation ($\approx 5 \times 10^{-7}$ M). Levels of PR appear to remain elevated in human myometrium throughout gestation (18). Although levels of immunoreactive PR in the uterus have been reported to decline modestly in women in labor (19), such differences are difficult to interpret because of the existence of different PR isoforms. It is therefore likely that in women, alterations in PR transcriptional activity play a far more important role in facilitating transition of the myometrium from a quiescent to an activated state.

In light of the importance of coregulators in transcriptional activity of nuclear receptors, we postulated that changes in expression of key PR coactivators and/or corepressors in myometrium toward the end of gestation may compromise progesterone action and increase the sensitivity of the uterus to contractile stimuli. Members of the SRC family interact with and enhance transcriptional activity of nuclear receptors. SRC-1, which was isolated in a two-hybrid screen by using PR ligand-binding domain as bait, increases transactivation by PR and other nuclear receptors (20). The interaction of SRC-1 with PR, as well as its coactivating function, is progesterone dependent (21). Other members of the SRC family include SRC-2 (22) and -3 (23), which increase transcriptional activity of a variety of nuclear receptors. Although there is significant sequence similarity among SRC family members, there are notable functional differences. For example, whereas SRC-1 and -3 contain intrinsic HAT activity, SRC-2 does not. Interestingly, SRC-3 is unique in that it manifests regulated translocation between the cytoplasm and the nucleus (24). Also, SRC-3 interacts with a wider variety of transcription factors than do SRC-1 and -2 (5). Knock-out mice for SRC-1, -2, and -3 manifest a variety of phenotypes, including partial steroid hormone resistance and compromised reproductive function (25). These studies indicate that, although there is some functional redundancy among family members, the coactivators also serve distinct roles (24, 26, 27).

In the present study, we analyzed expression levels of SRC family members in human myometrial samples obtained from the uterine fundus of women before and after the initiation of labor and in uteri of pregnant mice on days 16–19 (in labor) of gestation. We found that SRC-1 expression at both the mRNA and protein levels was relatively unchanged in human myometrium before and after the onset of labor. By contrast, expression levels of SRC-2 and -3 were markedly reduced in myometrium after the initiation of spontaneous labor. Similarly, SRC-1 and -2 expression was decreased at term in uteri of pregnant mice. SRC-1 and -2 protein levels progressively increased from gestational days 16–18 and declined markedly to almost undetectable levels during labor (day 19). SRC-3 levels remained relatively constant from gestation days 16–18, with a modest decrease in expression during labor.

CBP and its structural homologue p300 serve to coactivate transcription mediated by a number of transcription factors, including steroid receptors (28, 29). CBP/p300 also interact with all members of the SRC family of coactivators (5). In this study,

we observed pronounced decreases in CBP expression at the mRNA and protein levels in human fundal myometrium obtained after the initiation of spontaneous labor. In uteri of pregnant mice, CBP levels increased dramatically toward term with a marked reduction in expression on day 19 (in labor). Other coactivators and corepressors studied in human myometrium at the mRNA level, including steroid receptor RNA activator, nuclear receptor corepressor, and silencing mediator for retinoid and thyroid hormone receptor, Alien and RIP-140 and proteins reported to be required for PR DNA binding (high-mobility group proteins 1 and 2), manifested considerable variability with no obvious trend between the in-labor and not-in-labor samples.

CBP/p300 and SRC family members are considered to be "cointegrators," because they are present in limiting amounts and interact with a number of different transcription factors activated by diverse signaling mechanisms. In light of the limiting cellular levels of these coactivators, our findings suggest that the decline in expression of SRC-2 and -3 and CBP in myometrium of women in labor and of SRC-1 and -2 and CBP in the mouse at term may play a key role in reducing PR functional activity. The marked decline in CBP mRNA levels is likely to have a pronounced inhibitory effect on uterine PR function, because humans (30) and mice (31, 32) heterozygous for targeted deletion of the CBP gene exhibit abnormalities in growth and development. Such findings suggest that a 50% decline in CBP expression levels can have a pronounced effect. It has also recently been demonstrated that a 50% decline in endogenous SRC levels in cultured human breast cells virtually eliminated receptor-induced histone acetylation (33).

As mentioned above, CBP and SRC-1 and -3 possess HAT activity (5). Coactivators possessing HAT activity have been shown to cause postranslational acetylation of the N-terminal tails of nucleosomal core histones, resulting in local opening of chromatin structure (34). This opening of chromatin structure, in turn, allows binding of transcription factors to the promoter, stable assembly of the basal transcription complex with recruitment, and transcription initiation by RNA polymerase II. Biochemical and genetic studies support the concept that hyperacetylation of core histones is a characteristic of gene activation, whereas histone deacetylation represses gene expression (35). In the present study, we also observed a marked decrease in histone H3 acetylation in myometrium obtained from women in labor and in the pregnant mouse uterus at term. These provocative findings suggest that the marked decrease in coactivator levels within the uterus at term in both mice and humans leads to a global decline in histone acetylation, thereby closing chromatin structure and preventing general transcription factor interaction with the promoters of target genes that maintain uterine quiescence.

To analyze the functional importance of this decline in levels of coactivators and of histone acetylation in the pregnant uterus at term, pregnant mice were injected with TSA on days 15–19 of gestation. TSA caused hyperacetylation of uterine histone H3 on gestation day 19 and delayed the initiation of spontaneous labor for 24–48 h. These findings suggest that in species as diverse as humans and mice, a global decline in histone acetylation within the pregnant uterus at term caused by a marked decrease in expression of uterine coactivators with intrinsic HAT activity may serve a key role in the loss of PR function and may lead to the initiation of labor.

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