Distension of the uterus induces HspB1 expression in rat uterine smooth muscle

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White BG, MacPhee DJ. Distension of the uterus induces HspB1 expression in rat uterine smooth muscle. Am J Physiol Regul Integr Comp Physiol 301: R1418–R1426, 2011. First published September 7, 2011; doi:10.1152/ajpregu.00272.2011.—The uterus musculature, or myometrium, demonstrates tremendous plasticity during pregnancy under the influences of the endocrine environment and mechanical stresses. Expression of the small stress protein heat shock protein B1 (HspB1) has been reported to increase dramatically during late pregnancy, a period marked by myometrial hypertrophy caused by fetal growth-induced uterine distension. Thus, using unilaterally pregnant rat models and ovarioctomized nonpregnant rats with uteri containing laminaria tents to induce uterine distension, we examined the effect of uterine distension on myometrial HspB1 expression. In unilaterally pregnant rats, HspB1 mRNA and Ser15 phosphorylated HspB1 (pSer15 HspB1) protein expression were significantly elevated in distended gravid uterine horns at days 19 and 23 (labor) of gestation compared with non gravid horns. Similarly, pSer15 HspB1 protein in situ was only readily detectable in the distended horns compared with the non gravid horns at days 19 and 23; however, pSer15 HspB1 was primarily detectable in situ at day 19 in membrane-associated regions, while it had primarily a cytoplasmic localization in myometrial cells at day 23. HspB1 mRNA and pSer15 HspB1 protein expression were also markedly increased in ovarioctomized nonpregnant rat myometrium distended for 24 h with laminaria tents compared with empty horns. Therefore, uterine distension plays a major role in the stimulation of myometrial HspB1 expression, and increased expression of this small stress protein could be a mechanoadaptive response to the increasing uterine distension that occurs during pregnancy.

Heat shock protein (HSP) B1 (HspB1), previously known as Hsp27, is one of 11 members of the mammalian small HSP (sHSP) family (13, 22). All members of this class have a molecular weight of 15,000–40,000 and share a structural domain in their COOH-terminal halves, named the α-crystallin domain, that spans two putative actin-binding domains (36). Furthermore, these proteins have demonstrated ATP-independent chaperone activity (14, 22).

Phosphorylation of sHSPs is a posttranslational modification that is very important for regulation of sHSP structure and function. For example, HspB1 phosphorylation results in dissociation of large oligomers of HspB1 and loss of chaperoning activity (15, 17). Two phosphorylation sites have been reported for HspB1 in rodents, Ser15 and Ser86 (homologous to Ser82 in humans and Ser98 in hamsters) (8). The latter site is necessary for the dissociation of large sHSP multimers, but at the cellular level, it is not always sufficient (9, 16, 19). Ser15 phosphorylation of HspB1 may produce a conformational change in HspB1 that aids the direct binding of HspB1 with actin microfilaments (19). A number of previous reports have indicated a role for HspB1 in actin polymerization, remodeling, stabilization, and actin-myosin cross-bridge cycling (1, 10, 19, 23, 24, 29). The facilitation of actin formation and actin-myosin interaction by HspB1 has been shown to be essential for the contraction of colonic smooth muscle (2).

Many different cellular stress signals, such as heat shock and oxidative stress, initiate HspB1 expression and phosphoryla-
tion (16, 20), and HspB1 expression is reportedly induced by stretch of epithelial cells and heavy resistance training of skeletal muscle (27, 31). Recently, Chaudhuri and Smith (6) reported that cyclic mechanical stress of airway smooth muscle cells resulted in increased HspB1 phosphorylation, yet the role of mechanical stress on HspB1 expression within the uterine musculature during pregnancy remains unknown.

We previously reported that HspB1 gene expression is highly upregulated in the rat myometrium during late pregnancy and labor (49); this finding parallels the increase in mechanical stress or distension exerted on the myometrium due to growing fetuses. It was also reported that, during late pregnancy, Ser^{15}-phosphorylated HspB1 (pSer^{15} HspB1) had a predominantly membrane-associated localization in situ within cells of the circular and longitudinal muscle layers. We have thus suggested that HspB1 is a potential CAP, but the regulatory mechanism(s) underlying myometrial HspB1 expression during pregnancy remain(s) unknown. Utilizing a unilaterally pregnant rat model, we examined the effect of uterine distension on myometrial HspB1 expression. We hypothesized that this stress would increase HspB1 expression within the uterine musculature.

MATERIALS AND METHODS

Animals and Tissue Collection

Sprague-Dawley rats were maintained in the Animal Care Unit at the Health Sciences Centre (Memorial University of Newfoundland) under standard environmental conditions (12:12-h light-dark cycle), and the Institutional Animal Care Committee approved all experiments under protocols 06-02-DM–08-02-DM. Animals were fed Purina Rat Chow 5012 (Purina Mills, St. Louis, MO) and water ad libitum. Pregnant rat samples were obtained by mating virgin female rats (~220 g) with stud males. Day 1 of pregnancy was determined by the observance of vaginal plugs on the next morning. Under this timing scheme, the rat gestational period was 23 days.

CO₂-induced asphyxiation was used for euthanization of all animals prior to sample collection. Uterine horn samples to be used for immunocytocchemical analysis were fixed in 4% paraformaldehyde in 1× PBS overnight with agitation and then washed in 1× PBS for 24 h. Tissue samples were subjected to a graded ethanol series and xylene penetration and then embedded in paraffin wax. In other cases, excised uterine tissue was placed in ice-cold PBS (pH 7.4) and opened longitudinally for collection of the myometrium, as previously described (49). The endometrial layer was removed from all samples by gentle scraping with a scalpel blade prior to flash-freezing in liquid nitrogen and storage at ~80°C.

Experimental Design

Unilaterally pregnant rat model. Virgin female rats (~220 g) were anesthetized by injection of ketamine (100 mg/kg im; Ketaset, Wyeth Animal Health, Guelph, ON, Canada) and xylazine (20 mg/kg im; Rompun, Bayer, Toronto, ON, Canada) and then subjected to unilateral tubal ligation through bilateral flank incisions (dorsal surface), ~1 cm distal of the spine, as described elsewhere (34, 35). Animals were monitored postoperatively and subsequently allowed to recover for ~5 days before matings were attempted. Samples of gravid and nongravid horns were collected on gestational days 19 and 23 (n = 4).

Nonpregnant ovariec tomized rat models. To investigate the role of uterine distension on HspB1 expression in the absence of any endocrinologic contributions from the fetoplacental units or ovarian steroids, we utilized a nonpregnant ovariec tomized rat model. Prior to experiments using laminaria tents, female rats (~220 g) were anesthetized by injection of ketamine (100 mg/kg im) and xylazine (20 mg/kg im). Rats were then bilaterally ovariec tomized as previously described in detail (34). Animals were allowed to recover for ~5 days postoperatively before laminaria tent insertion.

To generate a dynamic uterine distension over 24 h in the uterine horns of these rats, we used extra-small (2 × 50 mm) laminaria tents (catalog no. 021002, MedGyn, Lombard, IL). These tents consist of dried, sterilized seaweed stems that are hygroscopic and gradually expand over 24 h. Larger versions of these tents can be used clinically as disposable devices for gentle dilation and softening of the cervix. After the rats were anesthetized (see above), bilateral flank incisions (dorsal surface), ~1 cm distal of the spine, were made to expose the uterine horns. Laminaria tents were surgically inserted into the lumen of one of the uterine horns of the ovariec tomized rats (n = 4) through a small distal incision in the horn, which was sutured closed. To control for the presence and effect of an intrauterine device (IUD), polyethylene tubes of the same size and diameter (~2 mm OD; catalog no. 7446, Clay Adams) as preexpanded laminaria tents were also inserted into some uterine horns (n = 4) in place of the laminaria tents. Samples were subsequently collected from empty, polyethylene tube- and laminaria-containing horns 24 h postinsertion.

Northern Blot Analysis

For each experimental model studied, four independent sets of RNA samples (n = 4) were used. TRIzol Reagent (Invitrogen, Carlsbad, CA) was used for RNA extractions, which were completed according to the manufacturer’s instructions. Spectrophotometric analysis of RNA was performed using a Shimadzu Bio-Mini spectrophotometer (Mandel Scientific, Guelph, ON, Canada) to assess RNA purity and concentration before storage at ~80°C.

Northern blot preparation and hybridization were performed as previously described (49). Briefly, RNA (10 μg) was prepared, separated on a 1% agarose-formaldehyde-MOPS gel, transferred to a nylon membrane (Hybond-XL nylon membrane, GE Healthcare, Little Chalfont, Buckinghamshire, UK), and stored at ~20°C. Prehybridization of membranes for 1–2 h at 42°C was followed by hybridization with radioabeled ([³²P]dCTP) probes overnight at 42°C. Probes were produced utilizing a hamster HspB1 cDNA template, following instructions provided in the Megaprime DNA labeling kit (GE Healthcare). Blots were subsequently washed, and multiple exposures of X-ray film were produced to confirm the linearity of the film response. After detection of HspB1 mRNA, Northern blots were stripped and reprobed for 18S rRNA for use as a loading control. The hamster HspB1 cDNA (GenBank accession no. X51747) and rabbit 18S ribosomal cDNA (GenBank accession no. X06778) were kind gifts from Dr. J. Landry (Laval University, Laval, QC, Canada) and Dr. I. Skerjanc (University of Ottawa, Ottawa, ON, Canada), respectively. 18S rRNA is constitutively expressed in rat myometrial cells and has been utilized as a loading control for analysis of myometrial gene expression (33, 39, 49).

Immunoblot Analysis

Immunoblot analysis was performed on at least four independent sets of samples, as previously described in detail (49). Flash-frozen myometrial samples were used to extract total protein in RIPA lysis buffer containing Complete Mini EDTA-free protease inhibitors and PhosSTOP phosphatase inhibitors (Roche Molecular Biochemicals, Laval, QC, Canada). Protein concentrations were determined using the Bradford assay (4). Protein samples (100 μg/lane) were separated under reducing conditions in 12% SDS-polyacrylamide gels (18) and transferred to nitrocellulose membranes (0.45 μm, Pierce membranes, Fisher Scientific, Ottawa, ON, Canada; 0.45 μm, Pall, East Hills, NY).

Membranes were first probed for detection of pSer^{15} HspB1 with a pSer^{15} HspB1-specific rabbit polyclonal antibody (1 μg/ml final concentration; catalog no. PA1-016, Affinity BioReagents, Golden, CO) and then stripped with Restore Western blot stripping solution.
(catalog no. PI-21059, Fisher Scientific, Ottawa, ON, Canada) and reprobed for calponin, which served as a loading control, using a mouse monoclonal antibody provided as an ascites fluid (0.01 μg/ml final concentration, clone hCP, catalog no. C2687, Sigma-Aldrich, Oakville, ON, Canada). The appropriate horseradish peroxidase-conjugated anti-rabbit (catalog no. W4011, Promega, Madison, WI) and anti-mouse (catalog no. W4021, Promega) secondary antibodies were utilized in 1:10, 000 and 1:150,000 dilutions, respectively. The pSer15 HspB1- and calponin-specific antisera recognized protein bands at the predicted 27,000 and 34,000 molecular weights, respectively. Blots were developed with the Pierce SuperSignal West Pico chemiluminescence substrate detection system (catalog no. PI34080, Fisher Scientific) according to the manufacturer’s instructions. Multiple exposures were generated to ensure the linearity of the film exposures.

### Immunocytochemistry

Immunocytochemical analysis was performed on two independent sets of uterine tissue samples for each experimental design and repeated at least twice, as previously described (49). All paraformaldehyde-fixed samples were processed, embedded, and sectioned by the Histology Unit of Memorial University of Newfoundland School of Medicine. Tissue sections (5-μm thick) were adhered to silane-coated slides. Sections were dewaxed and rehydrated for immunocytochemistry, as previously described in detail (49). Briefly, the following steps were conducted at room temperature, unless otherwise stated. Antigen retrieval was accomplished by incubation of tissue sections in 0.125% trypsin in 1× PBS for 15 min. Sections were incubated in blocking solution (5% horse, 1% goat, and 1% fetal bovine serum in PBS) for 30 min and then in pSer15 HspB1-specific rabbit polyclonal antiseraum (5 μg/ml final concentration; catalog no. PA1-018, Affinity BioReagents) or a preimmune serum at the same effective concentrations for 1 h, as previously conducted (49). After they were washed with PBS, sections were incubated for 1 h with an FITC-conjugated anti-rabbit secondary antibody (20 μg/ml final concentration; catalog no. F7512, Sigma-Aldrich, St. Louis, MO). After additional washes with PBS + 0.02% Tween 20, sections were mounted in Vectashield (Vector Laboratories, Burlington, ON, Canada) antifade mounting medium and sealed around the edges with nail polish. Images were collected using a laser scanning confocal microscope (Fluoview 300, Olympus Optical, Melville, NY).

### Data Analysis

Differences in expression levels between samples within each experimental group were determined through densitometric analysis of X-ray films. After acquisition of digital scans of X-ray films with a Hewlett Packard Scanjet G4050 scanner, ImageJ (National Institutes of Health, Frederick, MD) was used to generate raw densitometric values for each sample lane. Densitometric measurements of HspB1 mRNA were normalized to those of 18S rRNA, while measurements of pSer15 HspB1 protein on immunoblots were normalized to those of calponin. Statistical significance between data sets was assessed by a two-tailed t-test using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, www.graphpad.com). P < 0.05 was considered significantly different.

### Results

#### HspB1 Expression in Unilaterally Pregnant Rat Myometrium

**HspB1 mRNA expression.** Northern blot analysis was employed to observe any change in myometrial HspB1 mRNA expression due to uterine distension. Initial investigation was performed using a unilaterally pregnant model consisting of rats at days 19 and 23 (labor) of gestation with one gravid (distended) and one nongravid (empty) horn (n = 4). HspB1 mRNA expression was significantly increased in the gravid horn myometrium from unilaterally pregnant rats on days 19 and 23 compared with samples from the nongravid horn (Fig. 1; P < 0.05). pSer15 HspB1 protein expression and localization in situ. Total myometrial protein extracts (n = 4) were utilized for immunoblot analysis to determine any distension-induced changes in HspB1 protein expression. Investigation with pSer15 HspB1-specific antibodies in unilaterally pregnant rats showed that pSer15 HspB1 expression in the myometrium was significantly induced with uterine distension at days 19 and 23 (Fig. 2; P < 0.05).

To investigate changes in subcellular localization and detection levels of pSer15 HspB1 between gravid and nongravid uterine horns, immunofluorescence analysis was utilized. At day 19, the gravid horn exhibited a high level of detection of pSer15 HspB1 in both muscle layers, and localization was primarily membrane-associated; however, some perinuclear localization was also observed (Fig. 3). In contrast, the nongravid horn exhibited virtually no pSer15 HspB1 immunostaining above the preimmune control. At day 23, pSer15 HspB1 was also highly detectable in both muscle layers of the gravid horn and localized at some membrane-associated regions but was much more detectable in the cytoplasm of myometrial cells (Fig. 4). The nongravid horn exhibited virtually no pSer15 HspB1 immunostaining above the preimmune control.

**HspB1 Expression in Nonpregnant Rat Myometrium**

**HspB1 mRNA expression.** It was possible that the increased myometrial HspB1 expression observed in the gravid horns during pregnancy could be, at least in part, to fetal or placent al paracrine influences absent in the nongravid horns.

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Fig. 1. Heat shock protein B1 (HspB1) mRNA expression in rat myometrium is significantly induced by uterine distension. A and B: representative Northern blot analysis of HspB1 mRNA and 18S rRNA expression in myometrium from nongravid (NG) and gravid (G) uterine horns at days 19 (d19) and 23 (d23) of pregnancy (top) and densitometric analyses of experimental data (bottom). Northern blot analysis was performed with a HspB1-specific hamster cDNA and an 18S-specific rabbit cDNA as templates for radiolabeled probe production. Values are means ± SE from 4 independent experiments. *Significantly different from nongravid (P < 0.05).
Thus, to more precisely confirm the induction of HspB1 expression by uterine distension, in the absence of any underlying influence from the fetoplacental unit or even ovarian steroids, a nonpregnant ovariectomized rat model was also employed for experiments. In this model, laminaria tents were surgically inserted into one of the two uterine horns of ovariectomized female rats for 24 h. The result was the production of uterine distension stress in the laminaria-distended horn compared with the contralateral empty horn (Fig. 5A). In other experiments, a polyethylene tube of similar length and diameter to the preexpanded laminaria tents (i.e., IUD control) was surgically inserted into one of the horns while the contralateral horn was empty.

Northern blot analysis (n = 4) revealed a significant increase in myometrial HspB1 mRNA expression in the laminaria-distended horn of ovariectomized rats compared with empty horns (Fig. 5B; P < 0.05). No significant difference was observed in myometrial HspB1 mRNA expression between uterine horns containing a polyethylene tube and empty uterine horns (Fig. 5C).

pSer15 HspB1 protein expression. After laminaria tent insertion, immunoblot analysis (n = 4) also demonstrated that the expression of pSer15 HspB1 in the myometrium of the distended horn was significantly increased compared with the empty horn (Fig. 6A; P < 0.05). In contrast, there were no significant differences in myometrial pSer15 HspB1 protein expression between uterine horns containing a polyethylene tube, mimicking a preexpanded tent, and empty uterine horns, demonstrating a lack of any IUD effect on protein expression (Fig. 6B).

pSer15 HspB1 localization in situ. Overall, we detected a decrease in pSer15 HspB1 immunolocalization in the nonpregnant compared with the pregnant state. However, there was an increase in pSer15 HspB1 detection in the myometrium of laminaria-distended horns compared with empty horns, particularly in the longitudinal muscle layer (Fig. 7A). Localization of pSer15 HspB1 in the distended horn appeared to be more cytoplasmic and perinuclear than the membrane-associated pattern observed in the unilaterally pregnant rat model at day 19 of gestation. As expected, there were no observable changes in myometrial pSer15 HspB1 detection levels or localization between the uterine horns containing a polyethylene tube and empty uterine horns (Fig. 7B).

DISCUSSION

In the myometrium during pregnancy, uterine distension has been reported to increase the expression and/or posttranslational modification of CAPs, focal adhesion proteins, and signaling kinases, such as connexin43, FAK, and ERKs, respectively (25, 26, 33–35). While investigation has been un-

![Fig. 2. Expression of Ser15-phosphorylated HspB1 [pSer15 HspB1 (pHspB1)] protein in rat myometrium is significantly induced by uterine distension. A and B: representative immunoblot analysis of pSer15 HspB1 and calponin protein expression in myometrium from nongravid and gravid uterine horns on days 19 and 23 of pregnancy (top) and densitometric analyses of experimental data (bottom). Values are means ± SE from 4 independent experiments. *Significantly different from nongravid (P < 0.05).](http://ajpregu.physiology.org/content/301/5/R1421)

![Fig. 3. Immunofluorescence detection of pSer15 HspB1 in rat myometrium was markedly increased by uterine distension at day 19 of pregnancy. A pSer15 HspB1-specific rabbit polyclonal antiserum was used to detect this phosphorylated form of HspB1 in longitudinal (Long) and circular (Circ) muscle layers of distended gravid and nongravid uterine horns. Gravid horns exhibited a high level of detection of pSer15 HspB1 in both muscle layers compared with the nongravid horns, and localization was primarily membrane-associated (arrows). Pre, preimmune serum control. Scale bar, 50 μm.](http://ajpregu.physiology.org/content/301/5/R1421)
The effects of stretch on the larger class of HSPs in smooth muscle, such as HSP70, research on sHSPs is still emerging (3). In particular, there has been limited investigation of the response of HspB1 to mechanical stress. Chaudhuri and Smith (6) showed that cyclic strain of airway smooth muscle cells resulted in significant and rapid increases in expression of phosphorylated HspB1, although the exact phosphorylated form studied was not specified. Thus we took the opportunity to investigate the effects of uterine distension on myometrial HspB1 expression in the unilaterally pregnant rat model, which, at the same time, might advance knowledge and stimulate research with additional types of smooth muscle.

Uterine Distension Induces Expression of HspB1 mRNA and pSer15 HspB1 Protein

We previously reported that HspB1 mRNA expression was significantly elevated in the pregnant rat myometrium at day 19 and then significantly decreased by day 23 (labor), while pSer15 HspB1 expression was significantly increased from day 19 to day 23 compared with earlier points in pregnancy (49). Our present results show that uterine distension induced expression of HspB1 mRNA and pSer15 HspB1 protein levels at days 19 and 23, demonstrating the important role of distension or mechanical stress on the regulation of expression, regardless of the day in late pregnancy. However, the increased expression in the gravid horns was observed when circulating levels of progesterone and 17ß-estradiol were reported to be elevated in the rat at days 19 and 23, respectively (37). As a result, it was still possible that these steroids, as well as other contributions from the fetoplacental unit, could contribute to the observed increases in HspB1 expression during late pregnancy. Examination of HspB1 expression in ovariectomized nonpregnant rats subjected to uterine distension with laminaria tents clearly showed that HspB1 mRNA and pSer15 HspB1 protein expression could be potently induced by uterine distension in vivo in the absence of fetal, placental paracrine, or ovarian steroid influences. However, we cannot exclude the likelihood that a substance(s) could leak out of the laminaria tents or that...
intrauterine and interstitial fluid could be excessively absorbed by the tents and contribute signals, in addition to uterine distension, leading to the observed increase in HspB1 expression.

Despite our findings, hormonal influences cannot be excluded as a regulatory mechanism underlying HspB1 expression during pregnancy. We did observe an overall decrease in detectable pSer15 HspB1 immunolocalization in ovariectomized nonpregnant rats compared with pregnant rats. This could be a result of the loss of the specific hormone environment of late pregnancy. Lastly, marked differences in levels of pSer15 HspB1 immunolocalization were also observed between the two uterine muscle layers following laminaria distension in ovariectomized nonpregnant rats and gravid uterine myometrium. Laminaria tents cannot completely simulate the timing and extent of fetal growth-induced stretch of the uterus during pregnancy, and it is conceivable that, as a result, uterine muscle layer-specific responses to the distension produced by the laminaria tents are different from those produced by growing fetuses.

It was previously reported that the rat HspB1 gene promoter contains overlapping specificity protein 1 and activator protein 2 transcription factor binding sites and a heat shock element (32). Thus it is possible that specificity protein 1, activator protein 2, and/or heat shock factor (HSF) 1 or 2 could induce transcription of HspB1 mRNA in response to uterine distension. Importantly, HSF1 has been found to be necessary for the regulation of HspB1 expression.

Fig. 7. Immunofluorescence detection of pSer15 HspB1 in ovariectomized nonpregnant rat myometrium was increased in laminaria-distended uterine horns. Laminaria tents (A) or polyethylene tubes of the same size and diameter as preexpanded laminaria tents (B) were surgically inserted into one of the uterine horns of nonpregnant ovariectomized rats. Samples were subsequently collected from empty (E), polyethylene tube-containing (P), and laminaria-containing (Lam) horns 24 h postinsertion. A: increase in pSer15 HspB1 detection in the myometrium of laminaria-distended horns compared with the myometrium of empty horns, particularly in the longitudinal muscle layer. B: pSer15 HspB1 detection was very low within circular and longitudinal muscle layers, and no changes were noted in the muscle layers from empty uterine horns compared with horns containing polyethylene tubes (plastic). Pre, preimmune serum control; C, circular muscle layer; L, longitudinal muscle layer. Scale bar, 50 μm.
stress-induced HspB1 upregulation in mouse embryonic fibroblasts (33), and Xu and colleagues (52) reported that HSF1 activation was induced by cyclic mechanical stress in vascular smooth muscle cells. However, the expression of HSF1 has yet to be determined in pregnant rat myometrium.

HspB1 is a substrate for phosphorylation by the p38 MAPK pathway, utilizing members of the MAPK-activated protein kinase family, although phosphorylation of Ser15 and Ser26 does not appear to occur in any specific obligate order (17, 21, 46). Static stretch of primary cultures of rat myometrial smooth muscle cells in vitro strongly induces activated p38 MAPK expression, and uterine distension also led to increased phosphorylated p38 MAPK expression, beginning at about day 19 of pregnancy in the rat myometrium and reaching significantly elevated levels by day 22 (33). Thus the increased expression of pSer15 HspB1 protein at days 19 and 23 may be due, at least in part, to some contributions from the p38 MAPK signaling pathway.

HspB1 and the Actin Cytoskeleton

The dynamic modulation of actin microfilament formation likely plays a large role in smooth muscle contraction (48). For example, Shaw et al. (38) reported that agonist-induced constriction of nonpregnant rat myometrium was reduced by inhibition of actin polymerization with cytochalasin D. All three actin isoforms (α, β, γ) are expressed in rat myometrium, but only γ-actin appears to undergo increased expression and changes in localization as full-term approaches (40). Analysis of full-term pregnant mouse uterus demonstrated that actin microfilaments were densely packed and ran parallel to the longitudinal axis of uterine smooth muscle cells (48). Smooth muscle dense plaques, or focal adhesions, are sites on the plasma membrane where clusters of integrins, signaling molecules, and adapters, such as FAK and vinculin, can provide a structural link between the ECM and the actin cytoskeleton. FAK activation is highly induced in rat myometrium during late pregnancy, where focal adhesion signaling may be necessary to remodel cell-ECM adhesion during myometrial hypertrophy (28). Using a stereological approach, Shynlova et al. (44) recently demonstrated that sizes of uterine smooth muscle cells were significantly increased at late pregnancy (day 19) and were not significantly decreased until postpartum. Myometrial hypertrophy was also significantly higher in gravid than nongravid uterine horns at day 19 and at full-term in unilaterally pregnant rats. Therefore, in this study, the increased expression of pSer15 HspB1 protein levels in distended uterine horns at day 19 and at labor and the immunolocalization of the protein to membrane-associated regions indicate that pSer15 HspB1 could be part of a mechanoadaptive response to regulate actin cytoskeleton dynamics at focal adhesion sites and support hypertrophy-induced focal adhesion reorganization during late pregnancy. Evidence to support this possibility can be found in recent reports. During et al. (7) demonstrated that HspB1 is a γ-actin-sequestering protein and that HspB1 phosphorylation enhances actin filament assembly. Jia et al. (12) also showed, with mass spectrometry analysis, that phosphorylated HspB1 coimmunoprecipitated with members of the actin regulatory complex Aρ2/3.

Since our results demonstrate significantly induced pSer15 HspB1 levels at day 23 (labor) upon uterine distension and immunolocalization of pSer15 HspB1 to the cell cytoplasm at this time, we cannot rule out a role for this protein in myometrial contraction. Increased myometrial stretch, as a result of multiple gestation pregnancies for instance, has been suggested as one factor that could lead to the increased incidence of premature uterine contractions (5). The facilitation of actin formation and actin-myosin interaction by HspB1 has been shown to be essential for the contraction of colonic smooth muscle (2), and it is postulated that the mechanism involves phosphorylated HspB1-mediated modulation of caldesmon association with tropomyosin, a thin-filament protein critical for actin-myosin interaction (2, 45). Taken with the recent findings of stretch inducing phosphorylation of caldesmon in the myometrium (25), a specific association or relationship of HspB1, caldesmon, and stretch in uterine smooth muscle contraction may exist.

Perspectives and Significance

Cellular stress signals, such as heat shock and oxidative stress, are known to initiate HspB1 expression and phosphorylation (16, 20). The results of this investigation provide novel insights into the mechanism of regulation of HspB1 expression in uterine smooth muscle during pregnancy and demonstrate that mechanical forces have a major role in regulating HspB1 expression. The data also add to a limited literature on the importance of mechanical stress in inducing HspB1 mRNA and phosphorylated protein expression in smooth muscle per se. The increasing identification of proteins, such as HspB1, that are highly expressed and regulated by uterine distension in the myometrium during late pregnancy also provides new potential targets for the development of effective therapeutic strategies to mitigate premature myometrial contraction. Further identification of the exact mechanism(s) of action of HspB1 on the myometrial cell actin cytoskeleton and on contraction will require a molecular approach at the cellular level.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

B.G.W. performed experiments; B.G.W. and D.J.M. analyzed data; B.G.W. and D.J.M. interpreted results of experiments; B.G.W. and D.J.M. prepared figures; B.G.W. and D.J.M. drafted the manuscript; B.G.W. and D.J.M. approved the final version of the manuscript; D.J.M. was responsible for conception and design of the research; D.J.M. edited and revised the manuscript.

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