Hypotonic Stress Increases Cyclooxygenase-2 Expression and Prostaglandin Release from Amnion-derived WISH Cells*

David W. Lundgren‡‡‡, Robert M. Moore‡, Patricia L. Collins¶ and John J. Moore†††‡‡‡

From the Labor Focus Research Group, Departments of ‡Pediatrics, ¶Obstetrics and Gynecology, and ‡Biochemistry, Case Western Reserve University School of Medicine, MetroHealth Medical Center, Cleveland, Ohio 44109

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This report examines the effect of cell volume expansion on cyclooxygenase-2 (COX-2) mRNA expression, COX-2 protein expression, and prostaglandin E₂ release from human amnion-derived WISH cells. Earle's balanced salts solution (EBSS) with limited NaCl concentration was utilized as the hypotonic medium. COX-2 mRNA was elevated 6-fold in cells incubated for 1 h in hypotonic EBSS. COX-2 mRNA expression was not increased when raffinose or sucrose were used to reconstitute low NaCl. Actinomycin D blocked COX-2 mRNA accumulation as the induction medium. COX-2 mRNA and protein concentrations increased as a function of decreasing media osmolality and incubation time in hypotonic EBSS. Hypotonic EBSS induced a 3-fold increase in prostaglandin E₂ release. WISH cells transiently transfected with a luciferase expression vector driven by the human COX-2 promoter for the COX-2 gene show a 3-fold increase in luciferase activity when incubated in hypotonic EBSS. COX-2 mRNA levels in primary human amnion cells were also increased by hypotonic stress. This study suggests that amnion cell COX-2 gene expression is regulated by cell volume expansion and/or increased plasma membrane tension.

Prostaglandins have a central role in regulating human parturition. Prostaglandin E₂ (PGE₂), the primary prostaglandin produced by fetal membranes during labor, may be directly involved in the initiation and maintenance of uterine contractions (1, 2). Untimely increases in prostaglandin biosynthesis early in gestation may be responsible for inducing preterm labor in some individuals (3). Although many hormones, growth factors, and cytokines have been reported to increase or decrease the synthesis and/or release of PGE₂ in fetal tissues, the physiological factor(s) that up-regulates prostaglandin biosynthesis during parturition has not been identified.

Metabolism of arachidonic acid to prostaglandin H₂ is a key and rate-limiting step in prostaglandin biosynthesis. The reaction is catalyzed by cyclooxygenase. Two isoforms of cyclooxygenase have been identified, designated as COX-1 and COX-2. Both enzymes have been characterized in considerable detail (for review, see Refs. 4–8). The genes for COX-1 and COX-2 are encoded on different chromosomes and have been sequenced. The gene for COX-1 is constitutively expressed, present in most tissues at low to nondeductible levels, and is generally considered to have "housekeeping" functions. In contrast, the gene for COX-2 has been characterized as an immediate early response gene. A wide range of mitogens, hormones, cytokines, and endotoxins increase the rates of COX-2 gene transcription and prostaglandin biosynthesis (4–8). COX-2 mRNA and COX-2 protein have been reported to increase near the onset of labor. A major site of PGE₂ synthesis during labor occurs in the amnion (9, 10). COX-2 protein concentrations (11) and mRNA levels (10) are higher in amnion from women in labor versus patients not in labor. The underlying mechanism(s) leading to enhanced COX-2 mRNA expression in amnion during labor remains to be identified.

Fetal membranes clearly undergo mechanical stretching as a result of several processes during gestation, including fetal growth, increased amniotic fluid volume, and labor. Increased membrane tension of fetal cells may also occur as a direct result of cell volume expansion; human amniotic fluid osmolality decreases as a function of advancing gestational age (12), raising the possibility that increased amnion cell plasma membrane tension might occur, in part, by changes in cell volume. Furthermore, as initially observed by Danforth and Hull (13) and as summarized by Alger and Pupkin (14), little if any amnion cell mitotic activity is observed in the latter part of gestation, and therefore, to accommodate the growing fetus, existing amnion cells must increase their size by stretching and hypertrophy. It has been recognized for some time that mechanical stretching of human cultured amnion cells increases the release of PGE₂ (15). Although the biochemical mechanism by which mechanical stretching increases prostaglandin release from these tissues is largely unknown, it was recently reported that mechanical stretching of cultured rat glomerular mesangial cells induces a number of immediate early response genes including the gene for COX-2 (16). Based on this knowledge, we hypothesize that an increase in the volume of amnion cells and the resulting increase in plasma membrane tension induce COX-2 gene transcription and promote PGE₂ release from fetal tissue. This study provides evidence that an increase in cell volume up-regulates COX-2 mRNA expression and elevates prostaglandin biosynthesis in amnion cells.

**MATERIALS AND METHODS**

**Culture and Treatment of WISH Cells—**Amnion-derived WISH cells were obtained from the American Type Culture Collection (Rockville, MD) at passage 167. Tissue culture medium, designated as DF10F, consists of a combination of Dulbecco's modified Eagle's medium plus Ham's F-10 (50/50) supplemented with sodium bicarbonate (14 mM), 15 mM Hepes buffer (pH 7.4) plus 10% fetal calf serum. DF10F contained the following antibiotics: penicillin (2 × 10⁵ IU/liter), streptomycin (2 × 10⁻⁵ M), and ampicillin (25 mg/liter). For each experiment, confluent WISH cell stock cultures were subcultured from a Falcon T75 tissue
culture flask into 60 × 15-mm Falcon culture dishes (1.5 × 106 cells/dish) containing 5.0 ml of DF10F. Unless otherwise stated, cultures were incubated at 37 °C in humidified air containing 5% CO2, fed on day 3 with DF10F, and used for experiments on day 5. Under these growth conditions, WISH cells reach confluence between the third and fourth days.

Cells cultured for 5 days in DF10F were washed with 2.0 ml of Earle's balanced salts solution (EBSS). Induction medium (5.0 ml, equilibrated to 37 °C), as defined under "Results," was added to cultures that were then incubated at 37 °C in humidified air containing 5% CO2. As described below, cells were then harvested for preparation of mRNA or COX-2 protein. In some experiments, spent incubation media were removed, frozen at −70 °C, and, as described below, analyzed for prostaglandin concentrations.

Preparation and Culture of Primary Human Amnion Cells—Human amnion cells were cultured using a modification of the method of Okita et al. (17). Placentae and associated membranes were obtained from women undergoing repeat cesarean section. All tissue manipulations were performed using aseptic technique and sterile (0.2 μm filtered) solutions. Amnion was stripped from choriodecidua and washed successively in 200-ml changes of ice-cold Ca2+- and Mg2+-free phosphate-buffered saline containing gentamicin (50 μg/ml) until clear of blood. Tissue was minced in 50 ml of TEP buffer (0.05% trypsin (SIGMA, Type II), 0.02% EDTA in phosphate-buffered saline) and then incubated at 37 °C on a shaking water bath. Tissue was minced in 20 ml of Terasman buffer (Tris plus 0.1 mM EDTA, pH 7.4), and then baked at 80 °C for 1.5 h. As previously demonstrated (19), the transfer of RNA from gel to filter was complete.

RNA dot blots were performed with a VacuDot-VS manifold (Amer-ican Bionetics). Nytran filters were pretreated with 6 × SSC prior to applying samples. RNA solutions were diluted with 4 volumes of denaturing solution and then heated at 65 °C for 15 min. Solutions of denatured RNA were chilled on ice, diluted with 1.5 volumes of 6 × SSC, and then loaded into manifold wells. The samples were allowed to drain completely for 30 min, and then vacuum-washed twice with 400 μl of 6 × SSC. Filters were air-dried and then heated at 80 °C for 1.5 h. Filters from both Northern blots and dot blots were then incubated for 2 h at 42 °C in prehybridization buffer as described previously (20).

The 32P-labeled probe for COX-2 mRNA was generated with a random primer labeling kit (Amersham International plc, Buckinghamshire, United Kingdom). The substrate was the 1.2-kilobase COX-2 cDNA insert purified from plasmid pCdNA COX-2. After prehybridization, the 32P-labeled COX-2 cDNA probe was denatured and then added directly to the prehybridization buffer (1.0 × 106 dpm/ml). Filters were incubated overnight at 42 °C. They were then incubated twice in 6 × SSC buffer plus 0.5% SDS and once in 1 × SSC plus 0.1% SDS for 15 min at room temperature. The final wash was carried out in 1 × SSC plus 0.1% SDS for 30 min at 56 °C.

Autoradiography and COX-2 mRNA Analysis—Nytran filters from Northern blots and dot blots were exposed to Kodak X-Omat RP film at −70 °C employing Loading Plus intensifying screens. Autoradiographs of 32P-hybridized RNA dot blots were scanned with a Microtek MSF-3000 frame grabber (Microtek, Palo Alto, CA) connected to a Macintosh IIsi computer. The image generated by the Microtek grayscale scanner was captured by Image Studio software (L extraset, Parmus, NJ) and then analyzed for intensity of grains relative to background by Scan Analysis software (Biosoft, Milltown, NJ). Quantitation of image intensities on film was carried out at less than maximal densities as described previously (20). When an image from a given dot blot was determined to be overexposed, autoradiography was repeated employing a shorter exposure time.

Western Blot Analysis—Monolayers of WISH cells were suspended in 0.5 ml of lysis buffer (20 mM Hepes (pH 7.2), 120 mM NaCl, 1% Triton X-100, 5 μg/ml aprotinin, 10 μg/ml antipain, 5 μg/ml leupeptin, 5 μg/ml pepstatin A, and 0.1 mM phenylmethylsulfonyl fluoride) and frozen at −70 °C. Cell homogenates were thawed on ice and maintained at 4 °C throughout manipulation. Homogenates were sonicated for 10 s (Heat Systems-Ultrasonics, Inc., model W225R with microtip), setting 4, 100% duty cycle, and then cleared by centrifugation at 12,000 × g for 5 min at 4 °C. Aliquots (25 μl) of homogenates were subjected to polyacrylamide gel electrophoresis on 8% gels. Gels were equilibrated in 20 mM Tris, 190 mM glycine, 20% methanol, pH 8.5 overnight at 10 °C. Resolved proteins were stained on transfer paper employing a 10% (He-Systems-Ultrasonics, Inc.) using 10% of the secreted alkaline phosphatase genetic reporter system (pSEAP-Control vector; CLON-TECH, Palo Alto, CA), and 1.0 ml of DF10F lacking serum and antibiotics. Cells were incubated for 16 h in the initial transfection medium, after which an additional 1.0 ml of DF10F containing 10% fetal calf serum was added to each plate. The transfection medium was removed after 24 h, and cells were then incubated for an additional 48 h in DF10F plus 10% fetal calf serum minus antibiotics. Induction media, as defined under "Results," were added to plates, and cells were incubated for designated times at 37 °C in 5% CO2. For alkaline phosphatase activity, cells were pelleted from the precipitation solution by centrifugation for 15 min at 5 °C, the supernatants were removed, and cells were liposome-mediated transfected employing the following: 50 μg of LipofectAMINE (Life Technologies, Inc.), 10 μg of the COX-2 pXP1 luciferase reporter vector, 2 μg of the secreted alkaline phosphatase genetic reporter system (pSEAP-Control vector; CLON-TECH, Palo Alto, CA), and 1.0 ml of DF10F lacking serum and antibiotics. Cells were incubated for 16 h in the initial transfection medium, after which an additional 1.0 ml of DF10F containing 10% fetal calf serum was added to each plate. The transfection medium was removed after 24 h, and cells were then incubated for an additional 48 h in DF10F plus 10% fetal calf serum minus antibiotics. Induction media, as defined under "Results," were added to plates, and cells were incubated for designated times at 37 °C in 5% CO2. For alkaline phosphatase assays, induction media (500 μl) were removed from each plate and then centrifuged at 12,000 × g for 5 min at 10 °C. The supernatants were removed, and cells were washed with 3 × 100 μl of lysis buffer plus 10 μg/ml leupeptin, 5 μg/ml pepstatin A, and 0.1 mM phenylmethylsulfonyl fluoride twice, and then lysed by sonication using a commercial system (CLONTECH) and a 96-well format. Samples were exposed to x-ray film for various time periods to assure that detection was within the linear range of the film. For luciferase activity, cells were scraped into 200 μl of luciferase assay lysis buffer (Promega) and then sonicated (Heat Systems-ultrasonics Inc.) for 5 s on ice at 50% output. Samples were centrifuged at 12,000 × g for 5 min at 10 °C.
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**Results**

**Hypotonic Stress and COX-2 mRNA Expression**—COX-2 mRNA expression was initially examined in WISH cells incubated for 1 h in hypotonic *versus* isotonic EBSS. Relative to the concentration of COX-2 mRNA from cells incubated in isotonic EBSS, COX-2 mRNA expression was markedly elevated in hypotonically stressed cells (Fig. 1, lanes 2, 5, and 8 *versus* lanes 3, 6, and 9, respectively). The 32P-labeled COX-2 cDNA probe hybridized to three mRNA species of approximately 5.8, 4.8, and 3.4 kb. All three mRNA species were elevated in hypotonically stressed cells. In contrast to COX-2 mRNA expression, no change in the level of COX-1 mRNA was detectable by Northern analysis employing a cDNA-specific probe for COX-1 (not shown).

The relative concentration of basal level COX-2 mRNA from untreated cells (i.e., cells harvested directly from spent tissue culture growth medium, DF10F) was also determined (Fig. 1, lanes 1, 4, and 7). Basal COX-2 mRNA levels were consistently less than levels from WISH cells incubated in isotonic EBSS (compare lanes 1, 4, and 7 to lanes 2, 5, and 8, respectively). During the course of these studies it was determined that, due to evaporation, the osmolarity of fresh DF10F tissue culture medium increased from 290–300 mosmol/liter to 315–320 mosmol/liter after 48 h of incubation. The osmolarity of standard isotonic EBSS routinely assayed between 280 and 290 mosmol/liter and, therefore, cells cultured for 48 h in DF10F medium and then incubated in isotonic EBSS were subjected to a decrease in osmolarity of between 25 and 40 mosmol/liter.

Thus, shifting cells from 48-h spent tissue culture medium to standard isotonic EBSS is sufficient to increase COX-2 mRNA expression. As shown in Fig. 3, very little if any difference in COX-2 mRNA concentrations from untreated cells *versus* isotonic EBSS-treated cells were observed when the osmolarity of EBSS was increased by 30 mosmol/liter with 30 mM raffinose.

As determined by autoradiography and grayscale scanning of RNA dot blots hybridized to the 32P-labeled COX-2 cDNA probe, the relative concentration of COX-2 mRNA from WISH cells incubated for 1 h in hypotonic EBSS was elevated approximately 6-fold above the COX-2 mRNA concentration from cells incubated in isotonic EBSS (Table I). Numerous reports have shown that EGF is a potent inducer of COX-2 gene transcription in a variety of biological systems, including human amnion cells. As a positive control in this study, and to compare the relative potency of hypotonic stress to a recognized inducer of COX-2 gene expression, the effect of EGF was also determined on WISH COX-2 mRNA expression. The relative concentration of COX-2 mRNA in cells incubated in isotonic EBSS plus EGF was elevated approximately 10-fold higher than that in cells incubated in isotonic EBSS (Table I).

**Effects of Increased Cell Volume versus Reduced NaCl on COX-2 mRNA Expression**—Hypotonic media in Fig. 1 and Table I were formulated by decreasing the amount of NaCl in EBSS. A previous study, employing rat papillary collecting tubule cells, reported that PGE2 was induced in this system by a reduction in extracellular Na+ (23). To determine if elevated WISH cell COX-2 mRNA expression is related to cell volume expansion or, alternatively, related to a reduction in extracellular Na+ and/or Cl−, cells were incubated in isotonic EBSS in which 116 mosM NaCl was reduced to 58 mosM (H), and isotonic EBSS plus 20 ng/ml EGF (I + EGF). RNA (5.0 µg) was dot-blotted and probed for COX-2 mRNA as described under “Materials and Methods.” An autoradiogram of the dot blot was then analyzed by grayscale scanning as described under “Materials and Methods.” Values are the mean ± S.D. of three replicate cultures.

**Table I**

| Media          | Relative COX-2 mRNA levels |
|---------------|----------------------------|
| I             | 29.6 ± 7.0                 |
| H             | 187.0 ± 9.4                |
| I + EGF       | 290.4 ± 4.8                |

**Effects of Actinomycin D and Cycloheximide on COX-2 mRNA Expression**—The effects of actinomycin D and cycloheximide on elevated COX-2 mRNA resulting from hypotonic stress were determined by dot blot analysis (Fig. 3). The addition of actinomycin D to hypotonic EBSS completely suppressed COX-2 mRNA concentrations to levels observed for untreated cells and cells incubated in isotonic EBSS. In contrast, the relative concentration of COX-2 mRNA in hypotoni-
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FIG. 2. Effect of increased cell volume versus decreased NaCl on COX-2 mRNA expression (A) and PGE₂ release (B). A, relative COX-2 mRNA concentrations in cells incubated for 1 h in the following media: standard isotonic EBSS (I); EBSS made hypotonic by reducing the concentration of NaCl from 116 mM to 58 mM (H); isotonic EBSS containing 116 mM raffinose in place of 58 mM NaCl (S); and isotonic EBSS (R). Total RNA (5 μg) was dot-blotted and probed for COX-2 mRNA as described under “Materials and Methods.” An autoradiogram of the dot blot was then analyzed by grayscale scanning as described under “Materials and Methods.” B, PGE₂ concentrations released into media from cells described in A. PGE₂ concentrations were determined by radioimmunoassay as described under “Materials and Methods.” Each treatment represents the mean ± S.D. of three replicate cultures.

COX-2 protein expression was delayed for at least 2 h, increasing between 3 and 6 h, and remained elevated for several hours prior to returning to basal levels between 6 and 18 h (Fig. 5).

Response of COX-2 Promoter-Luciferase Construct to Hypotonic Stress—To ascertain the effect of hypotonic stress on COX-2 gene expression, WISH cells were transiently transfected with a human COX-2 promoter-luciferase construct. This reporter construct contains an 899-base pair fragment of the human promoter for the COX-2 gene, progressing 5′ from the ATG start site, and coupled to the Pxp1 luciferase expression vector (22). Relative to cells incubated in isotonic EBSS, cells incubated in hypotonic EBSS were characterized by approximately a 3-fold increase in luciferase activity (Fig. 6). The level of luciferase induction observed for cells incubated in isotonic EBSS plus EGF was approximately 6-fold above cells incubated in isotonic EBSS (Fig. 6).

Hypotonic Stress and COX-2 Expression in Primary Human Amnion Cells—Because WISH cells are immortal and therefore transformed, it cannot be assumed that the activation of a given signal transduction pathway in this cell line is applicable to normal human amnion cells. Previous studies of primary human amnion cells in culture have show that they retain many of the biochemical and morphological properties of amnion cells studied immediately after parturition (17). For this reason the effect of cell volume expansion on COX-2 mRNA expression was determined on primary human amnion cells prepared from placentae of women undergoing repeat cesarean section (Fig. 7). Relative to cells incubated for 1 h in isotonic EBSS, COX-2 mRNA was markedly elevated in cells incubated in hypotonic EBSS. The increased concentration of COX-2 mRNA in primary human amnion cells resulting from hypotonic stress was approximately 50% of that induced by EGF (Fig. 7), a pattern that is consistent with WISH cell COX-2

FIG. 3. Effects of actinomycin D and cycloheximide on COX-2 mRNA expression induced by hypotonic stress. Treated cells were preincubated for 30 min in either EBSS or, as specified, in EBSS plus inhibitor. Untreated cells (U) were harvested directly from plates without prior treatment (basal COX-2 mRNA level). At time 0, treated cells were incubated for 1 h in the following media: isotonic EBSS (I); hypotonic EBSS (H); hypotonic EBSS plus actinomycin D (0.8 μM) (H+A); and hypotonic EBSS plus cycloheximide (4.5 μM) (H+C). Hypotonic medium was made by reducing the NaCl concentration in EBSS from 116 mM to 58 mM. To compensate for the difference in osmolarity between 48-h spent medium and isotonic EBSS, 30 mM raffinose was added to all induction media. Cells were harvested, and the relative concentrations of COX-2 mRNA were determined by dot blot analysis as described under “Materials and Methods.” Values represent the mean ± S.D. of three replicate cultures.
DISCUSSION

The central finding in this study is that hypotonic stress increases prostaglandin biosynthesis in amnion cells. Results in this report demonstrate that COX-2 mRNA expression, the amount of COX-2 protein, and the release of PGE2 from WISH cells originally derived from amnion are all enhanced by cell volume expansion.

The expression of three apparent COX-2 mRNA species in hypotonically stressed WISH cells was an unexpected finding (Fig. 1). Most earlier studies of COX-2 mRNA expression, employing a wide variety of biological systems, reported the presence of a single COX-2 mRNA species ranging in size from 4.2 to 4.8 kb (4–8). Recently, in an extensive study of COX-2 mRNA isoforms induced by cytokines in human lung and kidney cells (25), three isoforms of COX-2 mRNA were detected: two major isoforms of 4.6 and 2.8 kb and a minor species of 4.1 kb. In the same study it was shown that the different isoforms are a product of alternative polyadenylation and, surprisingly, that the 2.8-kb COX-2 mRNA had a significantly longer half-life than the 4.6-kb species. In the current study, as determined by Northern analysis, two major COX-2 bands of 5.8 and 3.4 kb were detected, with a less pronounced band of 4.8 kb (Fig. 1). In a previous communication, interleukin-18 induced a single COX-2 mRNA species of 5.5 kb in primary human amnion cells from term placenta (26). The physiological significance of several COX-2 mRNA bands in WISH cells is unclear. To our knowledge, the large 5.5–5.8-kb species of COX-2 mRNA has...
only been detected in WISH cells and primary amnion cells. It remains to be established if the various COX-2 mRNA species in hypotonically stressed WISH cells are a result of alternative polyadenylation or alternative splicing. Knowledge of potential changes in the turnover rate of COX-2 mRNA, as a result of increased cell volume and/or increased membrane tension, may provide important information concerning the regulation of prostaglandin metabolism in amnion cells.

The observation that hypotonic stress induces luciferase in WISH cells transfected with a human COX-2 promoter-luciferase vector (Fig. 6), coupled with the knowledge that actinomycin D prevents hypotonic stress from increasing COX-2 mRNA levels (Fig. 3), suggests that changes in prostaglandin biosynthesis resulting from cell volume expansion are due to an increase in the rate of COX-2 gene transcription. Hypotonic stress induced a large increase in COX-2 mRNA levels within 30 min after treatment (Fig. 4B). The increase in COX-2 mRNA associated with cell volume expansion was not suppressed by cycloheximide (Fig. 3). Collectively, results in this study demonstrate that the gene for COX-2 acts as an immediate early response gene when stimulated by cell volume expansion. This suggestion is consistent with previous reports, employing a variety of biological systems, that classified the gene for COX-2 as an immediate early response gene following treatment with different growth factors, mitogens, and cytokines (4–8).

The signal transduction pathway(s) that is activated in higher eukaryotes by cell volume expansion and leads to increased gene expression has not been identified. Nevertheless, recent studies employing yeast mutants have demonstrated that hypotonic stress activates the PKC1 pathway (27), one of four recognized yeast mitogen-activated protein kinase pathways (MAP kinase pathway). These studies demonstrated that a functional PKC1 pathway is required for the survival of yeast in a hypotonic environment. In contrast to hypotonic stress, hypertonic stress activates a different MAP kinase pathway in yeast, designated as the HOG pathway. This pathway is required for survival of yeast in a hypertonic environment. Interestingly, hypertonic stress of Chinese hamster ovary cells produces a marked increase in Jnk 1 (28), an enzyme similar to the yeast protein kinase HOG1. Furthermore, Jnk 1 was able to rescue yeast mutants lacking functional HOG 1 from hypertonic shock. Since yeast and mammalian systems appear to have similar MAP kinase pathways and these pathways may be important in cell volume regulation (27–29), it is possible that a PKC1-like pathway exists in amnion cells and that this pathway is activated by cell volume expansion. The end product of this pathway may, in turn, modify a factor that increases the rate of COX-2 gene transcription.

Cell volume expansion and membrane stretch have been shown to modify the expression of a wide variety of genes in a number of diverse biology systems. Previous studies have reported that increased cell volume resulting from hypotonic stress modifies the concentration or activity of a number of factors that impact, directly or indirectly, on the transcription rate of specific genes (for a review, see Ref. 30) including, for example, intracellular calcium, cyclic AMP, tyrosine kinases, inositol 1,4,5-trisphosphate, and protein kinase C. Although the mechanism by which cell volume expansion increases amnion cell COX-2 gene transcription remains to be characterized, the promoter region for the human COX-2 gene has been shown to contain a wide variety of potential regulatory elements, including CRE, NF-κB, Sp1, and AP2 sites (22). Recently, what appears to be a novel putative cis-element in the promoter region of rabbit aldose reductase has been shown to be necessary for increased expression of this gene during hypertonic stress (31). Perhaps a novel regulatory element, yet to be identified, is essential for regulating gene expression as a function of cell volume expansion and/or increased membrane tension.

It has been recognized for more than 35 years that prostaglandins provoke uterine contractions (for a historical review of prostaglandins and uterine contraction, see Ref. 32). Within the past 10 years, a vast array of studies have documented that prostaglandins have an important role in human parturition. It is well established that amnion is a major site of prostaglandin biosynthesis. Approximately 10 years ago it was documented that mechanical stretching of cultured human primary amnion cells increases the release of PGE2 (15). Recent studies have shown that prostaglandin biosynthesis increases in amnion prior to labor, and the increase is associated with elevated cyclooxygenase expression (10, 11, 33), a rate-limiting step in prostaglandin biosynthesis (4–8). Although more than 30 hormones, mitogens, and cytokines have been shown to increase prostaglandin production, the mechanism by which increased PGE2 biosynthesis occurs in fetal tissues remains unclear. Studies in this report raise the possibility that an increase in cell membrane tension induced by cell volume expansion and perhaps cell membrane stretching up-regulates the rate of COX-2 gene transcription, increasing PGE2 biosynthesis and release in cells derived from human amnion. These observations raise the additional possibility that increasing mechanical forces resulting from increased cell volume and/or membrane stretch during gestation may be a critical factor in the initiation of labor.

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