Localization and steroid regulation of prostaglandin E2 receptor protein expression in ovine cervix

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Abstract

Although prostaglandin E2 (PGE2) has been identified as a central mediator of the cervical ripening process, the mechanisms responsible for PGE2 ripening are still poorly understood, partly because of the lack of information concerning the precise cellular localization and regulation of PGE2 (EP) receptors in the cervix. To provide new insights into the mechanisms of cervical ripening, we used indirect immunofluorescence to localize cervical EP receptor protein expression in ovariectomized ewes and examined the effect of administration of progesterone or estradiol. EP receptors were widely distributed in cervical blood vessels, epithelium of the cervical canal, circular and longitudinal muscles, and stroma. Estradiol replacement decreased EP1 and EP3 receptor protein in blood vessel media (by 23 and 31% respectively, \( P < 0.05 \)) and decreased EP1 receptor protein expression in the longitudinal muscle layer (by 27%, \( P < 0.05 \)). Stromal EP1 and EP3 receptor protein expression was also reduced by estradiol (by 29 and 20% respectively, \( P < 0.05 \)). Progesterone replacement had no significant effect on EP receptor protein expression. The arterial changes would favor PGE2-induced vasodilatation, subsequent edema and leukocyte infiltration during the cervical ripening process whereas the muscular alterations would facilitate smooth muscle relaxation and cervical dilatation. Furthermore, estradiol provoked perinuclear localization of EP3 receptor protein in the longitudinal muscle layer. This latter result suggests that cellular EP receptor localization is regulated by estradiol and that PGE2 may also control smooth muscle contraction and regulate ovine cervical dilatation in an intracrine manner via EP3 receptors.

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Introduction

Cervical ripening is an active process, independent of uterine contraction (Stys et al. 1978), which ensures that the cervix becomes a soft and compliant tissue able to dilate to permit the passage of the fetus during labor. Softening of the cervix involves a complex combination of biochemical and structural changes affecting the cervical stroma, and leading to an extensible organ (Uldbjerg et al. 1983). Human cervical ripening is characterized by: edema (Leppert 1995); leukocyte infiltration (Junqueira et al. 1980); dispersion of the collagen network (Danforth et al. 1974) mainly resulting from collagen degradation by leukocyte-released matrix metalloproteinases; and an increase in total glycosaminoglycans (GAGs) (Osmers et al. 1991, 1993). Similar changes are observed in sheep cervix (Fosang et al. 1984, Fosang & Handley 1988).

Several lines of evidence support a pivotal role for prostaglandin E2 (PGE2) in the regulation of the cervical ripening process. First, cyclooxygenase-2 (COX-2) expression (Dong et al. 1996) and PGE2 (Mitchell & Flint 1977) synthesis increase at the time of parturition in the rat and sheep cervix. Secondly, treatment with COX-2 inhibitors delays this process (Mitchell & Flint 1978). Finally, local application of PGE2 has been used clinically for 25 years (Hughes et al. 2001) to induce softening of the cervix successfully at term human pregnancy. The changes in the composition of the cervical connective tissue after PGE2-induced cervical ripening are similar to those occurring in spontaneous cervical ripening (Danforth et al. 1974, Junqueira et al. 1980, Cabrol et al. 1987, Allen et al. 1988, Osmers et al. 1991, 1993, Leppert 1995, Kelly 2002).

Indeed, PGE2 increases human cervical collagenolytic activity (Osmers et al. 1991) and GAG synthesis in rat cervix (Cabrol et al. 1987), induces vasodilatation of human cervical arteries (Allen et al. 1988) and thus promotes subsequent edema and leukocyte infiltration (Kelly 2002).

PGE2 transduces its signal via seven-transmembrane domain, G protein-coupled receptors, called EP receptors...
Mechanisms controlling cervical ripening. Progesterone sponges were purchased from Carter Holt Harvey Plastic Products (Hamilton, New Zealand) and produced plasma concentrations in the range 1.5–2 ng/ml (provided by the manufacturer). All animals were infused with the same volume of physiological saline as the control group. Cervices were removed from the body of the uterus at necropsy under halothane general anesthesia, rinsed in physiological saline, fixed in 10% buffered formalin (Fisher Scientific, Pittsburg, PA, USA) and embedded in paraffin. One piece of cervical tissue from one control animal was flash frozen to provide protein to validate the antibodies by Western blot analysis.

**Western blot analysis**

Western blots were run under denaturing and reducing conditions. Approximately 0.1 g of cervical tissue was homogenized in 1 ml RIPA buffer (50 mmol/l Tris–HCl, pH 7.4, 1% NP40, 150 mmol/l NaCl, 0.1% SDS, 0.25% sodium deoxycholate) supplemented with a cocktail of protease inhibitors. Samples were centrifuged at 12 000 g for 15 min to pellet cellular debris. Protein extracts were quantitated using a modified Bradford technique and BSA as a standard. Protein extracts (10 μg) were dissolved v/v in 2 × Tris–glycine SDS sample buffer (126 mmol/l Tris–HCl, 20% glycerol, 4% SDS, 0.005% bromophenol blue, pH 6.8) (Invitrogen) supplemented with 10 mmol/l dithiothreitol (DTT) and heated at 95 °C for 5 min before analysis on a 7.5% SDS-PAGE. After migration, proteins were transferred to a PVDF membrane (Amersham). Membranes were blocked in 10% non-fat dried milk powder in Tris-buffered saline–Twee-20 (TBS-T) (25 mmol/l Tris, 150 mmol/l NaCl, 0.1% Tween-20, pH 7.6) for 1 h. For immunodetection, blots were incubated with rabbit polyclonal antibodies against human EP receptors (Cayman Chemicals, Ann Arbor, MI, USA; catalog numbers 101740, 101750, 101760 and 101775) for 90 min at a dilution of 1:1000 in TBS-T containing 5% non-fat dried milk powder. After five washes in TBS-T, blots were incubated with horseradish peroxidase-linked goat anti-rabbit (SantaCruz Biotechnologies, Santa Cruz, CA, USA; catalog number sc-2004) for 45 min in TBS-T containing 5% non-fat dried milk powder at a dilution of 1:2000. Immunoreactive proteins were detected using a chemiluminescent detection system (Amersham ECL reagents, Amersham) according to the manufacturer’s directions. Control experiments were performed by incubating the membranes with anti-human EP receptor antibodies pre-absorbed on the related blocking peptides at a dilution of 1:100 (Cayman Chemicals; catalog numbers 301740, 301750, 301760 and 301775).

**Indirect immunofluorescence**

Sections (5 μm thick) were cut from the paraffin-embedded tissues, mounted on ProbeOn Plus microscope slides (Fisher Scientific), deparaffinized in xylene and rehydrated in a graded ethanol series. Sections were

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**Materials and Methods**

**Animals and tissue collection**

Surgical procedures were performed at Cornell University (Ithaca, NY, USA) and were approved by the Cornell University Institutional Animal Care and Use Committee. The Cornell facilities are approved by the American Association for the Accreditation of Laboratory Animal Care. Eighteen non-pregnant ewes (*Ovis aries*) were ovarioectomized on the day of ovulation, 48 h after removal of progesterone sponges used to synchronize estrus. After 10 days, as previously described (Wu et al. 1996, 1997, 2000, 2003), ewes received one of the following treatments; saline infusion controls (*n* = 6), estradiol (Sigma) infused intravenously for 2 days (50 μg/day in 0.5 ml saline per hour, *n* = 6), or an intravaginal progesterone sponge for 10 days (containing 0.3 g progesterone, *n* = 6). Progesterone sponges were purchased from Carter Holt.
washed in deionized water, and antigen was retrieved by microwaving in a polypropylene Coplin staining jar (VWR International, Aurora, CO, USA) at full power for 10 min in DAKO target retrieval solution (pH 6.20) (DAKO Corporation, Carpinteria, CA, USA). All sections were washed in PBS then blocked for 30 min with 10% normal goat serum (Vector Laboratories, Burlingame, CA, USA). Sections were then incubated for 1 h at room temperature with the primary antibody diluted in DAKO antibody diluent with background reducing components (DAKO Corporation). Polyclonal primary antibodies were used at 1:250 dilutions. Sections were washed in PBS and then further incubated with the second antibody (biotinylated goat anti-rabbit IgG) (Vector Laboratories) diluted 1:200 in 2% goat serum in PBS for 30 min at room temperature. Sections were washed and the antigen was localized using 2.5 μg/ml fluorescein streptavidin (Vector Laboratories) in PBS for 15 min. Slides were extensively washed and then mounted with Vectashield hard-set mounting medium (Vector Laboratories). To control for variability in intensity of staining, slides from different groups were stained at the same time. Negative controls included slides incubated without the primary antibody and sections incubated with non-immune rabbit serum (Vector Laboratories) instead of primary antibody.

**Image analysis**

Fluorescence staining was observed using a Nikon TE300 inverted microscope (Nikon USA, Melville, NY, USA). Images were captured with a CoolSNAP HQ cooled charge-coupled device (CCD) digital camera (Roper Scientific, Trenton, NJ, USA) coupled to the computer image analysis software Metamorph (Universal Imaging Corporation, Downingtown, PA, USA). Evaluation of all slides was performed by an investigator who was blind to the animal treatment. For each of the five different tissue components studied (blood vessels, mucosa, longitudinal muscle layer, circular muscle layer and stroma), three different pictures corresponding at widely separated positions on the slide were taken at a magnification of ×100 (15 pictures per slide). Quantification of EP receptor immunoreactivity was performed with ImageJ v1.31 (http://rsb.info.nih.gov/ij/) image analysis software. The average gray level of fluorescence immunoreactivity was measured on the whole surface of the picture (696 × 520 pixels) for the stroma. For the endothelium, the media, the epithelium of the cervical canal and the longitudinal and circular muscle layers, the whole surface of each tissue present in the picture was selected using the freehand selection tool and the average gray level of fluorescence immunoreactivity was measured in the selected areas.

**Statistical analysis**

The average gray level of fluorescence immunoreactivity of each tissue component from each animal was taken as the mean of three different measurements. The gray level of fluorescence immunoreactivity (sum of the average gray level of fluorescence immunoreactivity (sum of the gray level of fluorescence immunoreactivity for each tissue component from each animal (i.e. control, progesterone and estradiol groups). The gray levels of fluorescence immunoreactivity were then expressed in simpler relative numerical values (1.30 or 0.21 rather than 1296 and 210) by dividing all the values by 1000.

Statistical comparisons between control, progesterone and estradiol groups were carried out using one-way ANOVA. Differences in EP receptor expressions were determined by post hoc tests using the Bonferroni correction. The level of significance was set at $P < 0.05$.

**Results**

**Specificity of rabbit polyclonal anti-human EP receptor antibodies in sheep cervix**

The specificity of the different anti-human polyclonal antibodies for all four sheep EP receptor proteins was assayed by Western blot analysis. As shown in Fig. 1, distinct proteins of different apparent molecular masses were identified by the various rabbit anti-human EP receptor antibodies. We observed a protein band of approximately 56 kDa by staining with anti-EP1 antibody, a 67 kDa band with the anti-EP2 antibody, a 62 kDa band with the anti-EP3 antibody and a 64 kDa band with the anti-EP4 antibody. Furthermore, pre-absorbing the primary antibodies, with the antigen to which they were raised, abolished the 56, 67, 62 and 64 kDa signals (Fig. 1).

**Cervical distribution of EP receptor proteins**

Indirect immunofluorescence demonstrated the expression of all four EP receptor proteins in ewe cervicis. EP receptor localization is shown in Fig. 2. The EP1, EP2, EP3 and EP4
EP₄ receptor proteins were expressed in all six different tissue components studied – i.e. the endothelium and smooth muscle of cervical blood vessels, the epithelium of the cervical canal, the circular and longitudinal muscle layers, and the stroma. The absence of detectable immunoreactive protein when the primary antibody was replaced by non-immune rabbit serum confirmed the specificity of the staining.

**Figure 2** EP receptor immunolocalization in cervical tissues of control ewes. EP₁ (A, E, I, M, Q), EP₂ (B, F, J, N, R), EP₃ (C, G, K, O, S) and EP₄ (D, H, L, P, T) receptor localization was assayed by indirect immunofluorescence as described in the Materials and Methods. Receptors were localized in blood vessels (A, B, C, D), epithelium of the cervical canal (E, F, G, H), circular muscle layer (I, J, K, L), longitudinal muscle layer (M, N, O, P) and stroma (Q, R, S, T). Negative controls were performed by replacing the first antibody by non-immune rabbit serum (U).
Steroid regulation of cervical EP receptor protein expression

Progestosterone replacement had no significant effect on the distribution of the four EP receptor proteins in the six tissue components compared with the control group (Table 1). Compared with the control group, the expression of EP1 receptor protein in the media, the longitudinal muscle layer and in the stroma was significantly reduced in the estradiol-replaced group ($P < 0.05$) (Table 1). The expression of EP3 receptor protein, in the media and in the stroma of the estradiol-replaced animals, was also significantly decreased compared with the control and progesterone-replaced groups ($P < 0.05$) (Table 1).

Estrogen-dependent alteration of EP3 receptor cellular localization

Immunofluorescent staining demonstrated perinuclear localization of EP3 receptor protein expression in the longitudinal muscle layer of all six estradiol-replaced ewes (Fig. 3), but not in any other tissue in this group.

Table 1 Effects of progesterone and estradiol replacement on cervical EP receptor protein expression.

<table>
<thead>
<tr>
<th>Cervical tissues</th>
<th>Control ($n = 6$)</th>
<th>Progesterone ($n = 6$)</th>
<th>Estradiol ($n = 6$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP1 expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelium</td>
<td>1.21 ± 0.10</td>
<td>1.26 ± 0.10</td>
<td>1.05 ± 0.07</td>
</tr>
<tr>
<td>Media</td>
<td>1.07 ± 0.06</td>
<td>1.03 ± 0.09</td>
<td>0.83 ± 0.05*</td>
</tr>
<tr>
<td>Epithelium</td>
<td>1.15 ± 0.11</td>
<td>1.15 ± 0.10</td>
<td>0.94 ± 0.07</td>
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<tr>
<td>Circular muscle layer</td>
<td>0.96 ± 0.09</td>
<td>0.97 ± 0.09</td>
<td>0.76 ± 0.09</td>
</tr>
<tr>
<td>Longitudinal muscle layer</td>
<td>1.27 ± 0.10</td>
<td>0.99 ± 0.08</td>
<td>0.88 ± 0.08*</td>
</tr>
<tr>
<td>Stroma</td>
<td>0.74 ± 0.07</td>
<td>0.68 ± 0.04</td>
<td>0.53 ± 0.05*</td>
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<tr>
<td>EP2 expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelium</td>
<td>1.35 ± 0.17</td>
<td>1.45 ± 0.23</td>
<td>1.29 ± 0.18</td>
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<tr>
<td>Media</td>
<td>1.10 ± 0.10</td>
<td>0.99 ± 0.12</td>
<td>0.95 ± 0.15</td>
</tr>
<tr>
<td>Epithelium</td>
<td>1.31 ± 0.20</td>
<td>1.61 ± 0.19</td>
<td>1.26 ± 0.18</td>
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<td>Circular muscle layer</td>
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<td>0.99 ± 0.11</td>
<td>0.77 ± 0.11</td>
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<td>Longitudinal muscle layer</td>
<td>0.93 ± 0.12</td>
<td>1.13 ± 0.14</td>
<td>0.81 ± 0.11</td>
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<tr>
<td>Stroma</td>
<td>0.73 ± 0.09</td>
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<td>EP3 expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelium</td>
<td>1.39 ± 0.07</td>
<td>1.52 ± 0.13</td>
<td>1.27 ± 0.07†</td>
</tr>
<tr>
<td>Media</td>
<td>1.23 ± 0.09</td>
<td>1.11 ± 0.09</td>
<td>0.90 ± 0.06*</td>
</tr>
<tr>
<td>Epithelium</td>
<td>1.25 ± 0.14</td>
<td>1.31 ± 0.15</td>
<td>1.20 ± 0.10</td>
</tr>
<tr>
<td>Circular muscle layer</td>
<td>0.98 ± 0.06</td>
<td>1.20 ± 0.10</td>
<td>0.99 ± 0.05</td>
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<td>Longitudinal muscle layer</td>
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<td>1.31 ± 0.15</td>
<td>1.16 ± 0.09</td>
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<td>Stroma</td>
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<td>0.86 ± 0.08</td>
<td>0.64 ± 0.02†</td>
</tr>
<tr>
<td>EP4 expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelium</td>
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<td>1.10 ± 0.09</td>
<td>1.03 ± 0.10</td>
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<tr>
<td>Media</td>
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<td>1.14 ± 0.11</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>Circular muscle layer</td>
<td>0.82 ± 0.10</td>
<td>0.91 ± 0.06</td>
<td>0.74 ± 0.03</td>
</tr>
<tr>
<td>Longitudinal muscle layer</td>
<td>0.88 ± 0.09</td>
<td>0.87 ± 0.08</td>
<td>0.77 ± 0.03</td>
</tr>
<tr>
<td>Stroma</td>
<td>0.79 ± 0.08</td>
<td>0.68 ± 0.05</td>
<td>0.65 ± 0.02</td>
</tr>
</tbody>
</table>

Results are expressed as means±S.E.M. of gray levels of fluorescence immunoreactivity. Statistical comparisons between groups were carried out using a one-way ANOVA. Differences in EP receptor expressions were determined by post hoc tests using the Bonferroni correction.

* $P<0.05$ versus control.
† $P<0.05$ versus progesterone.

Perinuclear localization of EP receptor proteins was not observed in the tissues of the control (Fig. 2) or progesterone-replaced ewes for any EP receptor subtype.

Discussion

Until recently, the lack of EP receptor antibodies has prevented any attempt to study the precise EP receptor cellular distribution at the protein level in tissues of the female reproductive tract. The recent development of EP receptor antibodies enables localization as well as examination of steroid regulation of EP receptor protein expression in the tissues that compose the cervix.

In the present study, we first checked the cross-reactivity of the available rabbit anti-human EP receptor antibodies with EP receptors in the sheep cervix by running Western blot analysis which demonstrated single bands for EP1, EP2, EP3 and EP4 receptors of approximately 56, 67, 62 and 64kDa respectively. These values agree with the molecular mass range reported in human tissues (Morath et al. 1999) and rat (Southall & Vasko 2001). The specificity of the immunoreactive detection was further confirmed by the absence of signal when the primary antibodies were pre-absorbed by the original peptide antigen. Furthermore, the homology between the human and published ovine protein sequences, in the highly conserved C-terminal region against which the antibodies were designed, is high (85–90%), suggesting a specific antigen–antibody interaction.

We demonstrated the presence of all four EP receptor proteins, confirming previous data obtained at the RNA level in baboons (Smith et al. 2001b), in all the tissue components of the ovine cervix. During cervical ripening, PGE2 is generally considered to act mainly as an inducer of stromal extracellular matrix protein and glycoprotein alteration. The localization of the four EP receptors in blood vessels emphasizes components of the vascular system as possible targets for PGE2. PGE2 exerts its effects on blood vessels through actions on multiple counterbalanced signaling pathways in vascular smooth muscle cells. EP1 and EP3 receptors induce vasoconstriction whereas EP2 and EP4 receptors provoke vasodilatation (Wright et al. 2001). It is not possible from our results to predict the overall effect that PGE2 exerts on cervical blood vessels. However, the fact that EP receptors are expressed on both the endothelium and smooth muscle of cervical blood vessels suggests that PGE2 can act as an important modulator of cervical vascular tone. Any modification of the contractile/relaxant EP receptor ratio will affect the ability of PGE2 to provoke either vasoconstriction or vasodilatation.

In order to evaluate the effects of steroid hormones on EP receptor expression, ovariectomized ewes received replacement therapy with either progesterone or estradiol; we have used this model extensively in previous work (Wu et al. 1996, 1997, 2000, 2003). EP1 and EP3 receptor
protein expression were both significantly decreased in the blood vessel media of estradiol-replaced ewes compared with control tissues (by 23 and 31% respectively). Estradiol concentrations rise in ovine maternal plasma at parturition (Challis 1971) and prostaglandins mediate premature delivery induced by estradiol in pregnant sheep and goats (Currie et al. 1976, Wu et al. 2004). Estradiol, by decreasing EP1 and EP3 receptor expression, would facilitate EP2 and EP4 receptor-dependent vasodilator effects of PGE2 and thus promote cervical ripening. Interestingly, it has been demonstrated by competition binding studies and measurement of vascular resistance, in newborn versus adult porcine choroidal vasculature, that fewer EP1 receptors (33 versus 55%) and more EP2 receptors (33 versus 8%) were responsible for the increased vasodilatation in the newborn (Abran et al. 1997). Therefore, even modest variation in cervical EP receptor expression might be sufficient to provoke physiological alterations in the vascular response to PGE2.

Both in vitro and in vivo studies suggest an important role for EP4 receptor in mediating PGE2 ripening effects. PGE2 induces GAG synthesis by human cervical fibroblasts via EP4 receptors (Schmitz et al. 2001, 2003), and EP4 receptor agonists increase pregnant guinea-pig cervical compliance (Kanayama et al. 2004). Therefore, the significantly decreased expression of EP1 and EP3 receptors (by 29 and 20% respectively) in the stroma after estradiol replacement might play a role by altering the balance of positive and negative influences. As a result, the activation of EP4 receptors by PGE2 in this tissue compartment would represent a second mechanism for estradiol to promote cervical ripening.

Estradiol provoked a significant 27% decrease in EP1 receptor protein expression in the longitudinal muscle layer of the estradiol-replaced animals in comparison with the control group. Hollingsworth and Isherwood (1978) demonstrated decreased contractile response of cervical strips to PGE2 at the end of rat gestation. Decreased expression of contractile EP receptor would contribute to a better dilatation of the cervix during parturition.

Progesterone replacement had no significant effect on EP receptor distribution in the ovine cervix. Conversely, a significant increase in EP2 receptor mRNA expression in ovariectomized rat myometrium replaced by progesterone has been demonstrated (Dong & Yallampalli 2000). We have previously demonstrated different regulatory effects of progesterone on COX-2 protein expression in endometrium and myometrium using the ovine model (Wu et al. 1997). Similarly, progesterone regulation of EP receptor expression is likely to be tissue and/or species dependent. Differences in transcription and translation of the different receptors may also explain species- and tissue-specific changes.

In addition to the changes in EP1 and EP3 receptor tissue distribution, our results clearly demonstrate that estradiol alters cellular EP receptor localization. After estradiol replacement, indirect immunofluorescence demonstrated perinuclear expression only of EP3 receptor protein in the longitudinal muscle layer. This localized distribution was not observed in the tissues from the control ewes. These findings are in accordance with recent observations demonstrating perinuclear localization of EP receptors in porcine cerebral microvascular endothelial cells (Bhattacharya et al. 1998, 1999), in fibroblast cell lines (Bhattacharya et al. 1998), in human embryonic kidney cells (Bhattacharya et al. 1999), in human ocular tissues (Schlotzer-Schrehardt et al. 2002), in human adult and fetal bone tissues (Fortier et al. 2004) and in human myometrium (Astle et al. 2005). To our knowledge, this is the first report of an estradiol-dependent regulation of perinuclear EP receptor expression. Activation of pig perinuclear EP receptors by PGE2 modulates nuclear calcium transient and transcription of genes such as inducible nitric oxide synthase (NOS) (Bhattacharya et al. 1999) and endothelial NOS (Gobeil et al. 2002). Therefore, PGE2 might increase NO synthesis and facilitate cervical ripening.

**Figure 3** Perinuclear expression of EP3 receptors in estradiol-replaced ovariectomized ewes. EP receptor localization was assayed by indirect immunofluorescence as described in the Materials and Methods. Immunofluorescent staining demonstrated perinuclear localization (arrows) of EP3 receptors only in the longitudinal muscle layer of estradiol-replaced ewes (B) and not in controls (A).
dilatation during parturition in an intracrine manner via the activation of perinuclear EP3 receptor in the longitudinal muscle layer. Finally, estradiol-dependent expression of perinuclear EP3 receptor might represent a novel indirect pathway for estradiol to regulate gene expression. These hypotheses merit study in *vitro* paradigms.

In conclusion, EP receptors are widely distributed within cervical tissues and predominantly expressed in blood vessels. Estradiol replacement in the ovariec-tomized ewes decreased EP1 and EP3 receptor protein expression in the blood vessel media and decreased EP3 receptor protein expression in the longitudinal muscle layer. These changes would favor PGE2-induced vasodilatation, subsequent edema and leukocyte infiltration during the cervical ripening process as well as facilitating smooth muscle relaxation during cervical dilatation. Furthermore, estradiol administration results in perinuclear expression of the EP3 receptor in the longitudinal muscle layer. This finding suggests that EP receptor locations are not only regulated by estradiol at the tissue level, but also at the cellular level, and that PGE2 may control smooth muscle contraction and regulate ovine cervical dilatation in an intracrine manner via EP3 receptors.

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