Changes in the expression of nitric oxide synthase in the human uterine cervix during pregnancy and parturition

Marie-Anne Ledingham1, Andrew J. Thomson, Anne Young, Lena M. Macara, Ian A. Greer and Jane E. Norman

Department of Obstetrics and Gynaecology, University of Glasgow, 10 Alexandra Parade, Glasgow G31 2ER, Scotland, UK

1To whom correspondence should be addressed at: Department of Obstetrics and Gynaecology, University of Glasgow, 10 Alexandra Parade, Glasgow G31 2ER, UK. E-mail: ml50y@udcf.gla.ac.uk

Nitric oxide (NO) has been proposed as a mediator of cervical ripening. We investigated the expression, using Western blotting, and localization, using immunohistochemistry, of the nitric oxide synthase (NOS) enzymes, inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (bNOS) in the human cervix during pregnancy and parturition. Cervical biopsies were obtained from non-pregnant women, women in the first trimester of pregnancy, and pregnant women at term before and after the onset of labour. Each of the NOS isoforms was localized in the cervices of both non-pregnant and pregnant subjects using immunohistochemistry. iNOS expression was significantly greater in early pregnancy compared with the non-pregnant state (P < 0.005). iNOS expression was up-regulated further in samples obtained in the third trimester compared with the first trimester. bNOS expression was greater in samples from the first trimester of pregnancy than in non-pregnant samples (P < 0.005), but showed no additional increase in late pregnancy or with the onset of labour. eNOS expression was increased in samples obtained in the third trimester both before (P = 0.002) and after the onset of labour (P < 0.002) when compared with non-pregnant samples. The increased expression of NOS isoforms in late pregnancy supports the hypothesis that NO is involved in the process of cervical ripening.

Key words: cervical ripening/cervix/nitric oxide/NOS/pregnancy

Introduction

Prior to the onset of labour, the cervix undergoes physical changes, which are necessary for vaginal delivery. These changes, which occur during the last weeks of pregnancy, include softening, effacement and dilatation of the cervix and are given the term ‘cervical ripening’. The timing of these changes requires careful regulation. Premature cervical ripening may lead to preterm labour, a condition that is associated with considerable morbidity and mortality (Olah and Gee, 1992). Alternatively, failure of ripening leads to delay in the onset of labour and an associated increase in the Caesarean section rate and birth asphyxia (Smith et al., 1984; Calder, 1986; Wigton and Wolk, 1994). In the human, cervical ripening is an inflammatory reaction involving leukocytic infiltration, changes in the water content of the extracellular matrix and rearrangement of collagen fibres (Junquiera et al., 1980; Liggins, 1981; Leppert, 1992; Owiny et al., 1995). The control of this process remains poorly understood although a number of mediators have been implicated, including progesterone withdrawal, prostaglandins, relaxin, and various inflammatory cytokines (Barclay et al., 1993; Chwalisz et al., 1994).

The inflammatory mediator, nitric oxide (NO), a reactive gas with a short half-life, has more recently been implicated in cervical ripening. NO is involved in a variety of physiological processes including smooth muscle relaxation, inhibition of platelet aggregation, neurotransmission and apoptosis (Anggard, 1994; Vallance and Collier, 1994; Moncada, 1997). NO production is dependent upon the action of the enzyme nitric oxide synthase (NOS) which catalyses the conversion of L-arginine to L-citrulline. This enzyme exists in three isoforms, the calcium-independent isofrom, inducible NOS (iNOS) and the calcium-dependent isofroms, endothelial NOS (eNOS) and neuronal or brain NOS (bNOS) (Forstermann et al., 1991). NO has been shown to play a crucial role in several aspects of female reproductive physiology (Chwalisz et al., 1996; Rosselli, 1997) including menstruation (Telfer et al., 1995; Cicinelli et al., 1996; Tschugguel et al., 1998, 1999a), control of gonadotrophin-releasing hormone (GnRH) release (McCann et al., 1998), ovulation (Ellman et al., 1993; Jablonka-Shariff and Olson, 1998) and implantation (Purcell et al., 1998; Chwalisz et al., 1999). Furthermore, in some non-human species, NO may control the timing of the onset of parturition (Ledingham et al., 2000).

Recent evidence suggests that NO may be involved in the process of cervical ripening prior to the onset of labour. Previous studies, in both animals and humans, have shown that NO donors can artificially induce cervical ripening following their local application (Chwalisz et al., 1997; Thomson et al., 1997, 1998). In studies using rat models, nitrate production together with iNOS and bNOS mRNA expression increases in the cervix during labour (Buhimschi et al., 1996).
Other authors (Tschugguel et al., 1999b) have also suggested a role for NO in human cervical ripening. iNOS expression was increased in the cervices of women in the postpartum period compared with the non-pregnant state. No alteration in the expression of bNOS or eNOS was observed. However, the design of the study was such that effects of cervical ripening on NO expression could not be separated from the possible effects of pregnancy, labour or the delivery of the term infant through the cervix.

We postulated that each of the three isoforms of NOS is expressed in the human cervix and that NOS expression is increased during pregnancy, consistent with the involvement of NO in physiological cervical ripening. To test this hypothesis, we used Western blotting to examine the changes in expression of each of the three NOS isoforms in the human cervix throughout pregnancy. In addition, each of the three isoforms was localized in tissue specimens using immunohistochemistry.

Materials and methods

Cervical tissue biopsies were obtained from women in four different groups: (i) women undergoing hysterectomy for benign indications \( (n = 8) \); (ii) women undergoing suction termination of pregnancy in the first trimester of pregnancy \( (<12 \text{ weeks gestation}) \ (n = 8) \); (iii) pregnant women delivered at term \( (>37 \text{ weeks gestation}) \) by elective Caesarean section prior to the onset of labour \( (n = 8) \); and (iv) pregnant women delivered at term \( (>37 \text{ weeks gestation}) \) undergoing emergency Caesarean section after the onset of labour \( (\text{cervical dilatation} > 4 \text{ cm}) \ (n = 8) \).

The study conformed to the Declaration of Helsinki and was approved by the local research ethics committee. Written informed consent was obtained from each woman prior to recruitment. Pregnant women were excluded from the study if they had a multiple pregnancy or evidence of intrauterine infection, as determined by temperature \( >38^\circ\text{C} \). The indication for Caesarean section in the labouring group was fetal distress. Women with dysfunctional labour or who had received prostaglandin or oxytocin were excluded. Cervical tissue specimens were divided in two, one half was fixed in formalin and embedded in paraffin for immunohistochemistry and the other half was flash-frozen in liquid nitrogen and stored at \(-70^\circ\text{C}\) for subsequent Western blotting.

In the non-pregnant group, cervical biopsies were obtained from the anterior cervical lip using a scalpel within 10 min of removal of the uterus. Cervical biopsies from the first trimester subjects were taken from the anterior lip of the cervix using a 6 mm biopsy needle (Stifle Laboratories, Wooburn Green, Bucks, UK) under general anaesthetic after evacuation of the uterus. In the pregnant women at term not in labour, biopsies were taken from the anterior lip of the cervix prior to delivery of the infant. Biopsies from the pregnant women in labour were obtained from the same site in the cervix following delivery of the infant.

Immunohistochemistry for eNOS, bNOS and iNOS

Immunohistochemistry was performed on paraffin-embedded cervical tissue sections as previously described (Thomson et al., 1997) using antibodies against eNOS, bNOS and iNOS as detailed in Table I. Briefly, tissue was fixed at the time of collection in 10% neutral-buffered formalin and embedded in paraffin. Sections were cut to 5 \( \mu \text{m} \) thickness, dewaxed, rehydrated and endogenous peroxide activity blocked in 0.5\% \( \text{H}_2\text{O}_2 \) (Sigma) in methanol for 30 min at room temperature. Sections were washed in phosphate-buffered saline (PBS) and the antigen was retrieved by microwaving in a pressure cooker (Lakeland Plastics Ltd, Cumbria, UK) if required (Table I). Tissue sections were then blocked in either 20\% normal horse (SAPU, Carluke, UK)/human serum (eNOS and iNOS) or 20\% goat/ human serum (bNOS) for 30 min at room temperature and incubated for 16 h at 4\°\text{C} with the primary antibody diluted in 2\% normal horse serum or 2\% normal goat serum as appropriate (Table I). Sections were washed in PBS then incubated for 30 min with biotinylated horse anti-mouse (Vector Laboratories) diluted 1:200 in 2\% normal horse serum with 5\% human serum added (eNOS, iNOS) or with biotinylated goat anti-rabbit (Vector Laboratories) at 1:200 in 2\% goat serum with 5\% human serum added. Sections were washed in PBS, then incubated with avidin DH/biotinylated horseradish peroxidase (HRP) H reagent (Vector Laboratories, UK) in PBS for 30 min before final washing. Antigen was localized using 1 mg/ml diaminobenzidine tetrahydrochloride (Sigma, UK), 0.02\% \( \text{H}_2\text{O}_2 \) in 50 mmol/l Tris–Cl, pH 7.6 and appeared as a brown end product.

The specificity of each of the antibodies was demonstrated by Western blotting on lysates of rat cerebellum, human umbilical vein endothelial cells (HUVECS) and lipopolysaccharide (LPS)/interferon \( \gamma \) (IFN\( \gamma \))-stimulated mouse macrophages using the primary antibodies as tabulated above. The positive controls for iNOS, eNOS and bNOS were sections of small bowel from a patient with ulcerative colitis (Kimura et al., 1997; Guslandi, 1998), umbilical cord (Myatt et al., 1997) and rat brain respectively. Negative control slides were set up for all three antibodies with omission of the primary antibody. Negative controls were also set up for eNOS and iNOS with immunoglobulin (Ig)G1 and IgG\_2\text{a} immunoglobulins respectively replacing the primary antibody.

Immunoistochemistry for CD45

In order to localize leukocytes in serial sections of each of the tissues, immunohistochemistry was performed on paraffin embedded cervical biopsies using an antibody directed against the common leukocyte antigen, CD45 (Dako; M 0701). Briefly, biopsies of cervix were prepared and microwaved as above. Sections were blocked in 20\% normal horse (SAPU, Carluke, UK)/human serum for 30 min at room temperature and incubated for 16 h at 4\°\text{C} with anti-CD45 at 1:100 dilution. Sections were incubated for 30 min with biotinylated horse anti-mouse (Vector Laboratories) at 1:200 dilution and then incubated with avidin DH/biotinylated HRP H reagent (Vector Laboratories, UK). Antigen was localized using diaminobenzidine tetrahydrochloride as before and counterstained with Harris’ haematoxylin. Negative controls included slides incubated without the primary antibody.

Western blot analysis for eNOS, iNOS and bNOS

Total protein was extracted from the cervical tissue biopsies using the TRIZOL™ method according to the manufacturer’s instructions (Life Technologies, Paisley, UK). Protein was quantified using the BCA protein assay reagent (Pierce, IL, USA) and UV spectrophotometry at 562 nm. Samples containing 60 \( \mu \text{g} \) protein were prepared in equal volumes of sample application buffer, separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to a nitrocellulose membrane (pore size 450 \( \mu \text{m} \) ) (Hybond; Amersham Life Sciences, Little Chalfont, Buckinghamshire, UK) by wet blotting (100 V for 1 h 50 min). Gels were stained with Coomassie Blue to check protein transfer. Membranes were blocked in 5\% Marvel™ in 0.05\% v/v Tween–Tris-buffered saline (TBS–T) for at least 1 h prior to antibody application. The antibodies were the same as those used for immunohistochemistry at the following concentrations: iNOS at 1:10 000, eNOS at 1:2500 and...
Table I. Nitric oxide synthase (NOS) antibodies for immunohistochemistry

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Type</th>
<th>Immunogen</th>
<th>Dilution</th>
<th>Pretreatment</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS</td>
<td>mouse monoclonal</td>
<td>amino acids 1030–1209 of human eNOS</td>
<td>1:10 000</td>
<td>microwave</td>
<td>Transduction</td>
</tr>
<tr>
<td>bNOS</td>
<td>rabbit polyclonal</td>
<td>amino acids 1414–1434 of human brain synthetic peptide</td>
<td>1:25</td>
<td>none</td>
<td>Serotec</td>
</tr>
<tr>
<td>iNOS</td>
<td>mouse monoclonal IgG₂</td>
<td>amino acids 961–1144 of murine iNOS</td>
<td>1:50</td>
<td>microwave</td>
<td>Transduction</td>
</tr>
</tbody>
</table>

eNOS = endothelial NOS; bNOS = neuronal NOS; iNOS = inducible NOS; IgG = immunoglobulin G.

bNOS at 1:10 000. Lysates of IFNγ/LPS-treated mouse macrophages (Transduction Laboratories), HUVECS (Transduction Laboratories) and rat cerebellum were used as the controls for iNOS, eNOS and bNOS respectively. Immunoreactivity was visualized using HRP-linked secondary antibodies against the appropriate species and the ECL detection system as per the manufacturer’s instructions (Amersham). Stained molecular weight markers (Bio-Rad) were transferred to the nitrocellulose membrane and used to identify and characterize the molecular weights of the NOS isoforms examined.

Data analysis
Transmission densitometry was used to quantify NOS activity in the Western blots (Bio-Rad Multi-Analyst/PC Version 1.1). Parallel background readings of equal area were obtained in order to calculate relative intensities from the autorads and calculated using dedicated software. Readings were only compared between samples run in parallel under exactly the same conditions (i.e. same electrophoresis run, same buffers, stains and incubation periods). All densitometric assessments were performed within the optimal range of sensitivity. Statistical analysis was performed using Kruskal–Wallis Test with Mann–Whitney U-test as a post-hoc test. \( P < 0.05 \) was considered to be statistically significant.

For immunohistochemical analysis, the slides were examined using light microscopy by two independent observers (M.A.L. and J.E.N.). Staining was localized but there was no attempt to quantify staining in the different groups.

Results

Immunohistochemistry

iNOS
In each of the cervical tissues sampled from pregnant and non-pregnant women, iNOS protein was localized to the vascular endothelium. Immunostaining for iNOS was absent from the cervical glands. In biopsies obtained from pregnant women at term (in labour and not in labour), iNOS was identified in cells within the cervical stroma. Co-localization with the common leukocyte antigen CD45, identified these cells as leukocytes. The positive control sections (small bowel in ulcerative colitis) showed appropriate localization for iNOS, whilst the negative control sections showed no staining (Figure 1).

eNOS
There was no difference in the immunolocalization of eNOS among the four groups of women. Immunostaining was identified in the vascular endothelium, the parabasal cells of the surface epithelium and the cervical glandular epithelial cells in each of the sections. The positive control sections (term placenta) showed appropriate localization for eNOS and the negative control sections showed no staining (Figure 2).

bNOS
bNOS was localized in each of the cervical biopsies from non-pregnant and pregnant women. Immunostaining for bNOS was identified in the intermediate and superficial cells of the surface epithelium. Staining for bNOS was absent in the cervical glands and on the vascular endothelium. In cervical biopsies collected from pregnant women at term, bNOS staining was

Figure 1. Immunolocalization of inducible nitric oxide synthase (iNOS) in cervical biopsies collected from (a) non-pregnant women, (b) women in the first trimester of pregnancy, (c) pregnant women at term before the onset of labour and (d) pregnant women at term during spontaneous labour. In each of the biopsies, iNOS localized to the vascular endothelium. (e) In biopsies collected from pregnant women at term iNOS was also identified within leukocytes. (f) The positive control slides, small bowel from a patient with ulcerative colitis, showed appropriate localization of iNOS. The negative control sections (see text) showed no staining. (a, b, c, d and f) Scale bars = 50 µm. Figure (e) was obtained using a high-powered (×100) oil immersion lens.
identified in leukocytes in the cervical stroma. The positive control sections (rat brain) showed appropriate localization for bNOS and the negative control sections showed no staining (Figure 3).

**Western blotting analysis**

Western blotting confirmed the presence of protein for each of the three NOS isoforms within cervical tissue biopsies.

**iNOS**

iNOS protein was detected at 130 kDa. There was a 2.7-fold greater expression of iNOS in the first trimester compared with cervix obtained from non-pregnant subjects \( (P < 0.005) \). There was a further 1.6-fold increase at term prior to the onset of labour \( (P < 0.01) \). iNOS protein expression did not change following the onset of spontaneous labour \( (P = 0.27) \) (Figure 4).

**eNOS**

No significant changes were observed in eNOS expression when comparing the non-pregnant and first trimester groups. However, there was an increase in eNOS expression in the cervical samples obtained from term subjects both before labour and in labour when compared with samples from non-pregnant subjects \( (P = 0.002 \text{ and } P = 0.0016 \text{ respectively}) \) (Figure 5).

**bNOS**

In the cervix, bNOS protein was represented by positive bands at 160 kDa. bNOS protein increased in the first trimester of pregnancy compared with the non-pregnant state \( (P < 0.005) \). There were no further significant changes in bNOS expression at term or with the onset of labour (Figure 6).

![Figure 2](image1.png)

Figure 2. Localization of endothelial nitric oxide synthase (eNOS) in the human uterine cervix. In each of the biopsies, eNOS localized to (a) the vascular endothelium, (b) the parabasal cells of the surface epithelium and (c) the cervical glands. The positive control slides, (d) term placenta, showed appropriate localization of eNOS. The negative control slides (e, see text) showed no staining. Scale bars (a, c, d and e) = 50 µm, and (b) = 100 µm.

![Figure 3](image2.png)

Figure 3. Immunolocalization of neuronal nitric oxide synthase (bNOS) in human cervix. bNOS protein was absent from (a) the cervical glands, but was identified (b) within the intermediate and superficial cells of the surface epithelium. (c) Staining for bNOS was identified within leukocytes in biopsies collected from pregnant women at term. (d) Sections of rat brain without primary antibody were employed as the negative controls and showed no staining and (e) the positive control slides (rat brain) showed appropriate staining for bNOS. (a, d and e) scale bars = 50 µm; (b) scale bar = 100 µm; and (c) scale bar = 20 µm.
Discussion

This is the first study to examine the localization and expression of the three NOS isoforms in the human uterine cervix in non-pregnant subjects and in pregnant women in the first trimester and at term, both before and after the onset of labour. We have shown that the three NOS isoforms are present in the human cervix in the non-pregnant and pregnant states. Expression of each of the NOS isoforms is increased in pregnancy compared with the non-pregnant state. iNOS expression is further increased in the third trimester of pregnancy. None of the isoforms of NOS increases following the onset of labour. These results suggest that an increase in endogenous cervical NOS enzyme activity in the third trimester may be important in physiological cervical ripening in human pregnancy. The timing of these events would seem appropriate, given that the changes in the cervix during pregnancy are thought to occur at term prior to the onset of labour as part of a ‘conditioning phase’ before uterine activity can commence (Calder, 1994; Chwalisz and Garfield, 1997). At this time the cervix becomes soft and compliant secondary to alterations in its water content and constitutive connective tissue matrix components and the uterus undergoes changes which allow it to contract in a synchronous manner (Uldbjerg et al., 1985; Ekman et al., 1991; Calder, 1994; Garfield et al., 1998).

Previous studies in animals have provided evidence that NO may have a physiological role in cervical ripening. The NO generating system has been identified in the rat cervix where it may also be involved in regulating cervical extensibility (Shi et al., 2000). Increased expression of iNOS and bNOS has been demonstrated using Western blotting in the labouring cervix at term and iNOS expression is increased following onapristone-induced preterm labour (Buhimschi et al., 1996). Using reverse transcription–polymerase chain reaction (RT–PCR), one group of authors demonstrated an increase in iNOS mRNA in the cervix of labouring rats when compared with controls with only minor changes in the

---

**Figure 4.** Western blot analysis of cervical samples collected from non-pregnant and pregnant women using an antibody directed against inducible nitric oxide synthase (iNOS) (lanes 1–3 = non-pregnant; lanes 4–6 = pregnant first trimester; lanes 7–9 = term pregnant not in labour and lanes 10–12 = term pregnant in spontaneous labour). The putative iNOS band is seen at 130 kDa. The positive and negative controls are shown in lanes 13 and 14 respectively (see text). The figure shows representative bands from three out of the eight women in each group. Densitometric analysis showed a significantly greater expression of iNOS in the first trimester compared with non-pregnant subjects. There was a further increase by term with no change following the onset of labour (see text).

**Figure 5.** Western blot analysis of cervical samples collected from non-pregnant and pregnant women using an antibody directed against endothelial nitric oxide synthase (eNOS) (lanes 1–3 = non-pregnant; lanes 4–6 = pregnant first trimester; lanes 7–9 = term pregnant not in labour and lanes 10–12 = term pregnant in spontaneous labour). The putative eNOS band is seen at 135 kDa. The positive and negative controls are shown in lanes 13 and 14 respectively (see text). The figure shows representative bands from three out of the eight women in each group. Densitometric analysis showed a significantly greater expression of eNOS in cervical samples collected at term, compared with those collected from non-pregnant women (see text).

**Figure 6.** Western blot analysis of cervical samples collected from non-pregnant and pregnant women using an antibody directed against neuronal nitric oxide synthase (bNOS) (lanes 1–3 = non-pregnant; lanes 4–6 = pregnant first trimester; lanes 7–9 = term pregnant not in labour and lanes 10–12 = term pregnant in spontaneous labour). The putative bNOS band is seen at 160 kDa. The positive and negative controls are shown in lanes 13 and 14 respectively (see text). The figure shows representative bands from three out of the eight women in each group. Densitometric analysis showed a significantly greater expression of bNOS in the first trimester compared with non-pregnant subjects. There were no further changes in bNOS expression by term or following the onset of labour (see text).
constitutive NOS isoforms during gestation (Ali et al., 1997). In both human and animal studies, NO donors have been shown to ripen the cervix when artificially applied to this tissue (Qing et al., 1996; Chwalisz et al., 1997; Thomson et al., 1997, 1998). Furthermore, in rats the ripening process can be inhibited using L-nitro-arginine methyl ester (L-NAME), a NOS inhibitor (Buhimschi et al., 1996). Our data therefore support the hypothesis that NO is a pharmacological and physiological regulator of cervical ripening in the human.

Recent studies have described NOS expression within the human uterine cervix in samples obtained from non-pregnant women and in women following vaginal delivery at term (Tschugguel et al., 1999b). The authors found an increase in calcium-independent NOS activity (i.e. the inducible NOS activity) within the pregnant cervix following delivery using a commercially available arginine to citrulline conversion assay. However, there was no corresponding change in iNOS mRNA using RT–PCR. The authors suggested that the discrepancy between the RT–PCR and the enzyme activity was attributable to the fact that the iNOS mRNA may have been replaced by the more stable protein within the cervix thus accounting for these findings. A possible concern about this study is the effect of confounding variables such as pregnancy, labour and delivery of the baby on NOS expression. Our study has examined the expression of protein within this tissue during pregnancy and our results are in keeping with the above authors’ findings. We have demonstrated an increase in iNOS protein at term in the cervix prior to the onset of labour. However, we have clarified that the increase in NOS activity occurs prior to the onset of labour and is not merely attributable to factors associated with cervical dilatation or vaginal delivery of the fetus.

In contrast to studies performed on the rat (Buhimschi et al., 1996; Ali et al., 1997), our studies in the human cervix have demonstrated an increase in expression of iNOS towards the end of gestation with no further increase with the onset of labour at term. Similarly, eNOS expression is increased at term in pregnancy compared to the non-pregnant state. We failed to demonstrate an increase in bNOS expression at term although this isoform was increased in the first trimester of pregnancy. This would suggest that in the human NO is involved in the gradual changes in cervical extensibility and not in the acute process of cervical dilatation, which occurs with spontaneous labour, as has been demonstrated in the rat model. This supports the hypothesis that NO is involved in cervical ripening.

Using immunohistochemistry, we have demonstrated iNOS localization in the cervical vascular endothelium. This is in keeping with previous reports where iNOS has been identified in endothelial cells (Cheung et al., 1999; Purcell et al., 2000). We also demonstrated iNOS immunostaining in a proportion of cells within the tissue collected from labouring women. Using antibody directed against CD45, the common leukocyte antigen, these cells were identified as leukocytes infiltrating the tissue. These data are also in keeping with the findings of Marx et al., who have recently reported similar findings in the pregnant rat cervix (Marx et al., 2000). Tschugguel et al. also reported iNOS immunostaining in epithelial cells and in stromal spindle cells in cryosections of the human cervix. In comparison, our studies were carried out on paraffin-embedded sections of cervical tissue as this method of tissue fixation provides better morphological specimens for analysis. This however may account for the observed differences in tissue localization. The specificities of each of the NOS antibodies had been confirmed for the purpose of our studies using Western blotting analysis as previously discussed (Tschugguel et al., 1999b).

We demonstrated the presence of bNOS protein within the cervix of non-pregnant and pregnant women using both immunohistochemistry and Western blotting. Staining for bNOS was identified within the surface epithelium in both non-pregnant and pregnant subjects. bNOS was also localized to a proportion of leukocytes invading the cervix in labouring samples. This is in contrast to previous findings (Tschugguel et al., 1999b) who did not demonstrate either bNOS mRNA expression using RT–PCR or the presence of bNOS protein using immunohistochemistry. However our results are in keeping with studies recently performed in humans reporting the presence of bNOS mRNA and protein in the cervix of non-pregnant and pregnant subjects using RT–PCR and Western blotting respectively (Bao et al., 2000). Furthermore, localization of bNOS in cervical epithelium has also been confirmed previously in studies on rat uterus (Schmidt et al., 1992).

eNOS was confined to the vascular endothelium and the glandular and surface epithelium. Previous studies in rats have also shown eNOS immunoreactivity in endothelial and epithelial cells in non-pregnant animals (Chatterjee et al., 1996). Our findings support those of Tschugguel et al. (1999b), who have also reported the localization of endothelial NOS to the vasculature in non-pregnant and pregnant human cervix. However, these authors did not demonstrate eNOS immunostaining in glandular epithelia (Tschugguel et al., 1999b). The discrepancy in these results again may be accounted for by the differences in the tissue specimens studied (cryosections versus paraffin sections).

It is now acknowledged that cervical ripening is an inflammatory process involving leukocytic infiltration and release of inflammatory mediators, e.g. interleukin (IL)-1, IL-8 and tumour necrosis factor α (TNFα) (Liggins, 1981; Barclay et al., 1993; Chwalisz et al., 1994). It is therefore not surprising that the inflammatory mediator NO should be involved in these events. The exact role that NO plays in the ripening process is unclear. However, it is interesting to speculate as to the mechanism whereby NO is involved. During ripening the collagen content of the tissue decreases secondary to the action of degradative enzymes known as matrix metalloproteinases (MMPs) (Junquiera et al., 1980; Osmers et al., 1995). The composition of the constituent proteoglycans is also altered (Von Maillot et al., 1979; Uldbjerg et al., 1983). NO is capable of stimulating MMP activity and suppressing proteoglycan synthesis in other tissues (Hauselmann et al., 1994, 1998; Murrell et al., 1995; Trachtman et al., 1996; Sasaki et al., 1998) and hence may function in a similar manner in the cervix. We have shown previously that NO does not influence the activity of MMP-2 or MMP-9 in the human cervix, however...
other MMPs may be responsible for the cervical effects of NO (Ledingham et al., 1999b). NO may also contribute to the ripening process via induction of apoptosis (Nicotera et al., 1997; Brune et al., 1998), another mechanism which may be important in this physiological process (Leppert, 1992). The effects of NO may also be mediated via cross-talk with cyclooxygenase and an increase in prostaglandin synthesis which in turn would stimulate other inflammatory mediators, e.g. IL-8 and secretory leukocyte protease inhibitor (Denison et al., 1999; Ledingham et al., 1999a).

In conclusion, we have shown that all three NOS isoforms are present in the human uterine cervix in non-pregnant and pregnant individuals. Each of the isoforms of NOS is upregulated during pregnancy and a further increase in the expression of iNOS is detected prior to the onset of labour at term. This supports a role for NO in the remodelling of the cervix that constitutes the ripening process. We speculate that, in the future, it may be possible to influence cervical ripening when it occurs prematurely or is delayed, using agents directed against the NO generating system.

Acknowledgements

Dr Marie-Anne Ledingham is funded by SHERT grant 1442 for which we are grateful. We would also like to thank Dr C.B. Luan for his assistance in obtaining cervical biopsies from the first trimester subjects.

References


NOS in the human cervix during pregnancy


Received on May 17, 2000; accepted on July 24, 2000