Comparison of the effect of oestradiol, tamoxifen and raloxifene on nerve growth factor-α expression in specific neonatal mouse uterine cell types using laser capture microdissection

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Abstract

Oral dosing of CD-1 mice on days 2–5 after birth with tamoxifen but not raloxifene disrupts the development of the myometrium, resulting in adult uterine adenomyosis. Using laser capture microdissection and RT-PCR we have investigated nerve growth factor (NGF) and cognate receptor expression in uterine cells of 6-day-old pups that may be important in early developmental changes that give rise to adenomyosis. NGF down-regulation is known to occur during terminal myogenic differentiation.

NGF was found exclusively in endometrial luminal epithelium of controls. It was up-regulated 18-fold in the luminal epithelium following dosing with tamoxifen but not raloxifene. Western blotting for NGF protein in the whole uterus showed a 25-fold increase after tamoxifen treatment. Expression of the low affinity p75 neurexin receptor (p75NTR) was twofold higher in the myometrium compared with luminal epithelium or stroma. This was not altered following tamoxifen treatment. There was no detectable expression of high affinity tyrosine kinase receptor (trkANGFR).

This study shows luminal epithelial cells of the endometrium primarily form NGF. This suggests that NGF normally regulates the differentiation of the mesenchyme into uterine myocytes through paracrine mechanisms and that an early disturbance of this process plays a key role in the subsequent development of adenomyosis.

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Introduction

Oral dosing of newborn mice with tamoxifen on days 2–5 after birth results in a high incidence of adenomyosis, a benign condition characterised by ingrowth of the endometrium into the uterine musculature, sometimes associated with an overgrowth of the latter (Parrott et al. 2001). This seems to be associated with defects in the formation of the myometrium in the neonatal period. Using microarray analysis of RNA extracted from the whole uterus we have previously identified several genes which are modulated by tamoxifen during this critical phase of uterine development (Parrott et al. 2001). One key gene up-regulated in this tissue by tamoxifen is nerve growth factor (NGF)-α (Parrott et al. 2001). This may contribute to the repression of myometrial differentiation in the uterus. Besides being a key neurotrophic factor in neuronal cells, NGF proteins have a role in non-neuronal tissues, being mitogenic in both the human breast cancer MCF-7 cell line (Descamps et al. 1998, Chiarenza et al. 2001) and prostate cancer cells (Djakiew et al. 1991). This effect appears to be mediated via a high affinity tyrosine kinase receptor (trkANGFR). Tamoxifen inhibits NGF-induced proliferation of MCF-7 cells and receptor phosphorylation (Chiarenza et al. 2001). NGF can also interact with a low affinity p75 neurotrophin receptor (p75NTR) as an anti-apoptotic factor (Descamps et al. 2001). The 7S NGF protein is a member of the neurotrophin...
polypeptide family and consists of a complex of αNGF together with the active neurotrophic factor βNGF and γNGF (Bax et al. 1997). Although inactive, the subunits do however overlap the regions on the βNGF where it engages with the NGF receptor (Bax et al. 1997). The role of NGF in the uterus remains to be elucidated.

Many studies have used whole tissue to determine the effects of drugs on gene expression in tissues. However, like most organs, the uterus consists of many cell types including the luminal epithelium, stroma and myometrium. It has long been recognised that both autocrine and paracrine interactions can occur within the uterus and therefore the study of separate cell populations is important (reviewed in Reis et al. 2000). Homogenisation of tissue results in the loss of the ability to assess cell-specific gene expression. There is also a dilution effect of cells that are present in a lower number, e.g. uterine luminal epithelium compared with stroma or myometrium (Martin et al. 1973), making the detection of low copy number genes more difficult. In situ hybridisation can overcome some of these problems but at best only gives a semi-quantitative analysis (Looi & Cheah 1992).

In this paper we report using a combination of laser capture microdissection (LCM) (Emmert-Buck et al. 1996) and RT-PCR to quantify gene expression in specific cell types of the neonatal mouse uterus. LCM allows the isolation of specific cells from tissue sections, without contamination from other cell populations, that can be subsequently used for molecular analysis (Bonner et al. 1997, Luo 1999, Sgroi et al. 1999, Shen et al. 2000). We compared expression of NGF following tamoxifen administration with oestradiol as well as raloxifene, another anti-oestrogen which was previously shown not to be associated with the development of adenomyosis in mice under similar conditions.

**Materials and methods**

**Chemicals**

17β-Oestradiol benzoate was from Sigma Chemical Co., Poole, Dorset, UK. Tamoxifen and raloxifene hydrochloride were gifts from Dr T C Orton, AstraZeneca, Macclesfield, Cheshire, UK.

**Animals and treatments**

Ovariectomised adult (3 months old, n=4) female CD-1 mice were from Charles River Ltd, Margate, Kent, UK. Animals were housed in negative pressure isolators with a 12 h light:12 h darkness cycle and allowed free access to RM1 diet (Special Diets Services UK Ltd, Witham, Essex, UK) and water. The study was conducted under the authority of the United Kingdom Home Office, Animals (Scientific Procedures) Act 1986. Groups of three 6-day-old female CD-1 neonatal mice (pregnant mice were from Charles River Ltd) were orally dosed on days 2–5 after birth (day of birth is day 1) with 5·3 nmol/kg oestradiol benzoate or 2·7 µmol/kg tamoxifen or raloxifene suspended in peanut oil/lecithin/condensed milk mixture (2:0·2:3, by volume) at a dose volume of 5 µl/g body weight. Controls received vehicle only. On day 6, mice were killed and uteri removed and either snap frozen in liquid nitrogen or fixed in 3·7% neutral buffered formalin at 4 °C.

**Mouse uterine sections**

Paraffin-embedded (5 µm) or frozen (8 µm) sections of mouse uterus were cut and mounted on clean glass slides. To minimise RNase action, all solutions were made with 0·1% diethylpyrocarbonate-treated water. Frozen sections were post-fixed in 70% ethanol for 10 min at 4 °C. Slides were rehydrated in water, then washed in 70% ethanol, and industrial methylated spirits (three times) and finally dehydrated in xylene for 10 min. Sections were usually left unstained or occasionally, where indicated, counterstained with haematoxylin, 0·1% methylene blue or Mayer’s haematoxylin using standard procedures.

**LCM**

Separate populations of fixed luminal epithelial, myometrial or stromal cells were isolated from uterine sections using the PixCell II LCM System (Arcturus Engineering, Santa Clara, CA, USA). Identification of specific cells from unstained sections was judged from parallel haematoxylin-stained sections. A 15 µm or 30 µm laser beam (with varying times of pulse power 20–100 mW) and pulse width (0·5–5·0 ms) was used. An average of 100 laser shots per sample were used to transfer cells onto a CapSure cap (Arcturus Engineering).
This relates approximately to a tissue volume of \(1.12 \times 10^{-7} \text{µm}^3\), calculated by the Arcturus software program on an estimated 90% transfer rate, yielding approximately 200–300 cells per cap.

### Total RNA extraction

Total RNA was extracted from each CapSure cap using the StrataPrep Total RNA MicroPrep Kit (Stratagene Europe, Amsterdam, The Netherlands) following the manufacturer’s instructions, except that a 10 µl volume of elution buffer was used which was passed through the column twice upon elution.

### Reverse transcription

cDNA was synthesised from total RNA using random hexamers (Promega, Southampton, Hants, UK) and Superscript II RNase H-reverse transcriptase (Life Technologies, Glasgow, Strathclyde, UK) according to the manufacturer’s instructions. Negative controls, where water was substituted for reverse transcriptase, were included.

### Semi-quantitative PCR

The expression of genes for cytokeratin 19 (Krt19), glyceraldehyde-6-phosphate dehydrogenase (Gapdh), NGF (Ngfa), p75NTR (Ngfr), smooth muscle α-actin (Acta), trkA NGFR (Trka) and ubiquitin (Ub) were determined using semi-quantitative PCR by amplifying 1µl cDNA with the primer sequences shown in Table 1. Expression of each gene was amplified in duplicate in a total volume of 20 µl using either AmpliTaq Gold (Applied Biosystems, Warrington, Cheshire, UK), FastStart Taq DNA polymerase (Roche, Lewes, Sussex, UK), HotStarTaq DNA polymerase (Qiagen Ltd, Crawley, West Sussex, UK), JumpStart AccuTaq (Sigma Chemical Co.) or Platinum Taq DNA polymerase (Life Technologies) following the manufacturer’s instructions using a Hybaid Touchdown thermal cycler (ThermoHybaid, Teddington, London, UK). An annealing temperature of 60 °C was used for all primers and the number of PCR cycles ranged from 30 to 40. A negative control, where water was substituted for cDNA, was included in each PCR experiment. The resulting PCR products for each sample were electrophoresed at 100 V for 30 min in duplicate and in parallel, with a 100 bp DNA ladder (Life Technologies) as a size marker, through a 2% agarose gel in 1 × TBE with 5 ng/ml ethidium bromide (Sigma Chemical Co.) or 1 × Gelstar nucleic acid stain (Novara, Ashby-de-la-Zouch, Leics, UK) and visualised under u.v. The band densities were determined using a Kodak Image Station 440CF (Eastman Kodak Company, Rochester, NY, USA) and gene expression was normalised against the density of the corresponding Gapdh PCR product. No amplification was seen in the negative controls. Representative PCR products were verified by DNA sequencing.

### Immunocytochemistry

Formalin-fixed, 5 µm paraffin sections of 6-day-old mouse uterus were dewaxed and stained using specific antibodies for cytokeratin 19 (Dako Ltd, Ely, Cambs, UK) at a dilution of 1:100 or monoclonal anti-mouse smooth muscle α-actin (Dako Ltd) at a dilution of 1:100 using methods previously described (Parrott et al. 2001).

### Western blotting analysis

Control or tamoxifen-dosed 6-day-old mice uteri \((n=4)\) were separately homogenised and lysed in 10 µl/mg tissue ice-cold H8 buffer (20 mM Tris, pH 7.4, 2 mM EDTA, pH 7.4, 2 mM EGTA, pH 7.6), complete mini-protease inhibitor cocktail (Roche) and 30 µg/ml phenylmethylsulfonyl fluoride (Sigma Aldrich Co.) and incubated on ice for

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<thead>
<tr>
<th>Table 1</th>
<th>Mouse primer sequences for RT-PCR</th>
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<tr>
<td><strong>Primer sequences</strong> (5‘–3‘)</td>
<td><strong>Product size (bp)</strong></td>
</tr>
<tr>
<td><strong>Gene</strong></td>
<td><strong>AATGGCTCTGGGCTCTGTAAA</strong></td>
</tr>
<tr>
<td><strong>Acta</strong></td>
<td><strong>GTTCAGTGTTGGCTCTGTCA</strong></td>
</tr>
<tr>
<td><strong>Gapdh</strong></td>
<td><strong>ACCCAGAGAGACTGTGGATGG</strong></td>
</tr>
<tr>
<td><strong>Krt19</strong></td>
<td><strong>GAGACAACCTGGTCTCCTCAG</strong></td>
</tr>
<tr>
<td><strong>Ngfa</strong></td>
<td><strong>CTGCTGTGGGCTCTGTCA</strong></td>
</tr>
<tr>
<td><strong>Ngfr</strong></td>
<td><strong>CAAGGCGTGTTTCTCTCTGCAA</strong></td>
</tr>
<tr>
<td><strong>Trka</strong></td>
<td><strong>AGCTTCCCTAAGTGAAGACACAG</strong></td>
</tr>
<tr>
<td><strong>Ub</strong></td>
<td><strong>ACTGGCAGAGGAGGACAG</strong></td>
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15 min. The homogenate was transferred to a microfuge tube and centrifuged at 13 000 \(g\) for 3 min at room temperature. The supernatant was removed and heated to 100 °C for 5 min. After cooling, the lysate was loaded on a 10% SDS-PAGE gel. High precision prestained molecular weight markers (Biorad, Hemel Hempstead, Herts, UK) were also included. Gels were run at a constant current of 20 mA at room temperature. The gel was blotted overnight at 50 mA onto hybond enhanced chemoluminescent (ECL) nitrocellulose membrane (Amersham International plc, Little Chalfont, Bucks, UK). The membrane was blocked with 5% defatted milk protein (Premier Brands UK Ltd, Moreton, Wirral, UK) in phosphate-buffered saline (PBS) containing 0·1% (v/v) Tween 20 for 1 h at room temperature. It was then rinsed once in 0·1% (v/v) Tween 20 in PBS. Blots were hybridised with polyclonal IgG anti-mouse NGF (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:1000 or monoclonal anti-mouse \(\beta\)-actin (Sigma Aldrich Co.) at a 1:5000 dilution for 2 h at room temperature. Following washing (three times) in PBS containing 0·1% (v/v) Tween 20 for 10 min, the membrane was incubated with a secondary antibody of either anti-mouse-HRP (Sigma Aldrich Co.) at a dilution of 1:5000 for 1 h. The chemoluminescent signal was developed using an ECL Western blotting kit (Amersham International plc) according to the manufacturer’s instructions and quantitated over 5–10 min exposure using a Kodak Image Station 440CF.

**Statistical analysis**

Differences between groups were tested using analysis of variance with Fisher’s exact test for significance at the 5% level.

**Results**

**LCM optimisation**

Preliminary results showed that RNA from cells could be successfully isolated by LCM from sections fixed in 3·7% or 10% neutral buffered formalin, 70% or 90% ethanol or frozen. However, evaluating the efficiency of total RNA extraction showed cryostat sections resulted in the best quantity and quality of RNA, as judged by absorbance at 260 nm and 280 nm (Fig. 1). When using frozen sections, staining with methylene blue, haematoxylin or Mayer’s haematoxylin resulted in poorer recovery of cells using LCM, compared with unstained sections run in parallel. Increasing laser pulse power or width did not enhance cell recovery from the fixed stained sections (data not shown). It was therefore concluded that the use of unstained frozen sections was optimal for both LCM and subsequent RNA extraction. The use of unstained sections also eliminates any possible interference of the counterstain with downstream molecular analyses (Burton et al. 1998). Figure 2A shows a representative haematoxylin-stained section of a 6-day-old mouse uterus. The subsequent isolation of sub-populations of uterine cells by LCM onto CapSure caps is shown in Fig. 2B–D.

**Semi-quantitative RT-PCR optimisation**

The successful amplification of \(\text{Gapdh}\) over 40 PCR cycles demonstrated sufficient quality and quantity of RNA extracted from LCM samples for detection using gel electrophoresis. The addition of a negative control, where water substituted the reverse transcriptase during cDNA synthesis, showed no DNA contamination. The use of Gelstar nucleic acid stain resulted in fivefold greater sensitivity compared with ethidium bromide in agarose gels and therefore was used for all quantification by densitometry. The optimal number of cells from uterine tissue, i.e. LCM laser shots, needed in order to afford amplification of
Gapdh by RT-PCR was determined. Up to 100 laser shots/sample resulted in a detectable level of the gene (Fig. 3A). To compare the efficiency of several reverse transcriptases and Taq DNA polymerases, total RNA was extracted from an epithelial and stromal LCM sample of an ovariectomised adult mouse uterus and divided equally into three. cDNA synthesis was conducted using three different reverse transcriptases and Gapdh was amplified using PCR in parallel with five different Taq DNA polymerases, as listed in the Figure 3 Comparison of (A) Gapdh gene amplification by RT-PCR of tissue samples from frozen sections collected with an increasing number of LCM laser shots and (B) reverse transcriptase and Taq DNA polymerase efficiency in RT-PCR of Gapdh over 40 cycles of mouse uterine luminal epithelium cells isolated by LCM up to 100 shots). Lane 1 = AmpliTaq Gold; 2 = FastStart Taq DNA polymerase; 3 = Platinum Taq DNA polymerase; 4 = HotStar Taq DNA polymerase; 5 = JumpStart AccuTaq; 6 = 100 bp ladder. (C) Representative gel-electrophoresed genes amplified by RT-PCR from mouse uterine cells isolated using LCM. Lane 1 = Gapdh; 2 = Ub; 3 = Krt19; 4 = Acta; 5 = Ngfr; 6 = Ngfa; 7 = 100 bp ladder.

Figure 2 Representative 8 µm frozen tissue sections of a neonatal 6-day-old CD-1 mouse uterus. le, luminal epithelium; s, stroma; m, myometrium. (A) Stained with haematoxylin. Visualisation of specific sub-populations of cells transferred onto CapSure caps using LCM. (B) Luminal epithelium, (C) stroma and (D) myometrium. Original magnification × 20.
Materials and methods, using 40 cycles. Gapdh gene expression was amplified but with a varying degree of efficiency when comparing both reverse transcriptases and Taq DNA polymerases (Fig. 3B). Superscript II reverse transcriptase resulted in the highest amplification of Gapdh with all DNA Taq polymerases except Jumpstart AccuTaq (Fig. 3B). The combination of Superscript II reverse transcriptase and FastStart Taq DNA polymerase was superior in terms of amplification and specificity and therefore all subsequent PCR amplifications were conducted using Superscript II reverse transcriptase and FastStart Taq DNA polymerase. The optimal number of PCR cycles needed for quantification (i.e. linear phase of amplification) of all gene targets was similar at 32 cycles using FastStart Taq DNA polymerase (data not shown).

Representative amplified genes of interest using RT-PCR from uterine cells isolated by LCM and visualised by agarose gel electrophoresis are shown in Fig. 3C.

There was no difference in the amplitude of PCR product for the housekeeping Gapdh between the uterine cell types. There was a relatively lower gene expression of Ub in the myometrium (Ub/Gapdh, 9%) compared with either the luminal epithelium (35%) or stroma (38%). It was therefore decided to use Gapdh as the sole housekeeping gene for gene quantification in the uterus.

Cell marker expression

Gene expression of cell markers for epithelial (Krt19; Bartek et al. 1986) and myometrial (Acta; Skalli et al. 1987) cells was investigated to demonstrate LCM selectivity in 6-day-old and ovariectomised adult mice uteri. The results showed Krt19 to be higher in the luminal epithelium of both neonatal and adult mice compared with the myometrium (Fig. 4A). As expected, the majority of Acta expression was found in the myometrium (Fig. 4B). The pattern of Krt19 and Acta expression was emulated with immunohistochemistry using specific antibodies (Fig. 5A and C).

Uterine gene expression and regulation by oestrogen and SERMs

Using the system described above, expression for genes for Krt19 and Ngfa was investigated in the specific cell types of the 6-day-old mouse uterus in response to oestradiol, tamoxifen or raloxifene.

Cytokeratin 19

Following tamoxifen treatment, Krt19 expression was increased 4±6-fold in the luminal epithelium but not in the other cell types compared with the control (Fig. 6A). This change was emulated using
Figure 5  Immunohistochemical localisation in 6-day-old neonate CD-1 mouse uterus of cytokeratin 19 in (A) control mice, (B) tamoxifen-treated mice and (C) smooth muscle α-actin in control mice; haematoxylin counterstain. Original magnification × 25.
immunohistochemistry (Fig. 5A and B). No significant change in expression of Krt19 was observed with oestradiol or raloxifene.

**NGF**

Expression of Ngfa was exclusively found at a low level in the luminal epithelial cells of a 6-day-old mouse uterus (Fig. 6B). There was no expression of Ngfa in either the stroma or myometrium. After dosing with oestradiol, expression of Ngfa could not be detected in the luminal epithelium. In contrast, following tamoxifen treatment there was a 17-fold increase in Ngfa expression compared with the controls (Fig. 6B). Expression of Ngfa with tamoxifen treatment was also seen within the stromal layer. There was no change in Ngfa expression after raloxifene treatment (Fig. 6B). In whole uterus of the 6-day-old mouse, using an antibody directed at the mature NGF protein, Western blots show a low expression of NGF (Fig. 7). After tamoxifen treatment for 2–5 days after birth, there was a marked increase (25-fold) of NGF protein (Fig. 7).

**NGF receptor**

Expression of the low affinity p75<sup>NTR</sup> and high affinity trk<sup>NGFR</sup> for NGF were determined in the separate cell populations of 6-day-old mice uteri using LCM and RT-PCR. The low affinity p75<sup>NTR</sup> was expressed in the luminal epithelium and stroma at similar levels (Ngfr/Gapdh, 5%). The myometrium expressed twice the level of receptor mRNA (Ngfr/Gapdh, 10%). Upon tamoxifen treatment, levels of p75<sup>NTR</sup> expression were not altered in any of the cell types of the 6-day-old mouse uterus compared with controls. No expression of trk<sup>NGFR</sup> mRNA could be detected in any of the uterine cell types at 6 days although a PCR product was successfully amplified from the mouse submaxillary gland (data not shown).

**Discussion**

In this paper we describe the use of LCM to isolate luminal epithelium, stroma and myometrium from cryostat sections of newborn mice uterine tissue to determine the response of αNGF and other oestrogen-regulated genes to tamoxifen compared...
with oestradiol or raloxifene. Since the introduction of LCM in 1996 (EmmertBuck et al. 1996), it is becoming more widely used to isolate specific cell types from heterogeneous histological tissue sections and has been well analysed and demonstrated (Kitahara et al. 2001, Mariani et al. 2001, Tanji et al. 2001). Optimisation of tissue section preparation showed frozen sections best for isolating RNA from the mouse uterus, confirming a previous report (Goldsworthy et al. 1999). We found that nuclear counterstaining of tissue sections hampered the lifting of cells by the LCM system. This could be a result of further dehydration of the section causing it to adhere more firmly to the slide. Other fixatives and nuclear counterstaining may be more beneficial in tissues where the composition of cells may differ and alternative downstream applications are applied (Kohda et al. 2000, Ehrig et al. 2001, Tanji et al. 2001). The present results also showed there to be a surprising difference in the efficacy of reverse transcriptases and Taq DNA polymerases used in the RT-PCR of the samples. The difference in overall performance of the enzymes might be due to the original RNA template quality and quantity. While highly expressed mRNAs such as Ub and Gapdh were readily determined from uterine cells isolated using LCM, the present study showed that relatively weakly expressed genes including Ngfa could also be detected and quantitated using RT-PCR. In this paper we further describe the use of LCM to isolate separate sub-populations of luminal epithelial, stromal and myometrial cells from both newborn and adult mice uterine tissue. Such preparations have been used to determine expression of several genes by RT-PCR in response to tamoxifen and raloxifene.

The development of adenomyosis at 3 months of age after dosing from 2–5 days after birth with tamoxifen suggests that key genetic changes occur during these crucial days to permanently affect the events downstream by tamoxifen, but not by raloxifene. We have previously identified several gene changes by microarray studies that are altered with tamoxifen treatment in the whole uterus (Green et al. 2001, Parrott et al. 2001). We now further describe the specific cell distribution of αNGF and its response to oestradiol and SERMs in the neonatal mouse uterus using LCM.

The expression of NGF in the uterus has previously been described (Varol et al. 2000, Parrott et al. 2001). In this paper, we have established the localisation of the expression of NGF protein and the gene in the neonate mouse uterus and show it to be exclusively located in uterine luminal epithelial cells. The role of NGF in the uterus is unclear although it has been suggested that NGF is an important developmental regulator in the uterus and implicated in uterine reinnervation after pregnancy (Brauer et al. 2000, Varol et al. 2000). There is increasing evidence that NGF is under hormonal control in various tissues (Jehan et al. 1993, Veenstra et al. 1998, Pan et al. 1999) and we further demonstrate this with oestradiol in the mouse uterus. Although NGF is under positive control by oestradiol in the rat brain (Pan et al. 1999), the present results show it not to be up-regulated by oestradiol in the mouse uterus. NGF is down-regulated during differentiation of myotubes (Seidl et al. 1998) and therefore oestradiol could be a critical factor during the differentiation process in the myometrium of the uterus. Conversely, tamoxifen up-regulated NGF in the uterus of our mouse model, and continues up to 6 weeks of age after birth (authors’ unpublished observations). There is an AP1 enhancer element situated on the NGF promoter sequence which may be responsible for the NGF regulation by oestradiol and tamoxifen (Veenstra et al. 1998). Interestingly, the fact that the αNGF gene was also exclusively up-regulated by tamoxifen in the luminal epithelium similar to that of cytokeratin 19 and the AP1 sited on each gene promoter could suggest a comparable mode of induction of these two genes by tamoxifen (Veenstra et al. 1998, Choi et al. 2000). The mechanism of opposing actions of oestradiol and tamoxifen is unknown but may be due to oestrogen receptor (ER) levels; both ERα and ERβ are expressed in the 6-day-old mouse uterus (Parrott et al. 2001), which can influence the stimulation or repression of gene expression via the AP1 site (Webb et al. 1995).

To understand the possible pathways of NGF action in the neonatal uterus, we investigated the expression of the two receptors involved in NGF signalling, p75NTR and trkANFR. Although trkANFR is found in the secretory phase of the human endometrium (Shibayama & Koizumi 1996) and uterine carcinoma (Koizumi et al. 1998), we found no evidence of expression of trkANFR in the 6-day-old mouse uterus. p75NTR expression was shown in all cell types of the uterus. The highest level of p75NTR was found in the

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myometrium, confirming a previous report (Lommatzsch et al. 1999). p75NTR is involved in the paracrine action of NGF in the prostate (Graham et al. 1992) and our results suggest that a similar action could occur in the uterus. With the expression of NGF in the luminal epithelium there is also a possibility of an autocrine loop existing. As previously mentioned, the action of NGF depends on receptor expression. NGF signals via p75NTR leading to either apoptosis or cell survival; a potential role in the uterus. Down-regulation of NGF and p75NTR occurs during terminal myogenic differentiation (Erck et al. 1998, Seidl et al. 1998) and NGF enhances fibroblast migration (Micera et al. 2001). The precise mechanism by which NGF is responsible for adenomyosis is not yet fully clarified. It is clear from the present results that the NGF is produced primarily in the luminal epithelial cells and this action is up-regulated by certain ER modulators such as tamoxifen. It is proposed that paracrine signalling prevents differentiation of uterine myocytes in the mesenchyme and, over a period of time, this may permit downgrowth of the endometrium into the myometrium.

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