Steroids mediate the expression of cytoplasmic and membrane-linked components in human myometrial cells

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It is well known that the smooth muscle of the human myometrium is a target for the steroid hormones progesterone (P4) and estrogen. Progesterone is believed to participate in the maintenance of pregnancy, while estrogen is possibly involved in the process of parturition by promoting cervical dilatation. We examined the combined effects of P4 and 17β-estradiol (E2) on components of signalling pathways in human myometrial cells in vitro by immunoblotting. Long-term treatment of myometrial cells with a series of concentrations of P4 and E2 in combination caused a change in the phosphorylation status of p42/44 mitogen-activated protein kinase and of c-Jun N-terminal kinase (SAPK/JNK). P4 and E2 caused a decrease in protein expression of Gqα, Gαz, Gi1z/2 and, to a lesser extent, Gαα. The two steroids caused a decrease in the expression of the two small Gαα isoforms. Cyclo-oxygenase-2 expression was increased by 2.5-fold after steroid treatment, while proliferating cell nuclear antigen expression levels remained unchanged. These observations show that the combination of P4 and E2 influences intracellular and membrane-bound components of signal transduction pathways in human myometrial cells. The implications of the two steroid hormones on intracellular signalling pathways in the human myometrium merits further investigation.

Key words: G-proteins/kinases/myometrium/steroids

Introduction

The human uterine smooth muscle and the uterine vasculature are targets for estrogen and progesterone (P4), two known modulators of the myometrial contractile state (Maggi et al., 1992; Vagnoni et al., 1998; Rupnow et al., 2001). P4 is considered to be necessary for pregnancy maintenance and its withdrawal in many species is a key event in parturition, whereas 17β-estradiol (E2) augments the capacity for uterine contraction and cervical dilatation and therefore promotes parturition by causing myometrial contractions (Mesiano, 2001). Existing evidence on the effects of P4 and E2 on processes such as formation of gap junctions supports the concept that these steroids play a role in human parturition. While the involvement of estrogens and P4 in pregnancy is widely acknowledged, little is known about the steroid-mediated mechanisms that control the expression of key proteins regulating the excitability and contractility of the human myometrium. Previous work has shown that P4 increases intracellular calcium concentrations in myometrial cells in vitro (Fomin et al., 1999) and that estrogen promotes calcium influx into myometrial cells (Wehling, 1997).

Mitogen-activated protein (MAP) kinases are ubiquitous serine/threonine kinases that are activated by a wide variety of extracellular signals and are essential in triggering cell cycle progression (Seger and Krebs, 1995). It has been demonstrated that oxtocin acutely activates MAP kinase through an islet-activating protein-sensitive G-protein in human uterine myometrial cells, suggesting that MAP kinase may be involved in the modulation of human and rat myometrial contractility by oxtocin (Ohmichi et al., 1995, 1997; Nohara et al., 1996). Another well characterized subfamily of the MAP kinase superfamily are the stress-activated protein kinases (SAPK), also referred to as Jun N-terminal kinases (JNK), that function in a protein kinase cascade transducing cellular stress signals (Kyriakis and Avruch, 1990; Hibi et al., 1993; Derijard et al., 1994).

The presence of the rate-limiting enzyme in prostaglandin synthesis, prostaglandin endoperoxide-H synthase (cyclo-oxygenase; COX), in the non-pregnant uterus has been previously reported (Moonen et al., 1984). Two isoforms of COX exist, namely COX-1 and COX-2 (Hla et al., 1992). COX-2 is localized in human myometrial cells and it has been shown that human labour is associated with the up-regulation of prostaglandins within the uterus, synthesised via COX-2 (Moonen et al., 1985; Slater et al., 1999a,b; Sparey et al., 1999). COX-2 expression can be induced through multiple signalling pathways involving protein kinase A, protein kinase C, tyrosine kinases and lipopolysaccharides (Xie and Hershman, 1995). It is known that E2 and P4 regulate prostaglandin synthesis in bovine endometrium during the estrus cycle (Xiao et al., 1998), but the effects of these steroids on human myometrial COX-2 are unknown.

Similarly, very little is known about the effects of estrogen and P4 on G-proteins in the human myometrium. In rat myometrium, it has been demonstrated that Gi1/2 and Gqα subunits are physiological targets for both steroids in vivo (Cohen-Tannoudji et al., 1995). It is known that estrogen and P4 modulate the expression of G proteins in lactotropes (Livingstone et al., 1998).

We have previously reported on the synergistic effects of P4 and E2 on nitric oxide synthase expression in isolated myometrial cells (Zervou et al., 1999a). These observations suggest that ovarian steroids may have a profound influence on the myometrial intracellular microenvironment with important physiological implications.
Figure 1. Detection of p42/44 MAP kinase by immunoblotting in MCF-7 cells (a) and in cultured human myometrial cells (b). An antibody recognizing total p42/44 MAP kinase was used. (c) and (d): Densitometric analysis of total p42/44 MAP kinase protein levels in MCF-7 (corresponding to a) and in myocytes (corresponding to b). NS = no supplement; P+E = treatment with the combination of progesterone and 17β-estradiol, each at 5 µmol/l; A23187 = treatment with calcium ionophore. Data are expressed as mean ± SE.

Figure 2. Detection of p42/44 MAP kinase by immunoblotting, in MCF-7 cells (a) and in cultured human myometrial cells (b), as in Figure 1. An antibody recognizing the phosphorylated p42/44 MAP kinase was used. (c) and (d) Densitometric analysis of phosphorylated p42/44 MAP kinase protein levels in MCF-7 cells (corresponding to a) and in myocytes (b). NS = no supplement; P+E = treatment with the combination of progesterone and 17β-estradiol, each at 5 µmol/l; A23187 = treatment with calcium ionophore. Data are expressed as mean ± SE. *P < 0.05; **P < 0.01.
In our attempt to make progress in identifying further actions of P₄ and E₂ upon the human myometrium, we sought to investigate their combined action on cultured myometrial cells by determining their effects on the phosphorylation status of the family of p42/44 MAP kinases and SAPK/JNK kinases, on the expression of G-proteins, and on the expression of the enzyme COX-2. Unlike most other animal species including sheep, 'P₄ withdrawal' does not occur in human or primate pregnancies (Csapo and Pinto-Dantas, 1965; Mazor et al., 1993). P₄ and E₂ are present in high concentrations throughout human gestation, and no dramatic changes are seen towards the end of term. P₄ production by the placenta reaches ~300 mg per day at the end of pregnancy (Perusquia, 2001), while E₂ increases by almost 100-fold during pregnancy compared with non-pregnant levels (Mesiano, 2001). Therefore, we hypothesized that the
two steroid hormones modulate the expression of key proteins in the myometrial smooth muscle cells, causing an alteration to their profile, with an impact on uterine physiology and activity.

Materials and methods

Materials
Hanks’ Balanced Salt Solution (HBSS) with and without Ca²⁺/Mg²⁺, Dulbecco’s Modified Eagle’s Medium (DMEM), penicillin G, streptomycin, L-glutamine, fatty acid-free bovine serum albumin, P₄, calcium ionophore A23187 and E₂ were provided by Sigma (Poole, UK). Fetal calf serum (FCS) and non-essential amino acids were products from Labtech (East Sussex, UK) and Gibco Brl (Paisley, UK) respectively. Anti-phosphorylated p42/44 and anti-total MAP kinase antibodies were raised in rabbits, with a synthetic peptide corresponding to residues 345–358 of rat p42 MAP kinase (New England Biolabs, Beverly, MA, USA). The antibody against human proliferating cell nuclear antigen (PCNA) was provided by Zymed (San Francisco, CA, USA).

Antisera against the Gz α-subunit were purchased from Calbiochem (Nottingham, UK). Anti-G protein antibodies GC/2, AS/7, RM/1 and QL directed against the α-subunits, were obtained from New England Nuclear (NEN, Boston, MA, USA). All primary antibodies were raised in rabbits. AS/7, RM/1 and QL were polyclonal, whereas GC/2 and Gz antibodies were monoclonal. The anti-rabbit IgG antibodies were obtained from Sigma. All electrophoretic reagents were obtained from BioRad (Richmond, CA, USA). Anti-COX-2 polyclonal antibody was obtained from Santa Cruz Biotech (Santa Cruz, CA, USA). Polyclonal antibodies against human SAPK/JNK were produced by immunizing rabbits with a full length p54 SAPK/JNK fusion protein. The antibody was raised against a recombinant protein corresponding to amino acids 50–111 mapping near the C-terminus of COX-2 of human origin, non-cross-reactive with COX-1.

Selection of cells and experimental subjects
All tissue samples were collected from women undergoing a gynaecological operation at Women’s Hospital, University Hospitals of Coventry and Warwickshire, NHS Trust, Coventry, UK. The study was approved by the local ethics committee, and informed consent was obtained from each patient prior to operation. Myometrial biopsies were taken from the upper third of the uterine body ~5 mm away from endometrial or serosal surfaces, immediately after hysterectomy. All women recruited to the study were premenopausal and had not been exposed to steroid treatment for at least 3 months prior to the operation, and did not have either an intrauterine contraceptive device (IUD) or evidence of uterine pathology, such as fibroids or polyps. Human adenocarcinoma breast cancer MCF-7 cells were obtained from the European Collection of Animal Cell Cultures (CAMR, Centre for Applied Microbiology and Research, Salisbury, Wiltshire, UK). Although no positive controls were available for SAPK/JNK, COX-2, G protein α subunits or PCNA, MCF-7 cells were used as a positive control for MAP kinase experiments. This cell line was chosen because it expresses both estrogen and P₄ receptors. MCF-7 cells have been used in the past to characterize steroid effects on p42/44 MAP kinase. No such controls are available for the rest of the proteins studied here.

Establishment of primary myometrial cell cultures and maintenance of MCF-7 cells
Myometrial biopsies weighing ~3 g were collected from women undergoing hysterectomy for menorrhagia. Primary myometrial cell cultures were established as previously described (Phaneuf et al., 1993). Cells were re-suspended in DMEM, containing 10% FCS, 0.2% L-glutamine, 10 000 IU/ml penicillin G and 7610 IU/ml streptomycin, supplemented with P₄ and E₂. A ‘no supplement’ culture served as a negative control. Myometrial cells were plated into 25 cm² culture flasks at a density of 0.5–2×10⁶ cells/cm² and stored at 37°C in a humidified atmosphere (95% air and 5% CO₂) for up to 72 h. The purity of the myocyte cultures was assessed as previously described (Zervou et al., 1999a) using the ratio of the number of cells stained for α-actin to the total cell nuclei present. Analysis of large numbers of cells indicated that >95% of the cultured cells were identified as smooth muscle cells. All primary cultures were maintained for up to 4 days, and incubated with charcoal-stripped FCS and phenol red-free media for 24 h prior to steroid treatments. In this way, endogenous and exogenous steroids were eliminated. MCF-7 cells were maintained in phenol red-free minimum essential medium (Sigma) with 1% non-essential amino acids, 10% charcoal-stripped fetal bovine serum, 10 000 IU/ml penicillin (Sigma) and 7610 IU/ml streptomycin (Sigma).

Protein extraction from cultured cells
Media were removed and cell monolayers were washed with phosphate-buffered saline (PBS) at room temperature. A buffer containing PBS, 1% NP40 (Sigma), 0.5% sodium deoxycholate (Gibco), 0.1% sodium dodecyl sulphate (SDS; Gibco), 10 µg/ml polymethyl-sulphonyl fluoride, 30 µl/ml aprotinin and 10 µl/ml sodium orthovanadate (100 mmol/l) was added to the cultures. The cells were scrapped off the tissue culture surface with cell scrapers. The lysate was then centrifuged for 10 min at 13 000 g, at 4°C. The supernatant was removed and stored as the total cell lysate. The protein concentration was determined in all tissue extracts using the BioRad Reagent, according to the manufacturer’s instructions.

Immunoblotting
Myometrial cell lysates (80 µg) were solubilized with Laemmli buffer (5 mol/l urea, 0.17 mol/l SDS, 0.4 mol/l dithiothreitol and 50 mmol/l Tris–HCl, pH 8.0), mixed and placed in a boiling water bath for 5 min and allowed to cool at room temperature. Samples were separated on an SDS–10% polyacrylamide gel and the proteins were electrophoretically transferred to a nitrocellulose filter at 250 mA for 16–18 h in a transfer buffer containing 20 mmol/l Tris, 150 mmol/l glycine and 20% methanol. The filter was then blocked in PBS containing 0.1% Tween-20 and 5% dried milk powder (w/v) for 2 h at room temperature. After three washes with PBS–0.1% Tween, the nitrocellulose filters were incubated with each primary antibody against MAP kinase and JNK total and phospho-forms, PCNA, COX-2 and G-protein α-subunits as described previously (Karteris et al., 2000). All primary antisera were used at a 1:1000 dilution in PBS–0.1% Tween for 1 h at room temperature. The filters were washed thoroughly for 30 min with PBS–0.1% Tween before incubation with the secondary anti-rabbit HRP-conjugated Ig (1:2000) for 1 h at room temperature and further washing for 30 min with PBS–0.1% Tween. In order to detect the antibody complexes, solution A

Figure 5. Immunoblotting analysis of proliferating cell nuclear antigen (PCNA) protein levels in cultured human myometrial cells. A protein band at the size of 36 kDa was detected (a). (b) Densitometric analysis of PCNA protein levels in myocyte cultures, corresponding to (a). NS = no supplement; P+E = treatment with the combination of progesterone and 17β-estradiol, each at 5 µmol/l. Data are expressed as mean ± SE.
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Figure 6. Immunoblotting analysis of Gα protein levels in cultured human myometrial cells and corresponding densitometric analysis of expression levels. (a) and (b): Gq at 42.5 kDa; (c) and (d): Gz at 39 kDa; (e) and (f): Gs at 67, 54, 47 and 45 kDa; (g) and (h): G11/2 at 41 kDa; (i) and (j): G0 at 40.5 kDa. NS = no supplement; P+E = treatment with the combination of progesterone and 17β-estradiol, each at 5 µmol/l. Data are expressed as mean ± SE. **P ≤ 0.01.
Primary myocyte cultures were treated for up to 24 h with P₄ and E₂ combined, at a series of concentrations, optimized at 5 µmol/l or with the calcium ionophore A23187 at 10 µmol/l for 15 min. SDS-polyacrylamide gel electrophoresis was carried out using myometrial cell protein extracts from treated and untreated myocyte cultures, followed by immunodetection with antibodies against total and phosphorylated p42 and p44 MAP kinase. The ionophore A23187 caused an increase in p44 MAP kinase (Figure 2) and decreased the phosphorylated form of p42 and p44 MAP kinase (both at P < 0.05), while total p42/44 MAP kinase remained unchanged. The effect of steroids on p42/44 MAP kinase in cultured myometrial and MCF-7 cells

Immunoblotting experiments were performed, following treatment of myocyte cultures for up to 24 h with the combination of P₄ and E₂, at a series of concentrations, optimized at 5 µmol/l. A single protein band of 62 kDa was obtained in all samples, corresponding to the expected size band of human COX-2 (Figure 4). This experiment was carried out using an anti-COX-2 antibody, which does not recognize COX-1. The COX-2 protein levels were substantially increased by 2.5-fold in the steroid-treated samples compared with the untreated control myocyte cultures (P < 0.01).

Effect of steroids on PCNA

Cultured myometrial cells were treated with the combination of P₄ and E₂. Immunoblotting of protein samples showed that cultured myometrial cells contained immunoreactive PCNA, with the expected molecular mass of ~36 kDa. Treatment for up to 24 h with P₄ and E₂ combined, at a series of concentrations, optimized at 5 µmol/l did not cause a noticeable change in PCNA protein levels (Figure 5).

P₄ and E₂ effects on G-protein α-subunits

Exposure of myometrial cell cultures for up to 24 h with the combination of P₄ and E₂, at a series of concentrations, optimized at 5 µmol/l, had profound effects on the expression of the α subunits of G-proteins (Figure 6).

Detection of Gq

Probing with QL, a specific antibody for α₂q, α₁₁ (C-term) region of Gq, detected a single band at 42.5 kDa in myometrial cells. Estrogen and P₄ treatment reduced the expression of Gq by 3-fold compared with the control (P < 0.01; Figure 6a,b).

Detection of Gz

A similar effect was found on the α subunit of Gz. Probing with a specific antibody that does not cross-react with any of the other G-proteins, we were able to detect α₃ as a 39 kDa protein, which was down-regulated by almost 3-fold following treatment with P₄ and estrogen (P < 0.01; Figure 6c,d).

Detection of Gs

Immunoblotting experiments, using a specific antibody against the RM1 (C-term) region of αₛ, detected four αₛ species of apparent molecular weight 45, 47, 54 and 67 kDa in both treated and untreated myometrial cells. P₄ and estrogen treatment had no apparent effect on the two large isoforms (54 and 67 kDa), whereas it reduced the expression of the small isoforms (47 and 44 kDa) by 2.5- (P = 0.01) and 3-fold (P < 0.01) respectively (Figure 6e,f).

Detection of Gi₁/2

For this study we used an antibody (AS/7) which recognizes both α₁ and α₂. This antibody detected a band of 41 kDa in myometrial cells, indicating the presence of the Gz, α₁₁ subunit. P₄ and estrogen treatment reduced its expression by 2-fold when compared with the control (P < 0.01; Figure 6g,h).

Detection of G₀

Immunoblotting with a specific α₀ antibody (GC/2) (N-term) detected a band of 40.5 kDa in the myometrial cell extract. P₄ and estrogen treatment caused a slight reduction in G₀ expression when compared with the control (Figure 6i,j).

Discussion

In the present study, we demonstrate that P₄ and E₂ influence the expression of important intracellular and membrane-bound signalling molecules in human myometrial cells in culture. During our experimental design, the authors agreed to use P₄ and E₂ in combination, to mimic the hormonal milieu during pregnancy, since the roles of the two steroids in human pregnancy are not fully explained. Due to limited evidence on the exact role of E₂ in human pregnancy and on the extensive cross-talk between P₄ and E₂ signalling pathways in all reproductive tissues, the steroids were used in combination, in this

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preliminary survey of potential responses, in an attempt to mimic the steroid hormone milieu of pregnancy.

Estrogen is required for synthesis of P4 receptors (Aronica and Katzenellenbogen, 1991) and estrogen receptor expression is estrogen-dependent in reproductive tissues (Ing and Ott, 1999). Cross-talk between estrogen and P4 receptors has been reported, suggesting interactions between the estrogen and P4 intracellular pathways (Migliaccio et al., 1998; Katzenellenbogen, 2000).

In our study, P4 and E2 were used at relatively high concentrations to mimic the hormonal milieu of pregnancy. P4 production by the placenta reaches ~300 µg per day at term (Perusquia, 2001), while E2 increases almost by 100-fold during pregnancy (Mesiano, 2001). The concentrations of P4 and E2 were optimized at 5 µmol/l after a series of dose–response experiments (data not shown). Similar effects were exerted by P4 and E2 at concentrations up to 10 µmol/l, whereas higher amounts of the two steroids appeared to have toxic effects.

A number of time-point experiments were also performed as part of this study (data not shown). Myometrial cells were treated with P4 and E2 for up to 16 h. The effects of these steroids on protein kinases remained unchanged over the 16 h period, although some effects were evident before this time-point. A short-term incubation of up to 20 min could be part of a study on rapid, non-transcriptional events of steroids. Although myocytes were treated with steroids for up to 24 h, it was decided that steroid treatment for >16 h was not necessary for the purpose of our study. The change in the phosphorylation state of p42/44 MAP kinase in MCF-7 cells is rapid (Migliaccio et al., 1996), and therefore the cell line was treated with steroids for only 15 min. Similarly, 15 min of treatment with A23187 is the time required to initiate membrane-linked, Ca2+-associated signalling (Chen et al., 1999).

Here we demonstrate that P4 and E2 treatment of cultured myometrial cells causes a down-regulation in the phosphorylation status of p42/44 MAP kinase. This was in direct contrast to the calcium ionophore A23187, which caused an increase of the two phosphorylated protein kinases, demonstrating the activation of MAP kinase in the presence of increased Ca2+ concentrations. The effect of P4 and E2 is surprising in view of the fact that P4 increases intracellular Ca2+ concentrations in myometrial cells in vitro (Fomin et al., 1999) and that E2 promotes Ca2+ influx into myometrial cells (Wehling et al., 1997) as part of the non-transcriptional events initiated by the two steroid hormones in myometrial cells. This suggests that the effects of P4 and estrogen on MAP kinase phosphorylation status are not due to direct effects on Ca2+ mobilization. MAP kinase can also be activated in myometrial cells by G-protein-coupled receptor ligands such as oxytocin, endothelin and uroctin (Kimura et al., 1999; Molnar et al., 1999; Grammatopoulos et al., 2000).

We used MCF-7 cells only for MAP kinase experiments due to the fact that the cell line provides a very appropriate positive control, as demonstrated in a plethora of studies (Migliaccio et al., 1996; Castoria et al., 1999, Mougdil et al., 2001). The use of MCF-7 cells also shows the considerable variety of effects steroids could cause in different cell types. The same cell line could not be used as a positive control for the study of either SAPK/JNK (Caristi et al., 2001) or G protein α-subunit expression. MCF-7 cells express COX-2 (Liu and Rose, 1996). However, these cells would not be an appropriate positive control for the study of steroid effects on COX-2 expression.

In previous studies, we have shown a steroid-mediated increase of COX-2 mRNA levels in human myometrial cells in culture (Zervou et al., 1999b). In this study we confirm that this increase in mRNA is translated into increased protein expression. Our findings support the pivotal role that COX-2 has in myometrial physiology, as already demonstrated by others (Slater et al., 1999a,b; Allport et al., 2001).

In our studies the combination of E2 and P4 did not cause a noticeable change in the proliferation events of isolated human myometrial cells, as revealed by PCNA immunodetection. Our findings complement the work by Matsuo et al. and Maruo et al. in terms of showing the combined effect of P4 and E2 on PCNA (Matsuo et al., 1999; Maruo et al., 2000).

Protein kinases such as SAPK/JNK and MAP kinase are believed to interact with a wide range of G protein α subunits. Examples are the activation of SAPK/JNK by Gqi in HEK293 cells (Yamauchi et al., 2000). MAP kinase is also activated by Gqα (Jo et al., 1997). Gqi and Gts are believed to interact with Src pathways, which are closely related to MAP kinase pathways (Ram and Lyengar, 2001). Some G protein-coupled receptors are able to activate JNK in certain cell types (Naor et al., 2000). The Gi-coupled m2 muscarinic acetylcholine receptor activates JNK in COS-7 cells (Coso et al., 1996). Gq13 has also been shown to stimulate the COX-2 promoter in NIH3T3 cells (Slice et al., 1999), but no data are available on the link between Gts subunits and COX-2 expression.

In the human myometrium, a steroid-mediated expression of cytoplasmic and membrane-linked components would cause a ‘shift’ in G protein coupling to certain G protein-coupled receptors as part of tissue remodelling. A number of signal transduction pathways can be either activated or inactivated, leading to a change in smooth muscle contractile state.

This study demonstrates for the first time that ovarian steroids regulate expression of the α subunits of G-proteins in primary human myocyte cultures. Treatment with P4 and E2 markedly decreased expression of Gαq2, Gz, Gqα and Gsα and to a lesser extent G0α. Interestingly, of the four isoforms of the Gsα-subunit, only the smaller isoforms appeared to be down-regulated by P4 and E2. It is attractive to speculate that one of the mechanisms that might contribute to their down-regulation would be the effects of P4 and E2. It is known that the levels of Gαq fall at the onset of parturition (Europe-Finner et al., 1994). Moreover the fact that short isoforms are affected provides further evidence for the importance of Gαq alternative splicing. Europe-Finner and colleagues have shown that alternative splicing of Gαq precursor mRNA has a primary role in regulating expression of Gαq protein isoforms during pregnancy and labour (Europe-Finner et al., 1997).

It appears, therefore, that the α subunits of G-proteins in human myometrial cells are physiological targets for P4 and E2 in vitro. Our data are in agreement with previous findings on steroid-mediated expression of G-protein α-subunits in other tissues. In lactotropes, combined treatment with P4 and E2 leads to decreased Gi/Gq amounts (Livingstone et al., 1998). Moreover, in-vivo administration of P4 in rat myometrium has been shown to significantly reduce the amounts of Gq (Cohen-Tannoudji et al., 1995).

Classically, steroids exert their effects transcriptionally through nuclear receptors. However, recent evidence shows that steroids can influence membrane physicochemical properties (Wehling, 1997). P4 treatment has been shown to influence calcium signalling evoked by ligand stimulation of G-protein coupled receptors expressed in several cell lines (Burger et al., 1999). Many signalling mechanisms initiated by peptide hormone receptors can also be activated by membrane actions of steroid hormones (Watson and Gametchu, 1999). Such membrane-initiated responses on G-protein coupled receptors expressed in several cell types (Burger et al., 1999; Perusquia, 2001) are closely related to MAP kinase pathways (Ram and Lyengar, 2001). Some G protein-coupled receptors are able to activate JNK in certain cell types (Naor et al., 2000). The Gi-coupled m2 muscarinic acetylcholine receptor activates JNK in COS-7 cells (Coso et al., 1996). Gq13 has also been shown to stimulate the COX-2 promoter in NIH3T3 cells (Slice et al., 1999), but no data are available on the link between Gts subunits and COX-2 expression.

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Collectively, our findings indicate a number of novel P4- and E2-mediated effects on signal transduction pathways involving intracellular as well as membrane-bound components of human myometrial cells. These two steroid hormones could potentially modify the protein levels of these components and may be linked to myometrial tissue remodelling due to pregnancy.
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