Effects of progesterone on prostaglandin E₂-induced changes in glycosaminoglycan synthesis by human cervical fibroblasts in culture

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Prostaglandins are known to induce cervical ripening and this effect may be mediated by an increase in glycosaminoglycan (GAG) concentration. The aim of this study was to assess the effects of progesterone on prostaglandin E₂ (PGE₂) induced changes in GAG synthesis by human cervical cells in culture. Human cervical fibroblasts were obtained by cervical biopsies in hormonally active women and cultured. Cells were submitted to an incubation with progesterone or control medium. A second incubation was then performed with increasing concentrations of PGE₂. GAG synthesis by the cervical cells was assayed after extraction, by incorporation of [³H]-glucosamine and [³⁵S]-sulphate into GAGs. It was found that progesterone alone induced a dose-dependent increase in GAG synthesis. After pre-incubation with progesterone, PGE₂ further increased [³H]-glucosamine and [³⁵S]-sulphate uptake. However, when expressed as percentage of stimulation, the stimulatory effect of PGE₂ on GAG synthesis was inhibited at high progesterone concentrations. Therefore we concluded that, although high concentrations of progesterone increase the overall synthesis of GAG, they may also play a preventative role against PGE₂-induced changes in GAG production during pregnancy.

Key words: cervix/glycosaminoglycan/progesterone/prostaglandin/parturition

Introduction

It is now widely accepted that the cervix plays an active role in the process of labour and is not just under the control of uterine contractions. Whereas the onset of uterine contractions seems to be a sudden event, the process of cervical ripening is slow under normal conditions and appears to start early in the course of pregnancy (Uldbjerg et al., 1983). Preterm premature cervical ripening may be responsible for preterm birth and its prevention would require precise knowledge about the intimate mechanisms of cervical maturation.

It has been previously demonstrated that softening of the cervix is associated with changes in glycosaminoglycan (GAG) concentration occurring before parturition (Uldbjerg et al., 1983; Cabrol et al., 1987). The most relevant changes are an increase in total GAGs, a relative decrease in sulphated GAGs (especially dermatan sulphate), an increase in hyaluronic acid (Danforth et al., 1974; von Maillot et al., 1979; Cabrol et al., 1985; Osmers et al., 1993) and tissue water content (Uldbjerg et al., 1983; Cabrol et al., 1985). These changes precede the more dramatic events associated with collagenase activity observed during labour (Uldbjerg et al., 1983).

Progesterone is necessary to maintain pregnancy and to prevent preterm birth in many mammalian species. Contrary to these animal species (i.e. sheep or rat), parturition in humans is not preceded by a drop in maternal plasma progesterone concentration (Anderson et al., 1985; Challis and Olson, 1988). However, the theory of progesterone withdrawal is still pre-eminent, since myometrial changes in hormone and/or receptor concentration (Ferré et al., 1978; How et al., 1995) and antiprogesterone compounds have demonstrated their ability to induce cervical ripening (Carbonne et al., 1995) and to favour labour induction (Cabrol et al., 1990a, 1991; Frydman et al., 1992). On the other hand prostaglandins, particularly prostaglandin E₂ (PGE₂), are effective agents for labour induction in humans when the cervix is unripe (Rayburn, 1989) and they have been shown to induce some of the biological features of cervical ripening, at least in animal models (Cabrol et al., 1987).

The aim of this study was to assess the effects of progesterone on PGE₂-induced changes in GAG synthesis by human cervical cells in culture.

Materials and methods

Cultures of human cervical fibroblasts

Cervical biopsies were obtained after hysterectomy in hormonally active women for non-malignant lesions of the uterus, not affecting the cervix. Special care was taken in removing exo- and endocervical epithelia. Explants were minced and plated out in 60 mm diameter plastic dishes in Dulbecco’s modified Eagle’s medium (DMEM; Gibco BRL Life Technologies, Eragny, France) containing 20% inactivated fetal calf serum (FCS), glutamine and penicillin–streptomycin (100 IU/ml-100 mg/ml). Cultures were placed in a 5% CO₂ humidified incubator at 37°C. The medium was replaced every third day. After 7 days, cells started growing out from the explants and the medium was then replaced by DMEM containing only 10% FCS. Cells reached confluence after ~3 weeks. Explants were then
removed after treatment with 0.1% trypsin/0.005% EDTA, and subcultures were established in DMEM containing 10% FCS.

**GAG synthesis assay**

Cervical cells were subcultured onto 24-well plastic culture plates at a density of \(7 \times 10^4\) cells per well. When cell confluence was obtained, cultures were rinsed with phosphate-buffered saline (PBS) and the culture medium was replaced by serum-free DMEM containing 2.5 mCi/ml \([1-^{3}H]\)-glucosamine (Amersham, Les Ulis, France) and 2.5 mCi/ml \([^{35}S]\)-sulphate (Amersham) for 24 h.

GAG extraction was conducted as previously described (Wasteson et al., 1973) and modified by Redini et al. (1991). After 24 h, monolayer cultures were washed with PBS and digested with pronase (1 mg/ml; Boehringer Mannheim; Meylan, France) in 0.1 mol/l Tris-HCl (pH 7.5)/5 mmol/l CaCl\(_2\). Proteolysis was continued for 24 h at 56°C. GAGs from media and cell extracts were precipitated with cetylpyridinium chloride (CPC) 1% w/v, in presence of carriers (hyaluronic acid and chondroitin sulphate 100 mg/ml). The complex GAG–CPC was dissolved in 2 mol/l MgCl\(_2\). GAGs were then precipitated with ethanol. The final pellet was dissolved in 75 mmol/l NaCl. Radioactivity incorporated into GAGs was measured by liquid scintillation (Beckman LS3801).

**Incubations**

Before the labelling period, cervical cell cultures were pre-incubated with progesterone (Sigma, France) at concentrations ranging from \(10^{-7}\) to \(10^{-3}\) mol/l in serum-free DMEM or with serum-free DMEM alone (control medium) for 48 h at 37°C. All incubation media were oestrogen-free. Then the radiolabelled GAG precursors were added either in the control medium alone, or together with increasing concentrations of PGE\(_2\) from \(10^{-10}\) to \(10^{-5}\) mol/l for the next 24 h. The media were then removed and GAG extraction was performed as described above. Experiments were conducted simultaneously in 4 wells for each experimental condition.

**Statistical analysis**

Results were expressed as mean ± SEM in cpm/10^6 cells. Comparisons were performed using analysis of variance (ANOVA). When adequate, post-hoc inter-group comparisons were made using Scheffe test.

**Results**

**Effects of progesterone on GAG production**

The effect of pre-incubation with progesterone on cultured cervical cells was a dose-dependent increase in the incorporation of both \([^{35}S]\)- and \([^{3}H]\)-into GAGs (Figure 1). Progesterone concentration in the culture media ranged from \(10^{-7}\) to \(10^{-3}\) mol/l. Despite high progesterone concentrations (\(10^{-3}\) mol/l), the dose–response curve did not reach a plateau. When the results were expressed as a percentage of increase in GAG production, the stimulating effect of progesterone alone was proportionally more important on the incorporation of \([^{3}H]\)-than that of \([^{35}S]\)-into newly synthesized GAGs (Figure 1B). The maximum stimulation, 90% for \([^{3}H]\)-glucosamine and 30% for \([^{35}S]\)-sulphate, observed at \(10^{-3}\) mol/l progesterone, was significant when compared with incubation in control medium. Higher progesterone concentrations were not tested since they were too far out of the range observed in human plasma during pregnancy (182–690 nmol/l) (Anderson et al., 1985).

**Figure 1.** Incorporation of radiolabelled precursors, \([^{3}H]\)-glucosamine (---) and \([^{35}S]\)-sulphate (-----) into newly synthesized glycosaminoglycans. Results are expressed as: (A) cpm/10^6 cells and (B) percentage of stimulation with \(10^{-7}\) to \(10^{-3}\) mol/l progesterone, compared to incubation with control medium. *P < 0.05 when compared with control group (analysis of variance and Scheffé test for inter-group comparisons).

**Effects of PGE\(_2\) alone on GAG production**

PGE\(_2\) alone (i.e. after pre-incubation in control medium without progesterone) induced a significant increase in the incorporation of both \([^{35}S]\]-sulphate and \([^{3}H]\)-glucosamine into GAGs in a dose-dependent manner (Figures 2 and 3). The effect was observed for concentrations from \(10^{-8}\) to \(10^{-5}\) mol/l PGE\(_2\). The experiments were not pursued above \(10^{-4}\) mol/l PGE\(_2\). A plateau was reached for \([^{3}H]\)-glucosamine and \([^{35}S]\)-sulphate uptake at concentrations above \(10^{-6}\) mol/l PGE\(_2\). The stimulatory effect of PGE\(_2\) was most prominent on \([^{3}H]\)-glucosamine uptake since the maximum stimulation of incorporation into GAGs was 150% for \([^{3}H]\]-glucosamine after incubation with \(10^{-3}\) mol/l PGE\(_2\), compared to incubation with control medium (Figures 2 and 3).

**Effects of progesterone on PGE\(_2\)-induced production of GAGs**

The administration of PGE\(_2\), after pre-incubation with progesterone, led to the highest levels of \([^{3}H]\) and \([^{35}S]\) uptake (Figures 2 and 3). However, when expressed as percentage of stimulation, pre-treatment with progesterone did not alter the stimulation of radiolabelled precursors uptake obtained with PGE\(_2\) alone until the highest progesterone concentrations were reached (Figures 2 and 3). The stimulatory effect of PGE\(_2\) on \([^{3}H]\)-glucosamine incorporation was significantly inhibited at the highest concentration of progesterone (\(10^{-3}\) mol/l) (Figure...
Effects of progesterone and PGE₂ on cervical glycosaminoglycans

Figure 2. [³H]-glucosamine uptake into newly synthesized glycosaminoglycans by human cervical cells in culture with increasing concentrations of prostaglandin E₂, after pre-incubation with control medium (○○○○), or progesterone 10⁻⁷ (-- ● --), 10⁻⁵ (----- O ------) or 10⁻³ (---- ● ----). Results are expressed as: (A) cpm/10⁶ cells and (B) percentage of stimulation, compared to incubation with control medium. *P < 0.05 when compared to control group. §P < 0.05 when compared with other concentrations of progesterone during pre-incubation (analysis of variance and Scheffé test for inter-group comparisons).

Figure 3. [³⁵S]-sulphate uptake into newly synthesized glycosaminoglycans by human cervical cells in culture with increasing concentrations of prostaglandin E₂, after pre-incubation with control medium (○○○○), or progesterone 10⁻⁷ (-- ● --), 10⁻⁵ (----- O ------), or 10⁻³ (---- ● ----). Results are expressed as (A) cpm/10⁶ cells and (B) percentage of stimulation, compared with incubation with control medium. *P < 0.05 when compared with control group. §P < 0.05 when compared with other concentrations of progesterone during pre-incubation (analysis of variance and Scheffé test for inter-group comparisons).

2). The stimulation of [³⁵S]-incorporation into GAGs was also reversed, although less dramatically, at high progesterone concentrations (Figure 3). The effect of progesterone on skin fibroblasts is being investigated in order to exclude a non-specific effect of progesterone.

Discussion

In many mammalian species, progesterone is considered a key hormone in the mechanism of uterine quiescence and in the prevention of preterm delivery (Challis and Olson, 1988). However, in our study, pre-incubation with progesterone increased synthesis of GAGs by human cervical cells in culture as shown by incorporation of [³H]-glucosamine and [³⁵S]-sulphate into total GAGs. Thus, progesterone does not prevent, and high concentrations even favoured, changes in GAG production which are usually considered to reflect cervical ripening, i.e. increased total amount of GAG and increased hydrogenation of GAGs, mainly represented by hyaluronic acid (Danforth et al., 1974; von Maillot et al., 1979; Cabrol et al., 1987). Although this result may seem paradoxical, it is noteworthy that progressive changes in cervical consistency and in cervical GAG content can be observed as early as the first trimester of pregnancy (Cabrol et al., 1991; Osmers et al., 1993) despite the increasing concentration of progesterone throughout pregnancy. On the other hand, progesterone is known to favour quiescence of the uterine body and this could play a role in preventing preterm birth (Carbonne et al., 1998).

The mechanisms involved in this direct effect of progesterone on GAG production by cervical cells is currently under investigation by reversal studies with the antiprogesterone compound mifepristone.

Prostaglandins are key factors in the mechanism of parturition since they induce both uterine contractions and cervical ripening in vivo as well as in vitro (Challis and Olson, 1988, Rayburn, 1989). In our study, PGE₂ alone induced an increase in newly synthesized GAGs in cervical cells in culture. The effect of PGE₂ was more important on hydrogenated GAGs than on sulphated GAGs, as observed by Osmers et al. (1993) in cervical biopsies obtained during cervical ripening at different stages of pregnancy. This effect was dose-dependent, reaching a plateau around 10⁻⁶ mol/l. Although incubation with progesterone alone also induced an increase in GAG production by cervical cells in culture, this effect was lower than that of PGE₂ alone at concentrations usually observed in human peripheral plasma during pregnancy, suggesting a
Weaker cervical ripening effect of progesterone. When incubations with progesterone and PGE₂ were successively performed, we observed an inhibition of PGE₂-induced stimulation of GAG production by progesterone at high concentrations (10⁻³ mol/l). This phenomenon could represent a protection by high concentrations of progesterone against premature cervical ripening and eventually against preterm birth. With this hypothesis, progesterone withdrawal (necessary to allow cervical ripening at term), could be achieved through local changes in progesterone concentration or receptors (Ferré et al., 1978; How et al., 1995; Stjernholm et al., 1996) since plasma progesterone concentrations do not decrease at the end of human pregnancy (Anderson et al., 1985). This hypothesis would also be consistent with the induction of cervical ripening and of parturition by antiprogestin compounds in humans (Cabrol et al., 1990a; Frydman et al., 1992; Carbonne et al., 1995). The mechanism involved in this effect of progesterone is also under investigation. Similar experiments on skin fibroblasts are being performed in order to exclude a non-specific response to progesterone.

Cervical ripening is a multifactorial process which is only partially controlled by prostaglandins and steroids and the in-vitro model used in this study cannot take into account all its aspects. In particular, the role of inflammatory cells and mediators, involved in the process of cervical ripening and the role of other steroids and paracrine regulations could not be explored by this model. Another activity of progesterone is its ability to inhibit interleukin 8 (IL-8) release by cervical cells in vitro (Denison et al., 1999) and this could play a preventive role on cervical ripening by this indirect mechanism. These results, together with our data, suggest that progesterone could have a weak promoting effect on some aspects of cervical ripening on the one hand while, on the other hand, preventing more dramatic cervical changes induced by inflammatory mediators, e.g. prostaglandins or IL-8.

In conclusion, PGE₂ induces increased in-vitro synthesis of sulphated and hydrogenated GAGs. Although pre-incubation with progesterone increases the total amount of newly synthesized GAGs, the stimulatory effect of PGE₂ is inhibited at a high progesterone concentration.

References

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