RU486 inhibits synthesis of an endogenous inhibitor of cell arachidonate release from choriodecidua tissue

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Gravidin is a potent phospholipase A2 inhibitor that may contribute to the maintenance of human pregnancy. The aims of this study were to determine firstly, the site of gravidin synthesis within placental membranes and secondly, whether the antiprogestin compound, RU486, regulates synthesis. Membrane explants were taken from placentae, dissected and cultured in the presence of RU486, progesterone or inhibitors of DNA or protein synthesis and gravidin production was measured by a specific enzyme-linked immunosorbent assay. Production of gravidin was consistently greatest from choriodecidua compared with amnion and decidua. The antibiotics, cycloheximide and actinomycin D inhibited production of gravidin. The progesterone receptor antagonist RU486 at 10^{-8} and 10^{-7} M reduced gravidin production to 55 and 39% of the control value in membranes cultured after and before the onset of labour respectively. Progesterone at 10^{-6} M stimulated gravidin production by 47% after the onset of labour. The increase in gravidin production was correlated with progesterone concentration \( r = 0.47, F = 6.38, P = 0.019 \) in labour tissue. We conclude that the data are consistent with the hypothesis that gravidin production may be partially regulated by progesterone.

Key words: gravidin/mifepristone/ phospholipase/RU486/secretory component

Introduction

Inhibition of prostaglandin synthesis by pharmacological agents plays a major role in the control of processes as diverse as inflammation and parturition. The endogenous mechanisms of regulation of prostaglandin synthesis remain undefined but probably involve the inhibition of phospholipase A2 (EC3.1.1.4), a key enzyme in the regulation of prostaglandin synthesis. Abortion can be induced with prostaglandin treatment at any time in pregnancy after implantation and RU486 (mifepristone), which is able to cause abortion, increases prostaglandin concentrations (Haluska et al., 1994). RU486 is particularly effective after the uterus has been primed with low dose prostaglandins implying increased sensitivity to prostaglandins (Avrech et al., 1991).

We have partially characterized a phospholipase inhibitor that we have named gravidin (for review see Wilson, 1993a). It is an 80 kDa protein that has N-terminal sequence homology with the secretory component of immunoglobulin (IgA) (Wilson and Christie, 1991). However, gravidin is not identical to IgA secretory component as it can be distinguished from it with polyclonal antibody (Wilson and Christie, 1992) at a concentration of 5×10^{-9} M. Using an enzyme-linked immunosorbent assay (ELISA) for gravidin, we found elevated serum gravidin/secretory component concentrations in pregnant women (Wilson and Ganendren, 1992).

Previous work has suggested that the site of gravidin synthesis may be the placental membranes (Wilson et al., 1986). We postulated that the interaction between RU486 and prostaglandins may occur through regulation of gravidin synthesis. We therefore set out to determine the site of gravidin synthesis and the effect of RU486 and progesterone upon its production.

Materials and methods

Collection of membranes

Following a protocol approved by the Tayside Ethical Committee, placentae were collected within 15 min of delivery from women who delivered vaginally by spontaneous labour (IL) at term, and from women who were delivered at term by elective Caesarean section (NIL). The mean age of delivery was 38.6 ± 0.33 weeks and 39.3 ± 0.34 weeks (mean ± SEM) for elective sections and normal deliveries respectively. Membranes were washed well to remove traces of blood and then separated by blunt dissection into amnion, choriodecidua and decidua. The choriodecidua was mainly chorion, but contamination with decidua could not be excluded. After thorough washing in G199 medium (Gibco, Paisley, UK) containing Hanks’ salts, the tissue was cut with scissors into pieces of approximately equal size (~0.05 g w/w) and incubated at 37°C in an atmosphere of 95% air, 5% CO2, in 24-well plates containing 2 ml of medium/well (G199
with Earle’s salts, plus penicillin and streptomycin). After 6 h the medium was removed and replaced by treatment or control medium.

**Treatments**

RU486 (mifepristone) was a generous gift from Roussel-Uclaf, Paris, France. Actinomycin D, cycloheximide, progesterone, dexamethasone and cortisol were obtained from Sigma Chemicals (Poole, Dorset, UK).

Progestrone, cycloheximide and RU486, cortisol and dexamethasone were dissolved in ethanol to form stock solutions of $10^{-3}$ M and controls with appropriate dilutions of ethanol were used throughout. All incubations with test substances were performed for 13 h. Six replicate pieces of tissue were set up for each treatment and compared with untreated control tissue pieces from the same placenta. The 6–19 h interval was used in investigations as this was judged sufficiently distant from dissection trauma, yet close to time of tissue acquisition. The medium obtained at 19 h was centrifuged at 10 000 g for 5 min at room temperature to remove cell debris. Actinomycin D (at 1 or 50 µg/ml) or cycloheximide (at 10 or 100 µg/ml) were included in the incubation medium of some groups of explants from the start of treatment.

**Western Blotting of secreted tissue products**

Medium from incubated choriodecidua was concentrated to one tenth its original volume and protein precipitated with 100% ammonium sulphate after the addition of bovine serum albumin (0.05 mg/ml). The precipitate was dissolved in buffer (0.1 M Tris–HCl, pH 7.4) and run on a 7% SDS gel according to Laemmli (1970) and using pre-stained molecular weight markers from BioRad (Hemel Hempstead, UK). Several gels were blotted onto nitro-cellulose membranes and developed with either polyclonal (Sigma S1640, raised in goat) or monoclonal antibody from Sigma (I66350, 1:1000 dilution in Tris–HCl 10 mM, NaCl 150 mM and 1% Marvel, pH 7.4) or our in-house antigravidin antibody (raised in rabbit against a purified band of gravidin cut out from a polyacrylamide gel). After blotting, membranes were rinsed in buffer (Tris–HCl 50 mM, pH 7.4) and then in blocking buffer (Tris–HCl 10 mM, NaCl 150 mM, 1% Marvel, pH 7.4).

**Gravidin measurement**

Gravidin was measured using a sandwich ELISA assay as described previously (Wilson, 1993b). Anti-secretory component antibody was used in the assay for two reasons: firstly, there is no commercial source of gravidin for use as standard and in-house gravidin is in short supply; and secondly, anti-gravidin antibody does not recognize commercially obtained secretory IgA (from breast milk). On the other hand, anti-secretory component antibody detects both gravidin and secretory component. Briefly, 96-well plates were coated with a monoclonal antibody to secretory component Sigma catalogue number S1640 (1:1000 dilution in Tris–HCl 10 mM, NaCl 150 mM and incubated overnight with 100 µl choriodecidual media (1:8000 dilution in Tris buffer (50 mM, pH 7.4) containing 0.15 M NaCl. The second antibody was polyclonal anti-secretory component antibody from Sigma (S1640), and it was detected with an anti-IgG peroxidase-bound antibody. Intra-assay variation was <10% and inter-assay variation was 13%. Serum IgA from colostrum was used as standard and the concentration ranged from 5 µg to 100 ng/ml. The lowest detectable level of standard was 50 ng/ml.

**Protein determinations**

Tissue was sonicated in 0.1 M NaOH until completely dispersed (as indicated by no precipitate on centrifugation) and an aliquot of the supernatant was assayed for protein determination using the method of Bradford (1976).
Table I. Gravidin production: ratios between placental tissues

<table>
<thead>
<tr>
<th>Placenta</th>
<th>Choriodecidua/amnion</th>
<th>Decidua/amnion</th>
<th>Gravidin production</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.38</td>
<td>0.99</td>
<td>Gravidin production</td>
</tr>
<tr>
<td>2</td>
<td>4.67</td>
<td>1.62</td>
<td>Gravidin production</td>
</tr>
<tr>
<td>3</td>
<td>5.46</td>
<td>2.67</td>
<td>Gravidin production</td>
</tr>
<tr>
<td>4</td>
<td>2.31</td>
<td>1.48</td>
<td>Gravidin production</td>
</tr>
</tbody>
</table>

Figure 2. Gravidin production by choriodecidua before (following elective Caesarean) and after the onset of labour. Pieces of choriodecidua were prepared and cultured under the conditions described in the Materials and methods section. The medium was replaced after 6 h incubation and the incubation continued for a further 13 h when gravidin concentrations were measured by enzyme-linked immunosorbent assay. Results are expressed as mean ± SEM and the numbers in brackets are the numbers of placentae.

Treatment with actinomycin D and cyclohexamide

To determine whether synthesis and secretion of gravidin were taking place, actinomycin D (an inhibitor of transcription) and cycloheximide (an inhibitor of translation) were added separately to the incubations. Actinomycin D (1 µg/ml) reduced gravidin production by 38% whereas cycloheximide (10 µg/ml) reduced gravidin production by 42% over 13 h (Figure 3). The effect of antibiotics was significant ($P < 0.02$; two-way analysis of variance), but the difference between antibiotics was not significant (Figure 3).

Treatment with RU486

Addition of RU486 to culture media resulted in a decrease in gravidin production as shown in Figure 4. Because of the variation between patients in the amount of gravidin produced, the results are expressed as a percentage of control production for each tissue. At a concentration of 10–8 M, RU486 reduced gravidin production by 31% in NIL tissue and 55% in IL tissue. In a two-way analysis of variance, the difference between IL and NIL tissue was not significant, but was significant between RU486 concentrations ($F = 5.58; P = 0.003$). Using Dunnett’s multiple comparison post-hoc test, both RU486 concentrations inhibited gravidin production significantly compared with the control ($P < 0.01$).

Discussion

RU486 increases prostaglandin concentrations; this may occur through inhibition of the enzyme prostaglandin dehydrogenase (Kelly and Bukman, 1990). However, it is generally agreed that the observed increases in prostaglandin production are insufficient for induction of labour (Kelly, 1992). Hence, inactivation of prostaglandin dehydrogenase may not be the only mechanism whereby RU486 causes induction. A different explanation is that RU486 may stimulate prostaglandin syn-
The effect of RU486 on gravidin production by choriod decidua before (NIL, elective Caesarean) and after (IL) the onset of labour. Pieces of choriod decidua were prepared and cultured under the conditions described in the Materials and methods section and incubated with RU486 for 13 h, after which gravidin concentrations were measured by enzyme-linked immunosorbent assay. Results are expressed as mean ± SEM and the numbers in brackets are the numbers of placentae. Results obtained with treated tissue are expressed as a percentage of those from untreated control tissue from each placenta. A two-way analysis of variance with Dunnett’s post-hoc test was performed. *Significantly different from control (P < 0.05); **significantly different from control (P < 0.001).

Addition of RU486 to the culture media caused a substantial inhibition of gravidin production (Figure 4). Concentrations of 10⁻⁸ M RU486 were effective in reducing production to 45% of the control in tissue taken after the onset of labour. This is of the same order as reported serum concentrations following pharmacological administration of RU486. Pharmaco-kinetic investigations have shown that 2 days after a 600 mg dose of RU486, serum values are of the order of 3 µM (Heikinheimo et al., 1990) of which it is estimated that 98% is bound to α₁-acid glycoprotein. This would leave an effective serum value of 2×10⁻⁸ M in women receiving 600 mg of RU486.

Surprisingly, progesterone was not as effective at stimulating, as RU486 was at inhibiting, gravidin production. However, a dose–response curve was obtained for IL choriod decidua and, compared with the control, a significant stimulation was found with a concentration of 10⁻⁶ M progesterone (Figure 5). It is possible that the effect of RU486 was not progesterone-related (e.g. acting through inhibition of the glucocorticoid receptor). It is also possible that progesterone concentrations in the tissue were already high and the steroid was not easily reduced in the control tissue. A different explanation is that the effects of progesterone on gravidin synthesis are somehow impaired at term.

The effects of progesterone and corticosteroids were not the same (see Results), indicating that RU486 did not act through the glucocorticoid receptor. In fact, the response to dexamethasone was similar to RU486 at 10⁻⁶ M although a lower concentration of dexamethasone was not investigated.

It has been suggested that RU486 may cause induction of labour through inhibition of prostaglandin dehydrogenase.

Figure 5. The effect of progesterone on gravidin production by choriod decidua before and after the onset of labour. Pieces of choriod decidua were prepared and cultured under the conditions described in the Materials and methods section and incubated with progesterone at different concentrations for 13 h after which gravidin concentrations were measured by enzyme-linked immunosorbent assay. Results are expressed as a percentage of those from untreated control tissue from each placenta. A regression of progesterone concentration was performed for IL and NIL (elective Caesarean) tissues. This was significant for IL tissues (r = 0.47, F = 6.38, P = 0.019).
Kelly and Bukman (1990) showed reduced metabolism of prostaglandins in chorion from guinea pigs given the high dose of 10 mg/animal. Subsequent immunohistochemical studies using lower doses of RU486 showed an insignificant reduction in chorion prostaglandin dehydrogenase concentrations, but a marked reduction in decidual concentrations (Cheng et al., 1993).

Gravidin is a potent inhibitor of phospholipase activity (Wilson et al., 1989). Inhibition of gravidin synthesis would not normally cause an increase in prostaglandin production unless a stimulator was present. RU486 has been shown to increase prostaglandin synthesis in several systems although the results have been variable and quite modest (Kelly, 1992). This is consistent with RU486 inhibition of a phospholipase inhibitor. Whether prostaglandin production did increase would depend on the presence of stimulators or the amount of damage to the tissue during dissection. The observation that the effect on prostaglandin synthesis is greater in vitro than in vivo (Kelly, 1992), is consistent with such a hypothesis.

In summary, the effect of RU486 on gravidin production is consistent with the observed stimulation of prostaglandin synthesis by fetal membranes and may provide a mechanism.

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References

RU486 and gravidin production


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