CONTROL OF FETAL MEMBRANE PROSTAGLANDIN E2 PRODUCTION BY BACTERIA

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Abstract. The effect of bacteria on the production of prostaglandin E2 (PGE2) and PGE2 metabolites by fetal membranes has been investigated. Live bacteria stimulated a large increase in the levels of PGE2 metabolites, but only a small increase in the levels of PGE2 on the fetal side of the membranes. No significant changes in the levels of PGE2 or its metabolites were found on the maternal side. Bacteria may therefore stimulate PGE2 production by fetal membranes during short-term incubations, but it seems that the metabolic capacity of the chorion was so high that no change in PGE2 levels was detectable on the maternal side of the fetal membranes. This was confirmed by the finding that < 1% of ³H-PGE2 added to the fetal side of the membrane reached the maternal side without being metabolized.

Key words: fetal membrane, amnion, chorion, decidua, prostaglandin E2, bacterial infection

A number of studies have shown an association between maternal infection and pre-term labor (1-3). It has been suggested that bacterial components, particularly phospholipase A2 (PLA2) may be involved, since higher levels of this enzyme seem to be present in some of the bacteria involved in maternal genital infections than in a normal vaginal commensural organism such as the lactobacillus (4). Furthermore, both PLA2 and bacterial sonicates have been demonstrated to increase PGE2 production from cultured amnion cells (5,6). However, very little attention has been devoted regarding whether this PGE2 can actually cross the intact fetal membrane. The chorion contains enzymes that convert PGE2 to the 13,14-dihydro-15-keto-metabolite (PGE2m) (7), which is not thought to be involved in the initiation of labor.

The aim of this study was to determine whether bacteria would stimulate PGE2 production by the intact fetal membrane, as predicted from the above results, and whether this PGE2 would be metabolized by the enzymes in the chorion. The effects of bacteria on the transfer of ³H-PGE2 from the fetal to the maternal side of the cultures were also investigated.

MATERIAL AND METHODS

Intact fetal membranes were obtained at the time of elective cesarean section from full-term pregnancies in which there was no evidence of infection. The tissue was collected into phosphate-buffered saline (PBS) which contained 10 units/ml of heparin to reduce the formation of blood clots. All the remaining procedures were carried out under sterile conditions. After extensive rinsing in PBS to remove the heparin, intact membrane was placed over the ends of small glass tubes and held in place with silicone rubber O-rings. Excess tissue was removed with a scalpel. Silicone rubber washers were used to support the tubes in the lid of a 12-place multiwell, in which holes had been drilled (Fig. 1). The membranes were cultured with 1.5 ml of medium 199 (containing 10% horse serum and 1% L-Glutamine (Gibco)) on each side, at 37°C in 95% air; 5% CO2. An intact multiwell lid was placed over the whole system to maintain sterile conditions (Fig. 1). The viability of the cells in the membranes was assessed by diaphorase histochemical staining (8). The orientation of the membrane did not affect the results,
covering lid
silicone rubber washer
supporting lid
glass tube
medium
silicone rubber ring
fetal membrane

Fig. 1. Diagram of the fetal membrane culture system.

although for consistency the membrane was usually cultured with the amnion downwards.

An overnight incubation was used to allow the cells in the membrane to recover from the trauma of delivery and the establishment of tissue culture. Fresh medium was then added to both sides of the membrane. Intact bacteria (10^6–10^7 organisms/well) were added at the same time as the fresh medium. The incubations were then continued for 6 h when the medium from both sides of the membrane was removed and stored separately at −20°C until assay for PGE2 and PGE2 metabolites.

In order to investigate the transfer of PGE2 from the fetal to the maternal side of the membrane under different conditions, 3H-PGE2 (0.5 µCi/well) was added to the fetal side of the cultures in the presence or absence of bacteria on both sides of the membrane, and the incubation continued for a further 4–8 h. The medium was removed from either side of the membrane and frozen separately at −20°C. The distribution of radioactivity into 3H-PGE2 and its metabolites were determined by HPLC after the samples had been prepared with Sep-paks (9). Automatic on-line radioactivity monitoring (Ramona LS-5) detected the major peaks and the number of counts in each peak was automatically recorded.

Standard strains of lactobacilli, e. coli and streptococci were obtained from the Department of Microbiology, R.P.M.S. Group B streptococci were also isolated from pre-term infants with sepsis and their infected mothers, together with three control strains isolated from mothers who had given birth at term to normal uninfected infants (strains stored at M.M.C., Aarhus, Denmark). Bacteria were grown under standard conditions on solid media (Mr D. Adams, Dept. of Microbiology, R.P.M.S.) and harvested after 24 h of culture. The bacteria were used immediately and numbers of intact bacteria were obtained by culture of serial dilutions of an aliquot of the sample.

PGE2 was measured with an in-house RIA (antibody: ICN Biomedical; label: Amersham International; standard: Sigma), which had inter- and intra-assay variations of 10% and 8%, respectively. Total PGE2 metabolites were measured as bicyclic PGE2 (11-Deoxy-13,14-dihydro-15-keto-1β,16β-cyclo-PGE2) with a commercial RIA (Amersham International). This kit measures both 13,14-dihydro-15-keto-PGE2 (PGE2m) and 13,14-dihydro-15-keto-PGA2 (PGA2m).

RESULTS

Most (>95%) of the PGE2 produced by the membrane was detected as PGE2 metabolites under the culture conditions used (Table I). Group B Streptococci were added at a concentration of 10^7/ml to the fetal, maternal or both sides of the cultured fetal membrane. Only when the bacteria were added to both sides was there a significant increase in PGE2 and PGE2 metabolites (Table I). Total fetal side

Table I. Production of PGE2 and PGE2 metabolites by human fetal membranes

<table>
<thead>
<tr>
<th>Bacteria added</th>
<th>Maternal side</th>
<th>Fetal side</th>
<th>Both sides</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.30±0.11</td>
<td>0.59±0.15</td>
<td>1.27±0.61</td>
</tr>
<tr>
<td>Maternal</td>
<td>1.24±0.39</td>
<td>2.33±0.53</td>
<td>3.87±0.16*</td>
</tr>
<tr>
<td>Fetal</td>
<td>75.4±3.8</td>
<td>69.1±15.6</td>
<td>63.2±7.7</td>
</tr>
<tr>
<td>Concentration of PGE2 metabolites (ng/ml)</td>
<td>80.4±7.4</td>
<td>95.7±17.6</td>
<td>99.0±4.6</td>
</tr>
</tbody>
</table>

Effects of Group B Streptococci on PGE2 and PGE2 metabolite levels after a 6 h incubation. Culture conditions were as described in the Methods section. All data are means±S.E.M. (n=3), and are typical of three experiments. * p<0.05 vs no addition of bacteria.

PGE levels (PGE2 + metabolite levels in 1.5 ml of medium) increased by over 90 ng in 6 h, but only about 5 ng of this was unmetabolized. Lactobacilli had no effect on the production of PGE2 or its metabolites.

The transfer and metabolism of $^3$H-PGE2 across the fetal membranes was also investigated. Very little $^3$H-PGE2 crossed from the fetal to the maternal side of the membranes without being metabolized (<1% of the total PGE2 added) (Fig. 2). In a series of experiments, the mean transfer of $^3$H-PGE2 was $0.7 \pm 0.1\%$ (mean ± S.E.M., n = 8) of the total added, after incubations of 4–8 h. In the presence of Group B streptococci or E. Coli, added to the maternal side of the cultures, $0.9 \pm 0.3\%$ (mean ± S.E.M., n = 8) of the original PGE2 was transferred. Pre-incubation of the fetal membrane with bacteria for up to 12 h did not affect the transfer of $^3$H-PGE2 across the membrane, compared with the control transfer of $0.8 \pm 0.3\%$ (mean ± S.E.M., n = 9).

**DISCUSSION**

Intact fetal membranes produce high PGE2 concentrations, but the bulk (95%) was found as inactive metabolites. The addition of bacteria to both sides of intact fetal membranes resulted in a significant increase in the production of PGE2 metabolites and PGE2 on the fetal side of the membrane. The increase in PGE production on the fetal (amnion) side of the cultures is consistent with earlier studies which showed that isolated amnion cells can respond to bacteria or bacterial sonicates by increasing PGE2 production (5, 6), but it seems that most of this PGE2 is metabolized by the chorion and is therefore not available to initiate labor. The addition of $^3$H-PGE2 to the fetal side of the fetal membranes confirmed that most PGE2 from the fetal side is metabolized during transfer to the maternal side of the cultures.

In conclusion, we found no evidence that bacteria increased PGE2 levels on the maternal side of our cultures. This suggests that bacteria and bacterial enzymes (e.g. PLA2) may not have a direct role in initiating labor by stimulating PGE2 production by amnion, but bacteria may be involved by activating decidual cells (10,11), allowing access of active PGE2 to the myometrium.

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**REFERENCES**


