DISTRIBUTION OF PROSTAGLANDINS E₂ AND F₂α WITHIN THE FOETOPLACENTAL UNIT THROUGHOUT HUMAN PREGNANCY

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SUMMARY

The concentrations of prostaglandin E₂ and F₂α have been measured by radioimmunoassay in portions of cord, placenta, amnion, chorion, decidua and myometrium. The samples were obtained at defined periods of pregnancy, and the results have been compared with those obtained from the analyses of endometrial and myometrial tissue removed from women during the secretory phase of a menstrual cycle.

The results showed that during pregnancy the mean concentration of prostaglandin E₂ was higher (27-518 %) than the corresponding value for prostaglandin F₂α in all tissues. At term the concentration of prostaglandin E₂ (ng/100 mg wet weight of tissue, mean ± S.D.) was higher in the umbilical cord (5.54 ± 0.88), decidua (4.02 ± 1.78) and myometrium (4.19 ± 1.06), than in the amnion (2.25 ± 1.27), chorion (1.64 ± 0.63) or placenta (1.04 ± 0.25). During labour there was a significant rise ($P < 0.0005$, Student's ‘t’ test) in the concentration in decidua (10.76 ± 4.45), and to a lesser extent ($P < 0.05$) in the myometrium (5.84 ± 2.65) and amnion (4.77 ± 2.51). The overall concentration in decidua during the first trimester (3.09 ± 1.02) was significantly lower ($P < 0.005$) than in endometrial tissue (16.82 ± 10.13). The concentration was lower in myometrial tissue from non-pregnant subjects (2.90 ± 2.21), than in the corresponding tissue removed at term (4.19 ± 1.06) or during labour (5.84 ± 2.65).

The results for prostaglandin F₂α showed a similar pattern, but the values were significantly lower in the umbilical cord, and the percentage changes in concentration in the decidua and myometrium were of a higher magnitude.

INTRODUCTION

The primary prostaglandins and some of their metabolites have been identified in various tissues and fluids adjacent to the foeto-placental unit. The initial investigations showed that lipid extracts of human amniotic fluid contained muscle-stimulating activity (Hanlon, Coquoin-Carnot & Pignard, 1955; Ferraris, 1958; Hawkins, 1962). Subsequent work on the composition of this fluid resulted in the tentative identification of prostaglandins E₁, E₂, F₁α and F₂α (Karim, 1967; Karim & Devlin, 1967). Further studies with bioassay methods showed the presence of prostaglandins in decidua (Karim & Devlin, 1967), umbilical cords and placental arteries (Karim, 1967; Hillier, 1972).

Recent investigations have confirmed that a large proportion of the prostaglandins are produced and metabolized at, or near, their site of action. This finding, together with the location of the foeto-placental unit, and the weight of tissue required for analysis has impeded comparative studies on the concentration of these compounds in tissues throughout
pregnancy. Accordingly, the aim of the present investigation was to develop sensitive, accurate methods for the measurement of prostaglandins $E_2$ and $F_2\alpha$ in six tissues at defined periods of pregnancy. In addition, the results have been compared with the corresponding levels in endometrial and myometrial tissue from non-pregnant women. The study was undertaken to obtain more information on the role of prostaglandins in parturition.

**MATERIALS AND METHODS**

**Subjects and tissues**

Specimens of decidua and placenta were removed from 55 women undergoing termination of pregnancy by vacuum aspiration of the uterus during the first trimester. The age of each specimen was calculated in weeks from the estimated day of ovulation. During late pregnancy (36–38 weeks), or labour, pieces of decidua, myometrium, cord, membranes and placenta were obtained from 17 patients, who were undergoing elective (nine) or emergency (eight) Caesarian section. In addition, samples of endometrium and myometrium were obtained during the secretory phase of the menstrual cycle from 33 subjects who were undergoing hysterectomy or curettage. The tissues were placed on ice and transferred to the laboratory. Excess blood was removed with blotting paper, and the specimens were stored at $-15\,^\circ\mathrm{C}$.

**Reference prostaglandins and labelled markers**

Prostaglandins $E$, $F$, $A$ and $B$, and the 13,14-dihydro-15-oxo metabolites of prostaglandins $E_2$ and $F_2\alpha$ were kindly donated by the Upjohn Company, Kalamazoo, Michigan, U.S.A. Standard solutions were prepared in methanol and stored in sealed ampoules at $-15\,^\circ\mathrm{C}$.

$[\text{5,6,8,11,14,15(\text{n})^3\text{H}]\text{Prostaglandin E}_2\text{, sp.act. 150 Ci/mmoll, and }[\text{9}^3\text{H}]\text{prostaglandin F}_2\alpha\text{, sp.act. 15 Ci/mmoll, were obtained from the Radiochemical Centre, Amersham, Bucks. Solutions containing 10 }\mu\text{Ci/ml were prepared in ethanol:water (7:3, v/v) and stored at }-15\,^\circ\mathrm{C}.$

The purity of both batches was checked initially by thin-layer chromatography on silica gel G in the system benzene:dioxane:acetic acid (20:20:1, by vol.). In addition, the binding of both labelled antigens to their respective antisera was monitored throughout the course of the study, and it was found that the overall reduction in both assays was less than 10% over a period of 3 months.

**Antisera**

Antisera were raised in rabbits to prostaglandins $E_2$ and $F_2\alpha$ linked at carbon 1 to bovine serum albumin. The specificity of the batches of antisera to prostaglandin $E_2$ was assessed by comparing the relative potencies of related substances to compete with tritiated prostaglandin $E_2$ for binding sites on the antibodies. The percentage of cross-reactions was calculated from the relative amounts of authentic prostaglandin $E_2$ and the test compounds required to reduce the initial binding of labelled prostaglandin $E_2$ by 10% and 50%. According to this method, the best batch of antiserum had a cross-reaction with prostaglandin $E_2$ of 28.3% and 36.7% respectively. The cross-reactions of prostaglandins $A_2$, $B_2$, $F_2\alpha$ and 13,14-dihydro-15-oxo-prostaglandin $E_2$ were all less than 2%. The antiserum to prostaglandin $F_2\alpha$-1-bovine serum albumin has been evaluated, and the results reported (Hennam, Johnson, Newton & Collins, 1974). Under the same conditions the cross-reaction with prostaglandin $F_2\alpha$ was 5.2% and 1.9% respectively.

**Extraction and radioimmunoassay**

An appropriate amount of tissue (100–500 mg) was weighed accurately, and homogenized with 4 volumes of ice-cold potassium phosphate buffer (0.1 mol/l), acidified to pH 3 with citric acid (2.5 mol/l) immediately before use. Internal standards were added (4000 d.p.m. denated with 3 with...
tritiated prostaglandin E\(_2\) or F\(_{2\alpha}\)), and the homogenate plus washings (0·5 ml) were centrifuged (0 °C; 2500 g; 15 min). The supernatant was extracted immediately with freshly distilled diethyl ether (1 × 10 ml; 1 × 5 ml). The combined organic layers were extracted (2 × 5·0 ml) with Tris–HCl buffer (0·05 mol/l) at pH 7·5. Arachidonic acid remained in the organic phase, and the buffer which contained the prostaglandins was adjusted to pH 3·5 with 0·25 M-citric acid (0·2 ml) and re-extracted with diethyl ether (1 × 10 ml; 1 × 5 ml). The ether was removed and evaporated at 37 °C.

The concentrations of prostaglandin E\(_2\) and prostaglandin F\(_{2\alpha}\) were determined in all samples by methods based upon the principles of radioimmunoassay. Details of the procedure and a complete evaluation for the measurement of prostaglandin F\(_{2\alpha}\) in peripheral venous plasma have been published (Hennam et al. 1974). The salient features of both methods, as applied to the various tissues analysed in the present study, are as follows.

The dried extract was dissolved in 1 ml of tricine buffer (pH 7·4). An aliquot (400 µl) was removed to determine the amount of label recovered, and the sample was diluted for assay (1:10 or 1:5 for prostaglandin E\(_2\), and 1:3 or 1:1 for prostaglandin F\(_{2\alpha}\)). Duplicate samples (100 µl) were equilibrated with 100 µl of suitably diluted antiserum in buffer for 30 min at 4 °C. Then 20000 d.p.m. of tritiated prostaglandin was added to a further 100 µl of buffer and the mixture incubated for an additional 30 min at 4 °C.

The antibody-bound material was precipitated by the addition of 1·0 ml of a mixture of ammonium sulphate (65 % saturated solution) and calcium sulphate dihydrate (40 mg of powder in suspension). After centrifugation (6 °C; 2500 g; 10 min) the supernatant was discarded and the precipitate resuspended in water (400 µl). Scintillation fluid (1·0 ml) was added to each tube. The tubes were capped and the contents mixed. The tubes were placed in counting vials, which were transferred to a liquid scintillation counter, and the absolute amount of radioactivity was determined in each sample by a channels ratio method.

Known amounts of the reference prostaglandins (from 0–1 ng) were treated in an identical manner, and standard curves were constructed by plotting the mass on the abscissa against radioactivity bound on the ordinate. The amount of both prostaglandins in every sample was determined and the value corrected for experimental losses, the aliquot taken for assay, and the initial weight of tissue. The results are expressed as ng prostaglandin/100 mg wet tissue.

**Evaluation of method**

The reagent blanks were determined by using an equivalent volume of phosphate buffer (0·1 mol/l) in place of tissue, under the least favourable conditions with respect to tissue weight and fraction taken for assay. Under these circumstances the value (ng/100 µl; mean ± S.D.) was 0·38 ± 0·28 for prostaglandin E\(_2\), and 0·18 ± 0·40 for prostaglandin F\(_{2\alpha}\). The maximal sensitivity of the procedures (defined as that value which is significantly different from the method blanks) may be calculated as 0·50 ng/100 mg wet tissue for prostaglandin E\(_2\) and 0·32 for prostaglandin F\(_{2\alpha}\). The recovery of internal standards, expressed as a percentage (mean ± S.D.), was 63·60 ± 6·22 and 73·57 ± 6·69 from buffer, and 54·99 ± 11·01 and 62·64 ± 11·84 from tissue, for prostaglandins E\(_2\) and F\(_{2\alpha}\) respectively. The percentage recovery (mean ± S.D.; 8 determinations) of known amounts (5 ng, 10 ng) of either prostaglandin E\(_2\) or F\(_{2\alpha}\) added to tissue that had been assayed previously was 108 ± 6. In addition, the intra- and inter-assay precision (coefficient of variation, %) for both compounds—as assessed from the analysis of samples in duplicate—was 9·3 and 14·2 respectively.

To test further the validity of the procedures as applied to tissue, different weights of endometrium (100, 200, 300 and 500 mg) were extracted, and the prostaglandin content determined. The values (ng/tissue) for prostaglandin E\(_2\) were 26·8, 62·6, 98·5 and 170·0, and
for prostaglandin $F_{2a}$ 22·3, 44·8, 67·3 and 112·0. The straight lines which best fitted these values were calculated according to the method of least squares, and the coefficients of linear regression were 0·998 for prostaglandin $E_2$, and 0·984 for prostaglandin $F_{2a}$.

**RESULTS**

The concentrations of prostaglandins $E_2$ and $F_{2a}$ in seven different tissues at defined times from non-pregnant or pregnant women are shown in Table 1.

*At term*

The analysis of tissue removed at term showed that the concentration of prostaglandin $E_2$ was higher than that of prostaglandin $F_{2a}$ ($P < 0·01$, Student’s ‘$t$’ test). The placenta contained the most similar amounts, while the umbilical cord had the most divergent.

The concentration of prostaglandin $E_2$ was higher in the umbilical cord ($P < 0·0005$), decidua ($P < 0·025$) and myometrium ($P < 0·0005$), than in the amnion, chorion or placenta. The concentration of prostaglandin $F_{2a}$ followed a similar pattern, in that the decidua and myometrium contained the highest concentration and the amnion contained more than the chorion ($P < 0·0005$) and placenta ($P < 0·025$).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Prostaglandin</th>
<th>Non-pregnant women</th>
<th>First trimester pregnancy</th>
<th>At term</th>
<th>During labour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placenta</td>
<td>$E_2$</td>
<td>—</td>
<td>1·26 ± 0·49</td>
<td>1·04 ± 0·25</td>
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<td></td>
<td>$F_{2a}$</td>
<td>—</td>
<td>0·76 ± 0·35</td>
<td>0·82 ± 0·34</td>
<td>0·96 ± 0·38</td>
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<td>Cord</td>
<td>$E_2$</td>
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<td>5·54 ± 0·88</td>
<td>5·58 ± 1·86</td>
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<tr>
<td></td>
<td>$F_{2a}$</td>
<td>—</td>
<td>1·07 ± 0·24</td>
<td>1·19 ± 0·41</td>
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</tr>
<tr>
<td>Amnion</td>
<td>$E_2$</td>
<td>—</td>
<td>2·25 ± 1·27</td>
<td>4·77 ± 2·51</td>
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<tr>
<td></td>
<td>$F_{2a}$</td>
<td>—</td>
<td>0·98 ± 0·45</td>
<td>1·12 ± 0·55</td>
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<tr>
<td>Chorion</td>
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<td>$F_{2a}$</td>
<td>—</td>
<td>0·54 ± 0·23</td>
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<td>4·02 ± 1·78</td>
<td>10·76 ± 4·45</td>
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<td></td>
<td>$F_{2a}$</td>
<td>13·89 ± 10·98</td>
<td>1·41 ± 0·76</td>
<td>1·54 ± 0·98</td>
<td>6·44 ± 3·53</td>
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<tr>
<td>Myometrium</td>
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<td>—</td>
<td>4·19 ± 1·06</td>
<td>5·84 ± 2·65</td>
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<tr>
<td></td>
<td>$F_{2a}$</td>
<td>0·77 ± 0·27</td>
<td>—</td>
<td>1·92 ± 0·72</td>
<td>3·26 ± 2·73</td>
</tr>
</tbody>
</table>

*During labour*

The concentration of both prostaglandins in decidua from women in labour was significantly higher ($P < 0·005$) than in the corresponding tissue from women at term. In addition, the concentration of prostaglandin $F_{2a}$ rose in the amnion ($P < 0·025$), and the mean values for both prostaglandins increased in the myometrium. Although the concentration of prostaglandin $F_{2a}$ was lower than that of prostaglandin $E_2$ in the decidua and myometrium, the percentage increases in the mean values during labour were higher.

*Early pregnancy and menstrual cycle*

The concentrations of prostaglandins $E_2$ and $F_{2a}$ in endometrial tissue from non-pregnant women were significantly higher ($P < 0·0005$) than in decidua removed during the first
trimester of pregnancy and at term. The pattern with regard to the levels in myometrium was different in that the concentrations were significantly lower ($P < 0.0005$) in tissue from non-pregnant women, and the mean value showed a progressive increase at term and during labour.

**DISCUSSION**

Other workers have shown the presence of prostaglandins in decidua, endometrium, cord and placenta (Pickles, Hall, Best & Smith, 1965; Karim & Devlin, 1967; Downie, Poyser & Wunderlich, 1974; Grøen & Hagenfeldt, 1975; Singh, Baccarini & Zuspan, 1975), but the present study is the first attempt to compare the concentrations of prostaglandins $E_2$ and $F_{2\alpha}$ in many tissues associated with the foeto-placental unit, at defined periods of pregnancy.

Previous studies have shown that there is a significant increase in the concentration of prostaglandins $E_2$ and $F_{2\alpha}$ in amniotic fluid from early pregnancy to term, and from term to late labour (Karim & Devlin, 1967; Karim & Hillier, 1970; Jouvenaz, Nugteren & Van Dorp, 1973; Keirse, Flint & Turnbull, 1974). Furthermore, an increase has been shown in the mean concentration of prostaglandin $F_{2\alpha}$ in uterine venous plasma (Johnson, Manning, Hennam, Newton & Collins, 1975). Similarly, the concentration of the major metabolites of prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ in urine have been shown to increase as pregnancy progresses (Hamberg, 1974), and there is a significant increase in the concentration of 13,14-dihydro-15-oxo-prostaglandin $F_{2\alpha}$ in peripheral venous plasma during labour (Grøen, Bygdeman, Toppozada & Wiqvist, 1974). The results from the present study complement these observations and show that the highest concentrations of prostaglandins $E_2$ and $F_{2\alpha}$ in tissue are found in the decidua, myometrium and cord, followed by the membranes and placenta. Furthermore, the amounts of both prostaglandins are lower in the decidua than in endometrial tissue removed during the secretory phase of the menstrual cycle. Previous work has shown that the concentrations during this period of the uterine cycle in non-pregnant women are higher than during the proliferative phase, but significantly lower than during the period of menstrual bleeding (Downie et al. 1974; Singh et al. 1975; Willman, Collins & Clayton, 1976). During pregnancy the levels of both prostaglandins are lowest during the first trimester, but there is an increase in the mean values before the onset of labour, and during parturition the increase is statistically significant.

The results from the present investigations have also shown that the concentrations of prostaglandins $E_2$ and $F_{2\alpha}$ are lower in myometrial tissue from non-pregnant than from pregnant women, and that there is a progressive increase in the levels at term, and during labour. This finding may be associated with the increase in uterine contractility that has been observed during late pregnancy (Turnbull & Anderson, 1968; Csapo & Sauvage, 1968). Furthermore, Vane & Williams (1973) have suggested that this activity in the rat is the result of increased production of prostaglandins, as the effect is abolished by the administration of indomethacin. It is also postulated that the local generation of prostaglandins contributes to the regulation of the tone of the umbilical artery, and may be involved in the closure of the vessel at birth (Standberg, Tevemo, Hamberg & Samuelsson, 1975). The relatively high concentration of prostaglandin $E_2$ found in the umbilical cord adds further support to this hypothesis.

An observation of probable importance is that the concentration of prostaglandin $E_2$ is significantly higher than that of prostaglandin $F_{2\alpha}$ in all tissues associated with pregnancy, whereas in endometrial tissue the levels of both compounds are similar. There is some evidence from studies 'in vitro' to suggest that the myometrium is more responsive to prostaglandin $E_2$, and in clinical practice the amount of this compound that is required to induce labour or abortion is usually less than 10% of the amount of prostaglandin $F_{2\alpha}$. This finding suggests that it may be possible at some later date to assign different functions.
to prostaglandins $E_2$ and $F_{2\alpha}$, but the biochemical mechanisms whereby these compounds stimulate uterine muscle is not understood at present.

In conclusion, it may be stated that the results from the present study suggest that the increasing amounts of prostaglandins found in amniotic fluid, mainly originate from the decidua and myometrium. Furthermore, it would appear that prostaglandins play a physiological role in the initiation and maintenance of labour, since there is an initial reduction in amount during early pregnancy, and then a progressive increase in the concentration in responsive tissues at term and during parturition. However, the factors which control the biosynthesis and metabolism under physiological conditions remain to be elucidated.

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**REFERENCES**


Prostaglandins in foetal-placental tissues

