Hormonal control of proteins synthesized and secreted by guinea-pig endometrium

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The amounts of protein synthesized and secreted (as indicated by [³H]leucine incorporation) by guinea-pig endometrium cultured for 24 h increased 2.1-fold between days 7 and 15 of the oestrous cycle. This increase did not occur if the guinea-pigs were pregnant. In ovariectomized guinea-pigs, oestradiol acting on a progesterone-primed uterus was the optimum stimulus for the maximum increase in endometrial protein synthesis and secretion. The two main proteins synthesized and secreted by day-15 guinea-pig endometrium had molecular masses of 99.8 and 192.1 kDa as determined on Sephacryl S-200HR. The production of the 99.8 kDa protein increased 5.2-fold between days 7 and 15 of the cycle. The 192.1 kDa protein was not detected in secretions produced by day 7 endometrium, and the 99.8 and 192.1 kDa proteins were not detected in secretions produced by day-15 pregnant endometrium. In ovariectomized guinea-pigs, progesterone did not stimulate the synthesis of secreted proteins of molecular masses above 77 kDa. Oestradiol stimulated the synthesis of secreted proteins with molecular masses of 87.8 and 192.1 kDa. However, oestradiol acting on a progesterone-primed uterus stimulated the synthesis of the secreted proteins with molecular masses of 99.8 and 192.1 kDa, which indicates that this combination of steroid hormones may be responsible for the increase in production of these two proteins by the day-15, nonpregnant guinea-pig endometrium. This stimulation by ovarian steroids of the synthesis and secretion of the 99.8 and 192.1 kDa proteins by the guinea-pig endometrium is apparently inhibited during early pregnancy.

Introduction

Oestradiol administration after progesterone treatment is the optimum stimulus for increasing prostaglandin F_{2α} (PGF_{2α}) output from the uterus of ovariectomized guinea-pigs (Blatchley and Poyser, 1974; Poyser, 1983a). In intact guinea-pigs, oestradiol output from the ovary increases after day 10 of the cycle (Joshi *et al.*, 1973) and this increase precedes the increase in PGF_{2α} output from the uterus by 24 h (Blatchley *et al.*, 1972; Earthy *et al.*, 1975; Antonini *et al.*, 1976). In guinea-pigs, oestradiol acting on a progesterone-primed uterus is therefore the physiological stimulus for increased synthesis of PGF_{2α} by the endometrium. Oxytocin has no stimulatory effect on endometrial PGF_{2α} production in this species (Poyser and Brydon, 1983; Riley and Poyser, 1987).

The major mechanism by which oestradiol exerts its effects (following combination with its nuclear receptor) is by stimulating the production of specific RNAs that carry the information required for the synthesis of new proteins which in turn bring about physiological changes in target cells (Brenner and West, 1975). Actinomycin D, cycloheximide, puromycin and sodium fluoride (all inhibitors of protein synthesis) significantly reduce the outputs of PGF_{2α}, PGE₂ and 6-keto-PGF_{1α} from day-15 guinea-pig endometrium when included in the culture

medium, and the output of PGF_{2α} is particularly affected during the first 6 h of culture (Riley and Poyser, 1989; Leckie and Poyser, 1990). The intrauterine administration of actinomycin D to guinea-pigs on day 10 of the cycle decreases the outputs of PGF_{2α} and PGE₂, but not of 6-keto-PGF_{1α}, from day-15 endometrium, but not myometrium, in culture and from the day-15 uterus superfused *in vitro* (Poyser and Riley, 1987; Riley and Poyser, 1989). The simultaneous administration of oestradiol does not overcome this inhibitory effect of actinomycin D (Poyser and Riley, 1987). The synthesis of secreted proteins, but not of cellular proteins, is significantly greater by day-15 than by day-7 guinea-pig endometrium in culture. Actinomycin D, cycloheximide, puromycin and sodium fluoride in concentrations that inhibit endometrial PG synthesis also inhibit endometrial protein synthesis (Riley and Poyser, 1989; Leckie and Poyser, 1990). In addition, the intrauterine administration of actinomycin D on day 10 significantly reduces the synthesis of proteins by day-15 endometrium in culture (Riley and Poyser, 1989). These findings indicate that the stimulation of endometrial PGF_{2α} synthesis in guinea-pigs by oestradiol acting on a progesterone-primed uterus depends on increased endometrial protein synthesis.

Previous studies have shown that the increases in the amounts of phospholipase A₂ (PLA₂) and PG endoperoxide synthase in the endometrium after day 10 of the cycle are not responsible for 'switching on' endometrial PGF_{2α} synthesis (Downing and Poyser, 1983; Poyser, 1983b). These findings

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indicated that the activation of PLA_2 , probably as a result of raising the intracellular free Ca^{2+} concentration was the rate-limiting step in endometrial $\text{PGF}_{2\alpha}$ synthesis (Downing and Poyser, 1983; Poyser and Brydon, 1983). Subsequently, it was suggested that oestradiol acts on a progesterone-primed uterus to cause the synthesis of a protein which directly or indirectly raises the intracellular free Ca^{2+} concentration and thereby activates PLA_2 (Poyser and Riley, 1987). In the present study, the production of secreted proteins by the endometrium has been examined (i) in guinea-pigs on day 7 and day 15 of the cycle (days of low and high $\text{PGF}_{2\alpha}$ output, respectively), (ii) in ovariectomized guinea-pigs treated with progesterone, oestradiol or both, and (iii) in day-15 pregnant guinea-pigs, since uterine $\text{PGF}_{2\alpha}$ production is suppressed in early pregnancy (Blatchley *et al.*, 1975; Antonini *et al.*, 1976; Poyser, 1984).

Materials and Methods

Animals and treatments

Virgin guinea-pigs weighing 500–950 g were examined daily and a vaginal smear was taken when the vagina was perforate. Day 1 of the cycle was defined as the day preceding the post-ovulatory influx of leucocytes when cornification was at a maximum. All guinea-pigs had exhibited at least two normal cycles before being used in the experiments described below. The animals were killed by stunning and incising the neck. In all experiments, the uterus was removed and small pieces of the endometrium were cultured in 4 ml Medium 199 (plus Earle's salts, and supplemented with glutamine, amphotericin B and kanamycin) for 24 h as described previously (Riley and Poyser, 1989). The endometrium remains viable during this period. After culture, the culture medium was retained for protein analysis, and the dry weight of the culture endometrium was determined by heating the tissue at 37°C in an oven for 24 h followed by weighing.

Materials

L-[4,5- ^3H]Leucine (sp. act. 122 to 186 Ci mmol^{-1}) and D-[6- ^3H]glucosamine (sp. act. 32 Ci mmol^{-1}) were purchased from Amersham International Ltd, Cardiff; MW-GF-200 calibration kit for molecular masses 12–200 kDa, DEAE-Sepharose CL-6B, CM-Sepharose CL-6B, Coomassie blue stain, oestradiol benzoate and progesterone were purchased from Sigma Chemical Co., Poole, Dorset; low molecular mass electrophoresis calibration kit (14.4 to 94 kDa), Blue Sepharose CL-6B, Sephacryl S-200HR and Columns PD-10 (for desalting) were purchased from Pharmacia LKB Biotechnology Ltd, Milton Keynes; Medium 199 (plus Earle's salts), glutamine, amphotericin B and kanamycin were purchased from Flow Labs, Irvine; peanut oil was purchased from BDH Chemicals Ltd, Poole.

Experiment 1: quantitative analysis of total protein released from guinea-pig endometrium

The uteri from five day-7 and five day-15 guinea-pigs were used. The endometrium obtained from each uterus was cultured in 12 dishes each containing 20–30 mg tissue. After culture, the

Fig. 1. Calibration curve obtained with standard proteins run on a Sephacryl S-200HR column (1.6 cm $\times$ 97 cm). The best line of fit was produced using simple regression ($r = 0.99$). The figures in brackets indicate the approximate molecular mass of the standard proteins.

media from the 12 dishes were pooled and dialysed four times using 5 l of 2 mmol Tris-HCl l^{-1} buffer at pH 8.2 over 48 h. The dialysed medium was lyophilized. The lyophilate was dissolved in 2.5 ml water and loaded onto a desalting column that contained 9.1 ml Sephadex G-25 swollen gel resin (Column PD-10) which had been equilibrated with 25 ml water. The desalted protein was eluted from the column by 3.5 ml water, and 0.1 ml of the eluate was subsequently used for protein measurement (Lowry *et al.*, 1951). The remaining 3.4 ml was lyophilized. The lyophilate was weighed, redissolved in 1 ml Tris-HCl buffer (50 mmol l^{-1} ; pH 7) containing 0.1 mmol KCl l^{-1} and 5% glycerol, and passed down an affinity column containing Blue Sepharose CL-6B to remove contaminating albumin from the sample. The albumin-free protein was eluted from the column by Tris-HCl buffer (50 mmol l^{-1} , pH 7), and the eluate was dialysed, desalted and the amount of protein present measured as described above.

Experiment 2: analysis of total secreted proteins synthesized de novo by guinea-pig endometrium

The uteri from five day-7 and five day-15 guinea-pigs were used. The endometrium obtained from each uterus was distributed among eight dishes. Each dish contained 30–50 mg tissue and 10 μCi [^3H]leucine. After culture, the medium from the eight dishes was pooled, and the amount of [^3H]leucine incorporated into endometrial secreted proteins was measured as described by Riley and Poyser (1989). The medium was then dialysed and lyophilized as in Expt 1. Lyophilates of the proteins secreted by day-7 and day-15 guinea-pig endometrium in culture were then combined for each day, desalted and re-lyophilized (as in Expt 1) to give two respective protein pools. Both pools of protein were then passed through a Blue Sepharose CL-6B column to remove albumin, and the eluate was dialysed, desalted and assayed for protein content as in Expt 1. The protein solution was lyophilized and weighed.

Portions (7–20 mg) of the day-7 and day-15 secreted proteins were subjected to gel filtration chromatography on Sephacryl S-200HR using Tris-HCl buffer (10 mmol l^{-1} , pH 8.2) containing 0.33 mol NaCl l^{-1} . The column had previously been calibrated with proteins of known molecular mass (Fig. 1). Two forms of elution profile were then generated for

each fractionated sample: (i) an 'A-280 nm elution' profile, which was constructed by measuring continuously the UV absorbance at 280 nm of the eluate as it passed through a UV monitor and which indicated proteins present, and (ii) a 'radioactivity' profile, which was constructed by measuring the amount of radioactivity present in 200 μ l aliquots removed from each 2 ml fraction of eluate and which indicates proteins synthesized by the endometrium. Fractions of eluate containing proteins of low (< 30 kDa), medium (30–150 kDa) and high (> 150 kDa) molecular masses were combined to give three respective pools of protein. Each pool was dialysed, desalted, the protein content measured, and lyophilized as in Expt 1. Portions of each pool of protein were then subjected to ion-exchange chromatography using DEAE-Sepharose CL-6B and Tris-HCl buffer (10 mmol l⁻¹, pH 8.2) with increasing concentrations of NaCl between 0 and 0.5 mol l⁻¹ as the eluant, or using CM-Sepharose CL-6B and 50 mmol l⁻¹ phosphate buffer (pH 8.2) with increasing NaCl concentrations between 0 and 0.5 mol l⁻¹ as eluant. Two profiles of the eluted proteins (i.e. 'A-280' and 'radioactivity') were obtained as described above.

The remaining day-7 and day-15 secreted proteins which were not subjected to gel filtration chromatography were analysed by polyacrylamide gel electrophoresis performed in the presence of sodium dodecyl sulfate (SDS-PAGE), as described by Laemmli (1970), using a Protean II Slab Cell (Bio-Rad Laboratories Ltd, Watford) for vertical electrophoresis. The protein samples and standard proteins were run simultaneously on homogenous polyacrylamide (12%) gels, which were subsequently visualized by Coomassie blue staining. 'Radioactivity' profiles were constructed for each electrophoresed sample using a gel slicing technique based on the method used by Basha *et al.* (1979). Each gel of each protein sample was sliced into 1.5–1.6 mm sections and each section was placed in a 6 ml scintillation vial followed by 0.3 ml hydrogen peroxide (30% v/v). The vials were then incubated at 70°C for 24 h, then filled with scintillation fluid, and the amount of radioactivity present estimated. Molecular masses of the radioactive proteins were calculated from their R_f values in comparison with the R_f values of the standard proteins.

This experiment was repeated using a further 10 guinea-pigs and 10 μ Ci [³H]glucosamine instead of [³H]leucine, but the analysis by SDS-PAGE was not included.

Experiment 3: effects of treatment with ovarian steroid hormones in vivo on endometrial protein secretion

Ovariectomized guinea-pigs (five per group) were injected s.c. once a day for 10 days with 0.5 ml peanut oil with the following additions: (i) no additions (control), (ii) 2.5 mg progesterone, (iii) no additions for 7 days, followed by 10 μ g oestradiol benzoate for 3 days, (iv) 2.5 mg progesterone for 7 days, followed by 2.5 mg progesterone plus 10 μ g oestradiol benzoate for 3 days.

The uterus was removed from each guinea-pig on the day after the last day of treatment. The endometrium obtained from each uterus was distributed among 12 culture dishes (15–30 mg tissue per dish). After culture, the medium obtained from each dish was pooled and subjected to the procedures described in Expt 1.

A further 20 ovariectomized guinea-pigs were treated as described above (five animals per group). The endometrium

Table 1. Mean ($\pm$ SEM, $n = 5$) amounts of protein secreted (μ g mg⁻¹ dry tissue) by day-7 and day-15 nonpregnant, and by day-15 pregnant endometrium cultured for 24 h before and after removal of serum albumin by Blue Sepharose CL-6B

Day	Nonpregnant		Pregnant
	7	15	15
Before removal of serum albumin	153.5 $\pm$ 7.9	182.5 $\pm$ 16.5	111.0 $\pm$ 6.9*
After removal of serum albumin	48.6 $\pm$ 6.5†	73.3 $\pm$ 8.6	14.8 $\pm$ 1.2*

*Significantly ($P < 0.01$) lower than corresponding day-7 and day-15 non-pregnant values.

†Significantly ($P < 0.05$) lower than corresponding day-15 nonpregnant value.

from each animal was distributed among eight culture dishes. Each dish contained 25–45 mg tissue and 10 μ Ci [³H]leucine. After culture, the media from the eight dishes were pooled, and purified by dialysis, desalting and chromatography on Blue Sepharose CL-6B and Sephacryl S200-HR as described in Expt 2.

Experiment 4: effect of pregnancy on endometrial protein secretion

Day 1 of pregnancy was defined as the day spermatozoa were present in the vaginal smear or there were external signs of mating. The uterus was removed from five guinea-pigs on day-15 of pregnancy. The endometrium from each uterus was distributed among 12 culture dishes (15–30 mg tissue per dish). After culture, the media from the 12 dishes were pooled and treated as in Expt 1.

The uteri from a further five day-15 pregnant guinea-pigs were removed, and the endometrium from each uterus was distributed among eight culture dishes. Each dish contained 25–45 mg tissue and 10 μ Ci [³H]leucine. After culture, the media from the eight dishes were pooled and purified by dialysis, desalting and chromatography on Blue-Sepharose CL-6B and Sephacryl S200-HR and the proteins were analysed by SDS-PAGE as described in Expt 2.

Statistical analysis

Results were analysed by Student's *t* test or, if the variances of the two groups were significantly different by the variance ratio *F* test, by a modified *t* test for unequal variances.

Results

Quantitative assessment of endometrial protein secretion

Total protein. The total amount of protein secreted by guinea-pig endometrium cultured for 24 h was higher on day 15 of the cycle than on day 7 of the cycle and on day 15 of pregnancy, although this difference was significant ($P < 0.01$) only for day 15 of the cycle and day 15 of pregnancy (Table 1).

Table 2. Mean ($\pm$ SEM, $n = 5$) amounts of protein secreted ($\mu\text{g mg}^{-1}$ dry tissue) by endometrium cultured for 24 h from ovariectomized guinea-pigs treated with peanut oil (control), progesterone, oestradiol, and progesterone plus oestradiol before and after removal of serum albumin by Blue Sepharose CL-6B

	Treatment			
	Control	Progesterone	Oestradiol	Progesterone plus oestradiol
Before removal of albumin	98.9 $\pm$ 9.7	127.7 $\pm$ 10.4	205.9 $\pm$ 22.9*	173.9 $\pm$ 11.5*
After removal of albumin	17.5 $\pm$ 1.6	26.0 $\pm$ 4.3	74.9 $\pm$ 10.5*	52.7 $\pm$ 4.3*

*Significantly ($P < 0.05$) higher than corresponding control and progesterone only values.

After the removal of albumin by Blue Sepharose CL-6B, the amount of protein secreted by guinea-pig endometrium was significantly higher on day 15 of the cycle than on day 7 of the cycle ($P < 0.05$) and on day 15 of pregnancy ($P < 0.01$; Table 1). In both instances the amount of protein secreted by the day-15 pregnant endometrium was significantly ($P < 0.01$) lower than the amount secreted by the day-7 endometrium.

In ovariectomized guinea-pigs, progesterone treatment alone had no significant effect on the amount of protein secreted by the endometrium before and after purification on Blue Sepharose CL-6B. Oestradiol treatment alone and in combination with progesterone significantly ($P < 0.05$) stimulated the amount of protein secreted by the endometrium before and after purification on Blue Sepharose CL-6B (Table 2).

Proteins labelled with radioactivity. The amount of [^3H]leucine incorporated into proteins synthesized and secreted by the endometrium was significantly ($P < 0.05$) higher on day 15 of the cycle than on day 7 of the cycle and on day 15 of pregnancy (Fig. 2a). The amounts of radioactivity incorporated into proteins secreted by the endometrium when cultured in the presence of [^3H]glucosamine were 5148 ± 881 and 3809 ± 644 d.p.m. mg^{-1} tissue per 10^{-6} standard d.p.m. added ml^{-1} on days 15 and 7 of the cycle, respectively, but this difference was not statistically significant.

In ovariectomized guinea-pigs, progesterone treatment caused a small, but significant ($P < 0.05$) increase in the amount of [^3H]leucine incorporated into proteins synthesized and secreted by the endometrium. Oestradiol treatment alone caused a much larger and significant ($P < 0.05$) increase in the amount of [^3H]leucine incorporated into secreted endometrial proteins, but treatment with progesterone and oestradiol produced the largest increase in the amount of [^3H]leucine incorporated into secreted endometrial proteins and this increase was significantly ($P < 0.05$) greater than with oestradiol alone (Fig. 2b).

Qualitative and quantitative analysis of endometrial protein secretion

Analysis by Sephacryl S-200HR

In intact guinea-pigs the absorbance (A_{280}) profile of day-7 endometrial proteins showed that most proteins were eluted in the void volume and had molecular masses > 250 kDa. How-

Fig. 2. Mean ($\pm$ SEM, $n = 5$) amounts of [^3H]leucine incorporated into secreted proteins synthesized *de novo* by endometrium cultured for 24 h from (a) day-7 nonpregnant, day-15 nonpregnant, and day-15 pregnant guinea-pigs, and (b) ovariectomized guinea-pigs treated with peanut oil (control), progesterone, oestradiol, and progesterone plus oestradiol.

*Significantly ($P < 0.05$) higher than day-7 nonpregnant and day-15 pregnant values.

†Significantly ($P < 0.05$) higher than control value.

‡Significantly ($P < 0.05$) higher than control and progesterone-treated values.

▲Significantly ($P < 0.05$) higher than control, progesterone-treated and oestradiol-treated values.

ever, there was a small peak at 99.8 kDa. Analysis of the radioactivity profile of [^3H]leucine incorporation showed small peaks of radioactivity at < 5.6 , 8.4, 10.9, 14.1, 18.4, 35.2, 59.4, 99.8,

Fig. 3. UV absorbance (280 nm) ($\square$) and radioactivity (% of total d.p.m. recovered; [3 H] leucine-incorporation) ($\blacklozenge$) profiles of proteins secreted by (a) day-7 and (b) day-15 non-pregnant guinea-pig endometrium cultured for 24 h, following analysis on Sephacryl S-200HR.

129.3, 218.8 and > 250 kDa. The major peak of radioactivity was at 218.8 kDa (Fig. 3a).

The absorbance (A_{280}) profile of day-15 endometrial secreted proteins contained two major peaks at 99.8 and 218.8 kDa, and a small peak of protein which eluted with the void volume (> 250 kDa). The radioactivity profile showed peaks at < 5.6, 7.4, 59.4, 99.8 and 192.1 kDa. There may also be a radioactive protein on day 15 equivalent to the 218.8 kDa protein seen on day 7, since the radioactivity profile increases at the same time as the UV absorbance profile of the 218.8 kDa protein (Fig. 3b). By comparing the areas under the radioactive peaks, the relative amounts of the 59.4 and 99.8 kDa proteins synthesized and secreted by the endometrium increased 2.2- and 5.2-fold, respectively, between day 7 and day 15 of the cycle. In addition, a 192.1 kDa protein is present on day 15 which is not seen on day 7. The 99.8 kDa protein showed a small degree of glycosylation on both days (Fig. 4).

In ovariectomized guinea-pigs, there was insufficient protein remaining from ovariectomized (control) guinea-pigs for analysis by Sephacryl S-200HR. The absorbance (A_{280}) profile of endometrial secreted proteins from progesterone-treated guinea-pigs showed peaks at < 5.6, 52.1, 99.8 and > 250 kDa. However, radioactive peaks were present only at < 5.6, 7.4, 20.9, 27.1, 35.2, 45.7 and 77.1 kDa (Fig. 5a). The A_{280} profile of endometrial secreted proteins from oestradiol-treated guinea-pigs showed peaks at 87.8, 218.8 and < 250 kDa. Radioactive protein peaks were present at < 5.6, 7.4, 16.1, 20.9, 67.6, 87.8, 192.1, 250 and > 250 kDa (Fig. 5b). The A_{280} profile of endometrial secreted proteins from guinea-pigs treated with progesterone and oestradiol showed peaks of 87.8 and > 250 kDa. Radioactive protein peaks were present at < 5.6, 9.6, 27.1, 99.8, 192.1 and > 250 kDa. There was also a small 'shoulder' on the 99.8 kDa peak between 77.1 and 87.8 kDa (Fig. 5c).

Fig. 4. UV absorbance (280 nm) ($\square$) and radioactivity (% of total d.p.m. recovered; [3 H]glucosamine-incorporation) ($\blacklozenge$) profiles of proteins secreted by (a) day-7 and (b) day-15 nonpregnant guinea-pig endometrium cultured for 24 h, following analysis on Sephacryl S-200HR.

In pregnant guinea-pigs, the A_{280} profile of proteins secreted by guinea-pig endometrium at day 15 of pregnancy showed peaks at 99.8 and >250 kDa. Radioactive peaks were present at <5.6, 7.4, 12.4, 18.4, 77.1, 114.0, 147.9 and 250 kDa (Fig. 6).

Analysis by ion exchange chromatography. Proteins of low, medium and high molecular masses secreted by day-7 and day-15 endometrium were retained on columns containing DEAE-Sepharose CL-6B but were not retained on columns containing CM-Sepharose CL-6B when the appropriate eluant buffer lacking NaCl was run through the columns. This shows that the proteins secreted by guinea-pig endometrium were acidic. The majority of the proteins retained on DEAE-Sepharose CL-6B were eluted by the eluant buffer containing 0.2–0.3 mol NaCl l^{-1} .

Analysis by SDS-PAGE. Analysis by SDS-PAGE of day-7 secreted endometrial proteins showed major bands of radioactivity at 54.1, 101.9, 108.4, and 126.5 kDa. There were also numerous smaller bands of radioactivity (Fig. 7a). The analysis of day-15 nonpregnant secreted endometrial proteins showed three major bands at 17.4, 74.3 and 81.3 kDa. There were also several smaller bands of radioactivity (Fig. 7b). The analysis of day-15 pregnant secreted proteins showed four major bands at 19.5, 83.8, 126.6 and 147.6 kDa, together with many small bands of radioactivity (Fig. 7c).

Discussion

The UV absorbance profile indicates total protein secretion by the endometrium (i.e. preformed proteins that are present in the

Fig. 5. UV absorbance (280 nm) ($\square$) and radioactivity (% of total d.p.m. recovered [^3H]leucine incorporation) ($\blacklozenge$) profiles of proteins secreted by endometrium cultured for 24 h from ovariectomized guinea-pigs treated with (a) progesterone, (b) oestradiol, and (c) progesterone plus oestradiol, after analysis on Sephacryl S-200HR.

endometrium and are secreted during the culture period plus proteins that are freshly synthesized and secreted by the endometrium during the culture period). The radioactivity profile indicates proteins that have been synthesized and secreted only during the culture period. This accounts for the discrepancy

between the UV absorbance and radioactivity profiles since not all proteins that are secreted have been freshly synthesized. The amounts of albumin-free protein secreted by guinea-pig endometrium during 24 h of culture increased approximately two-fold between day 7 and day 15 of the cycle. The increase did

Fig. 6. UV absorbance (280 nm) (□) and radioactivity (% of total d.p.m. recovered; [3 H]leucine incorporation) (◆) profiles of proteins secreted by day-15 pregnant guinea-pig endometrium cultured for 24 h, after analysis on Sephacryl S-200HR.

not occur if the guinea-pig became pregnant. In ovariectomized guinea-pigs, progesterone alone had no stimulatory effect on endometrial protein secretion, whereas oestradiol alone caused a threefold increase in the amount of endometrial protein released. Progesterone reduced this stimulatory effect of oestradiol to a twofold increase.

The amounts of proteins freshly synthesized and secreted by guinea-pig endometrium (as indicated by the incorporation of [3 H]leucine) increased 2.1-fold between day 7 and day 15 of the cycle, which is in agreement with results of Riley and Poyser (1989). This increase did not occur in pregnant guinea-pigs. In ovariectomized guinea-pigs, progesterone caused a 1.3-fold increase in the amount of [3 H]leucine-containing protein produced. Oestradiol alone stimulated the production and secretion of [3 H]leucine-containing proteins 2.9-fold, whereas oestradiol acting on a progesterone-primed uterus increased the output of [3 H]leucine-containing proteins 3.5 times. Oestradiol treatment therefore increased the amount of total protein secreted (i.e. 'preformed' plus 'freshly synthesized'), and increased the amount of freshly synthesized proteins secreted. However, progesterone treatment had two different actions. It had no effect on the amount of total protein secreted, and reduced the stimulatory effect of oestradiol on total protein secretion. However, progesterone increased the amount of freshly synthesized protein secreted, and when it was administered with oestradiol caused a further stimulation of the amount of freshly synthesized protein secreted. Proteins secreted by the guinea-pig endometrium were acidic. This result is in agreement with the study of Chaminadas *et al.* (1989) which showed that radiolabelled proteins secreted by guinea-pig endometrial cells in culture had acidic pI values.

Analysis of the [3 H]leucine-containing proteins secreted by day-7 endometrium by Sephacryl S-200HR showed that several proteins were present in relatively small amounts (including a protein of 99.8 kDa), plus a protein in a relatively larger quantity with a molecular mass of 218.8 kDa. Between day 7 and day 15 of the cycle, the relative amounts of the 98.8 kDa pro-

tein synthesized and secreted by the endometrium increased 5.5-fold, and another protein with a molecular mass of 192.1 kDa was synthesized. These two proteins were the major proteins synthesized and secreted (as indicated by [3 H]leucine incorporation) by the day 15 nonpregnant endometrium. The 99.8 kDa protein was glycosylated to a small extent. The 99.8 and 192.1 kDa proteins were apparently not synthesized and secreted by day-15 pregnant guinea-pig endometrium. However, labelled proteins with molecular masses of 77.1, 114.0 and 168.0 kDa were produced by the day-15 pregnant endometrium.

In ovariectomized guinea-pigs, progesterone treatment did not stimulate the synthesis and secretion of [3 H]leucine-containing proteins with molecular masses of 99.8 and 192.1 kDa. Oestradiol treatment did stimulate the synthesis of the 192.1 kDa protein, and also proteins of molecular mass 87.8 and 67.6 kDa. However, oestradiol treatment following progesterone administration stimulated the synthesis and secretion of [3 H]leucine-containing proteins with molecular masses of 99.8 and 192.1 kDa. Thus, oestradiol acting on a progesterone-primed uterus produced a similar profile of proteins synthesized and secreted by the endometrium to that produced by day 15 nonpregnant endometrium (which is also under oestradiol dominance following progesterone-priming by the endogenous hormones).

Analysis of the day-15 secreted proteins from nonpregnant endometrium by SDS-PAGE produced radioactive bands at 17.4, 74.3 and 81.3 kDa. These molecular masses are lower than the two main radioactive protein peaks seen on Sephacryl S-200HR at 99.8 and 192.1 kDa. This may be for several reasons, namely (i) the proteins separated by Sephacryl S-200HR consist of one or more polypeptides which dissociated and ran separately on SDS-PAGE, (ii) the proteins are unstable in SDS and breakdown into smaller polypeptide chains (the 81.3 and 17.4 kDa protein bands may be derived from the 99.8 kDa protein, since their combined molecular mass is 98.7 kDa, which is

Fig. 7. Radioactivity profiles (% of total d.p.m. recovered from gel; [3 H]leucine-incorporation) of proteins secreted by (a) day-7 nonpregnant, (b) day-15 nonpregnant, and (c) day-15 pregnant endometrium cultured for 24 h, after analysis by SDS-PAGE.

within the margin of error in calculating molecular masses), and (iii) assigning a molecular mass of 99.8 kDa to this protein on Sephacryl S-200HR may be an overestimate since, in the calibration curve for Sephacryl S-200HR (Fig. 1), the protein standard (i.e. bovine serum albumin) with a known molecular mass of 66.0 kDa consistently lay off the calibration curve and therefore had an apparent molecular mass of 83.7 kDa. If the same phenomenon occurs with other proteins of similar molecular mass, the protein at 81.3 kDa (or even at 74.3 kDa) may be the same protein that runs as the 99.8 kDa protein on Sephacryl S-200HR. Further studies are needed to investigate these three possibilities for the discrepancy between the Sephacryl-S-

200HR and SDS-PAGE profiles of the day-15 endometrial, secreted proteins. However, the 17.4, 74.3 and 81.3 kDa proteins were present in much smaller quantities, if at all, in the SDS-PAGE profile of [3 H]leucine-containing proteins synthesized and secreted by day-7 nonpregnant and day-15 pregnant guinea-pig endometrium.

Overall, this study has shown that the synthesis and secretion of proteins by the guinea-pig endometrium increases between day 7 and day 15 of the cycle, and that pregnancy suppresses this increase. These results are similar to those observed for $\text{PGF}_{2\alpha}$ synthesis and secretion by the guinea-pig uterus (Blatchley *et al.*, 1972, 1975; Earthy *et al.*, 1975; Antonini *et al.*, 1976). Oestradiol acting on a progesterone-primed uterus is the optimum stimulus for the greatest increase in endometrial protein synthesis and secretion, and this combination of ovarian steroids is precisely the same stimulus for maximum endometrial $\text{PGF}_{2\alpha}$ production in guinea-pigs (Blatchley and Poyser, 1974; Poyser, 1983a). Consequently, the present findings are consistent with the previous studies using protein synthesis inhibitors (Poyser, 1979; Poyser and Riley, 1987; Riley and Poyser, 1989; Leckie and Poyser, 1990) which indicated that increased endometrial $\text{PGF}_{2\alpha}$ synthesis depends on increased endometrial protein synthesis. Furthermore, the lack of increase in endometrial protein synthesis and secretion in early pregnancy in guinea-pigs is associated with a lack of increase in uterine $\text{PGF}_{2\alpha}$ production, which again suggests a cause and effect relationship. This is in agreement with the findings of Poyser (1984) that oestradiol administered to early pregnant guinea-pigs could not overcome the inhibitory effect of the conceptus on uterine $\text{PGF}_{2\alpha}$ production, nor could it stimulate an increase in the amount of prostaglandin endoperoxide synthase present in the endometrium. Normally, endometrial prostaglandin endoperoxide synthase concentrations increase between day 7 and day 15 of the cycle, an effect which is due to oestradiol stimulation (Poyser, 1983a, b). This increase in enzyme concentration does not occur in early pregnancy nor can it be induced by oestradiol in early pregnant guinea-pigs (Poyser, 1984). Thus it appears that, in the guinea-pig, the conceptus prevents oestradiol acting on a progesterone-primed uterus to stimulate endometrial $\text{PGF}_{2\alpha}$ synthesis and secretion by inhibiting protein synthesis.

In ovariectomized guinea-pigs, oestradiol acting on a progesterone-primed uterus stimulated the synthesis and secretion of two major proteins of molecular masses 99.8 and 192.1 kDa. These were also the two major proteins synthesized and secreted by day-15 nonpregnant guinea-pig endometrium, indicating that *in vivo* the same combination of ovarian steroid hormones is probably responsible for their synthesis. These two proteins were produced in very much smaller quantities, if at all, by day-7 nonpregnant and day-15 pregnant guinea-pig endometrium. Consequently, the 99.8 or the 192.1 kDa protein or both proteins may be involved in the activation of PLA_2 and the consequent stimulation of endometrial $\text{PGF}_{2\alpha}$ synthesis.

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References

- Antonini R, Turner TF and Pauerstein CJ (1976) The hormonal control of the guinea-pig corpus luteum during early pregnancy *Fertility and Sterility* **27** 1322-1325

- Basha SMM, Bazer FW and Roberts RM** (1979) The secretion of uterine specific, purple phosphatase by cultured explants of porcine endometrium dependency upon the state of pregnancy of the donor animal *Biology of Reproduction* **20** 431–441
- Blatchley FR and Poyser NL** (1974) The effect of oestrogen and progesterone on the release of prostaglandins from the uterus of the ovariectomised guinea-pig *Journal of Reproduction and Fertility* **40** 205–209
- Blatchley FR, Donovan BT, Horton EW and Poyser NL** (1972) The release of prostaglandins and progesterone into the utero-ovarian venous blood of guinea-pigs during the oestrous cycle and following oestrogen treatment *Journal of Physiology* **222** 69–88
- Blatchley FR, Maule Walker FM and Poyser NL** (1975) Progesterone, prostaglandin $F_{2\alpha}$ and oestradiol in the utero-ovarian venous plasma of non-pregnant and early, unilaterally pregnant guinea-pigs *Journal of Endocrinology* **67** 225–229
- Brenner RM and West NB** (1975) Hormonal regulation of the reproductive tract in female mammals *Annual Review of Physiology* **37** 273–302
- Chaminadas G, Remy-Martin J-P, Alkhalaf M, Propper AY and Adessi GL** (1989) Effect of progesterone on protein synthesis and secretion by cultured epithelial cells from guinea-pig endometrium *Cell and Tissue Research* **257** 129–136
- Downing I and Poyser NL** (1983) Estimation of phospholipase A2 activity in guinea-pig endometrium on Days 7 and 16 of the estrous cycle *Prostaglandins, Leukotrienes and Medicine* **12** 110–117
- Earthy M, Bishop C and Flack JD** (1975) Progesterone and prostaglandin F concentrations in utero-ovarian venous plasma of cyclic guinea-pigs *Journal of Endocrinology* **64** 11P–12P
- Joshi HS, Watson DJ and Labhsetwar AD** (1973) Ovarian secretion of oestradiol, oestrone, 20-dihydroprogesterone and progesterone during the oestrous cycle of the guinea-pig *Journal of Reproduction and Fertility* **35** 177–182
- Laemmli UK** (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage *Nature* **227** 680–686
- Leckie CM and Poyser NL** (1990) The effects of cholera toxin, pertussis toxin, sodium fluoride and α -interferon on prostaglandin production by the guinea-pig endometrium *Journal of Reproduction and Fertility* **89** 325–333
- Lowry OH, Roseborough NJ, Farr AL and Randall RL** (1951) Protein measurement with folin phenol reagent *Journal of Biological Chemistry* **193** 265–273
- Poyser NL** (1979) Effects of actinomycin D on uterine prostaglandin production and oestrous cycle length in guinea-pigs *Journal of Reproduction and Fertility* **56** 559–565
- Poyser NL** (1983a) Effect of treating ovariectomised guinea-pigs with estradiol and progesterone on basal and A23187-stimulated release of prostaglandins from the uterus superfused in vitro *Prostaglandins, Leukotrienes and Medicine* **11** 345–360
- Poyser NL** (1983b) Differential stimulation of prostaglandin and thromboxane synthesising capacities in guinea-pig uterus and ovary *Prostaglandins, Leukotrienes and Medicine* **10** 163–177
- Poyser NL** (1984) Prostaglandin production by the early pregnant guinea-pig uterus in relation to implantation and luteal maintenance, and the effect of oestradiol *Journal of Reproduction and Fertility* **72** 117–127
- Poyser NL and Brydon LJ** (1983) Prostaglandin release from the guinea-pig uterus superfused in vitro: effect of stage of estrous cycle, progesterone, estradiol, oxytocin and A23187 *Prostaglandins* **25** 443–456
- Poyser NL and Riley SC** (1987) Effect of actinomycin D on prostaglandin synthesis by and output from the guinea-pig uterus *Prostaglandins, Leukotrienes and Medicine* **28** 153–168
- Riley SC and Poyser NL** (1987) Effects of oestradiol, progesterone, hydrocortisone and oxytocin on prostaglandin output from the guinea-pig endometrium maintained in tissue culture *Prostaglandins* **34** 535–552
- Riley SC and Poyser NL** (1989) Is protein synthesis necessary for prostaglandin production by guinea-pig endometrium? *Journal of Reproduction and Fertility* **86** 73–89