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Is the Inhibitory Effect of Progesterone on Endometrial Prostaglandin $F_{2\alpha}$ Production due to an Inhibition of Protein Synthesis?

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ABSTRACT. **Progesterone and a high concentration of oestradiol (i) reduced the outputs of prostaglandin (PG) $F_{2\alpha}$ and, to a lesser extent, PGE_2 from Day-7 and Day-15 guinea-pig endometrium in culture, but had little or no effect on the output of 6-keto-$PGF_{1\alpha}$, (ii) prevented the increase in PGH synthase concentrations which normally occur in Day-7 and Day-15 guinea-pig endometrium during culture, and (iii) reduced the synthesis of secreted proteins by Day-15 guinea-pig endometrium in culture. These findings suggest that the inhibitory effect of progesterone and of high concentrations of oestradiol on endometrium $PGF_{2\alpha}$ synthesis is due to an inhibition of the syntheses of proteins involved in $PGF_{2\alpha}$ production.**

INTRODUCTION

In ovariectomised guinea-pigs and sheep, and in post-menopausal women, the administration of oestradiol following treatment with progesterone results in a large stimulation of prostaglandin (PG) $F_{2\alpha}$ synthesis by the uterus (1–3). In intact guinea-pigs, oestradiol output from the ovary increases after Day 10 of the cycle (4) which precedes the increase in $PGF_{2\alpha}$ output from the uterus by 24 h (5–7). Therefore, in the guinea-pig, oestradiol acting on a progesterone-primed uterus is the physiological stimulus for increased synthesis of $PGF_{2\alpha}$ by the endometrium. Oxytocin has no stimulatory effect on endometrial $PGF_{2\alpha}$ production in this species (8,9). In sheep, a combination of oestradiol, progesterone and oxytocin form the physiological stimulus for endometrial $PGF_{2\alpha}$ synthesis (10,11). However, progesterone has been shown to inhibit the basal and oestradiol-stimulated outputs of $PGF_{2\alpha}$ from human and guinea-pig endometrium maintained in culture (9, 12–15), and from human endometrial cells in culture (16, 17). In vivo plasma progesterone concentrations need to fall before maximum $PGF_{2\alpha}$ production by the uterus can occur (see 18, 19). Consequently, progesterone has inhibitory as well as facilitatory effects on endometrial $PGF_{2\alpha}$ synthesis. In addition, oestradiol in a high concentration inhibits $PGF_{2\alpha}$ output from guinea-pig endometrium maintained in tissue culture (9). Recently, we have reported (20) that increased endometrial $PGF_{2\alpha}$ synthesis depends upon increased protein synthesis, since inhibitors of protein synthesis greatly reduced $PGF_{2\alpha}$ production by guinea-pig endometrium. Consequently, the present study has investigated whether the inhibitory effects of progesterone and high concentrations of oestradiol on $PGF_{2\alpha}$ synthesis are due to an inhibition of protein synthesis.

METHODS

Virgin guinea-pigs weighing 650–950 g were examined daily and a vaginal smear was taken when the vagina was perforate. The first day of the cycle was taken as the day preceding the post-ovulatory influx of leucocytes when cornification was at a maximum. All guinea-pigs had exhibited cycles of normal length before being used on Day 7 or Day 15 of the cycle. The uterus from each guinea-pig was removed, and the endometrium was separated from the myometrium by cutting away 1 × 2 mm pieces

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of endometrium with a pair of fine scissors. Pieces of endometrium were placed on a raised platform in a Petri dish, which contained 4 ml Medium 199, plus Earle's salts, supplemented with glutamine, amphotericin B and kanomycin (21). Some of the Petri dishes contained 'treatments' as described in Experiments 1–3, and the Petri dishes were placed in modified Kilner jars. The endometrium was cultured for up to 24 h as previously described, and the tissue remains viable during culture (21, 22). The samples of culture medium obtained from the Petri dishes after culture were stored at −20°C before being assayed, without extraction, for $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ by radioimmunoassay. Equivalent volumes of culture medium were included in the 'standard' PG solutions as used in each assay.

Experiment 1: Effects of indomethacin on PG output from cultured endometrium (control experiments)

Six dishes of endometrium (12–20 mg/dish) were prepared from each uterus obtained from three Day-7 and three Day-15 guinea-pigs. Four dishes from each animal contained indomethacin (2 or 10 μg/ml) and two dishes were untreated (controls). The endometrium was cultured for 24 h, and the culture medium was replaced with fresh culture medium containing the same treatment every 6 h.

Experiment 2: Effects of progesterone and oestradiol on PG output from and PG synthesising ability of cultured endometrium

Twenty dishes of endometrium (12–20 mg/dish) were prepared from each uterus obtained from six Day-7 and six Day-15 guinea-pigs. The dishes were divided into 5 groups of 4 dishes, and each group was treated with one of the following: progesterone (10 and 100 ng/ml); oestradiol (10 and 1000 ng/ml); no treatment (controls). The endometrium was cultured for up to 24 h. and the culture medium was replaced with fresh medium containing the same treatment every 6 h.

After 6, 12, 18 and 24 h of culture, one dish from each group was removed and the endometrial tissue was collected. The tissue was homogenized in 5 ml Krebs' solution (for composition, see 23) containing 2 μg sodium arachidonate. Endometrium obtained at 0 h (i.e. just before culturing began) was treated in a similar manner. Each homogenate was gassed with 95% O_2 and 5% CO_2, and was incubated at 37°C for 60 min. PGs were extracted from the incubates as described previously (24), and were stored in 5 ml ethyl acetate at −20°C before being measured by radioimmunoassay.

Experiment 3: Effects of progesterone and oestradiol on endometrial protein synthesis

Five dishes of endometrium (30–60 mg/dish) were prepared from each uterus obtained from five Day-7 and five Day-15 guinea-pigs. Each dish contained 10 μCi [^{3}H] leucine and one of the following treatments: progesterone (10 and 10 ng/ml); oestradiol (10 and 1000 ng/ml); no treatment (control). The endometrium was cultured for 24 h. Immediately after starting the cultures, 30–60 mg of the remaining endometrium from each animal were placed in another Petri dish, rinsed with the [^{3}H] leucine-containing medium (10 μCi/ml), and left for 2 min at room temperature. These samples of endometrium and culture medium were used to measure the non-specific binding of [^{3}H] leucine. All samples of non-cultured and cultured, endometrium and medium were analysed for the amounts of [^{3}H] leucine incorporated into cellular and secreted proteins, by methods described previously (20). Non-specific binding of [^{3}H] leucine was subtracted from total [^{3}H] leucine incorporation, for both types of protein. All [^{3}H] leucine-containing samples were analyzed immediately after collection. There were no significant differences in the efficiency of counting among the samples so the results are as expressed as cpm.

Details of radioimmunoassay

$PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ were measured using antibodies raised in this laboratory and whose cross-reactivities have been reported elsewhere (see 25). The inter-assay and intra-assay coefficients of variation of all three assays were <10%. The limits of detection were 30–40 pg per assay tube for each assay.

Sources of materials

Medium 199 (plus Earle's salts), glutamine, amphotericin B and kanamycin were purchased from Flow Laboratories, Irvine, UK; sterile Petri dishes were purchased from Sterilin Ltd, Teddington, UK; oestradiol-17β and progesterone were purchased from Sigma Chemical Co., Poole, Dorset, UK; [^{3}H] leucine (sp.act. 160 Ci/mmol) was purchased from Amersham International Ltd, Bucks, UK; indomethacin was supplied by Merck, Sharpe & Dohme Ltd, Hoddesdon, Herts, UK.

Statistical tests

Changes in output of PGs with time were analysed by Duncan's multiple range test. Differences between two groups were analysed by Student's *t* test, which was used in a modified form if the variances

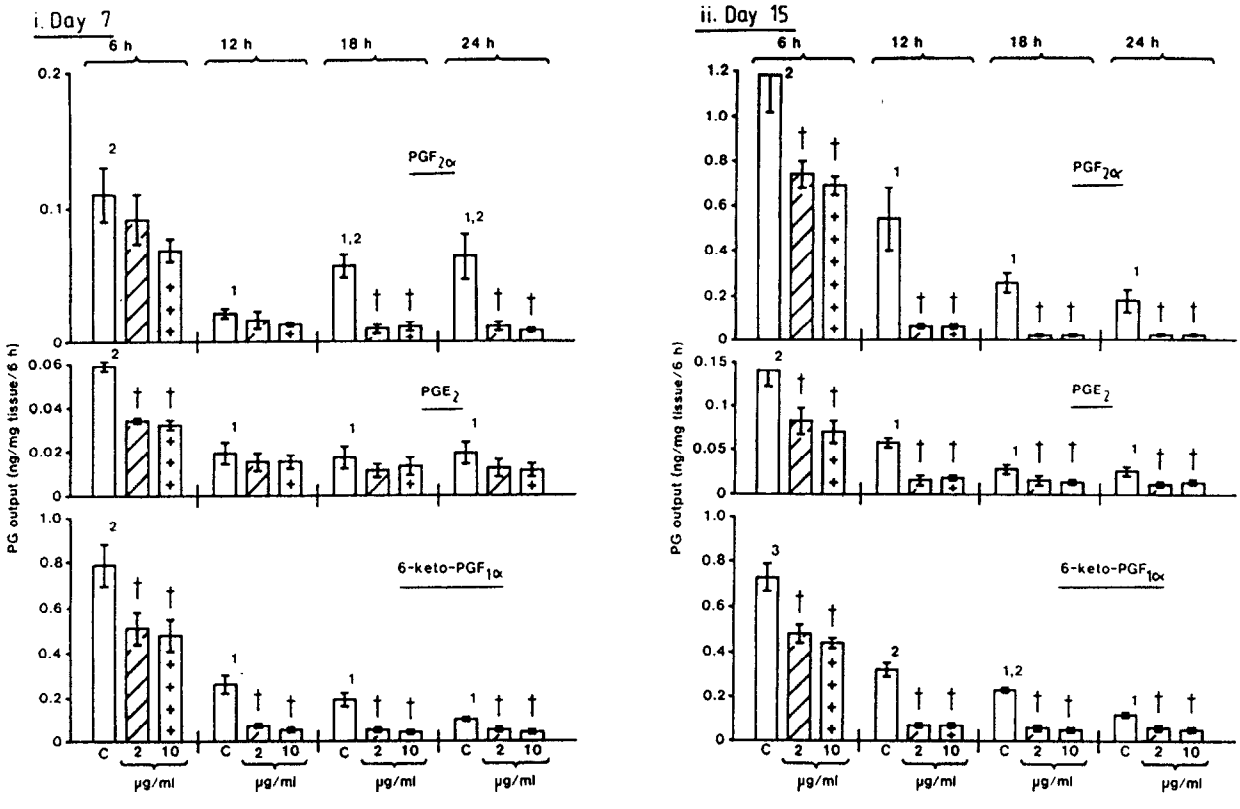

Fig. 1 Mean (± s.e.m., $n = 6$) outputs of prostaglandin (PG) $F_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ from (i) Day-7 and (ii) Day-15 guinea-pig endometrium cultured for 24 h, with sampling every 6 h, in the absence (C) and presence of indomethacin (2 and 10 μg/ml). For control (C) values, columns with the same number for one particular PG on one particular day are not significantly different ($P < 0.05$).
†Significantly ($P < 0.05$) lower than control (C) value for the same PG on the same day at the same time.

of the two groups were unequal by the variance ratio F test, or by the paired t test, as appropriate.

RESULTS

Experiment 1: Effects of indomethacin on PG output from cultured endemetrium (control experiments)

The outputs of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ from Day-7 and Day-15 guinea-pig endometrium significantly ($P < 0.05$) declined during the 24-hour period of culture, except for $PGF_{2\alpha}$ output from Day-7 endometrium which, after an initial decline, tended to increase (Fig. 1). The initial outputs of $PGF_{2\alpha}$ and PGE_2, but not of 6-keto-$PGF_{1\alpha}$, from Day-15 endometrium were significantly ($P < 0.05$) higher than from Day-7 endometrium. Indomethacin (2 and 10 μg/ml) significantly ($P < 0.05$) reduced the outputs of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ from Day-7 and Day-15 endometrium. However, the inhibitory effect of indomethacin on the endometrial outputs of $PGF_{2\alpha}$ after 6 and 12 h and of PGE_2 after 12, 18 and 24 h were less marked on Day 7 than on Day 15 due to the much lower basal outputs of $PGF_{2\alpha}$ and PGE_2 from Day-7 endometrium.

Experiment 2: Effects of progesterone and oestradiol on PG output from and PG synthesising ability of cultured endometrium

Progesterone (10 and 100 ng/ml) and oestradiol (1000 ng/ml) significantly ($P < 0.05$) reduced the output of $PGF_{2\alpha}$ from Day-7 and Day-15 guinea-pig endometrium after 12, 18 and 24 h of culture (Figs 2 and 3). Progesterone (both concentrations) and oestradiol (1000 ng/ml) tended to decrease PGE_2outputs but the decrease was significant ($P < 0.05$) only for Day-15 endometrium after 18 h. Progesterone and oestradiol had little effect on 6-keto-$PGF_{1\alpha}$ output although progesterone (100 ng/ml) significantly ($P < 0.05$) inhibited 6-keto-$PGF_{1\alpha}$ from Day-15 endometrium after 18 and 24 h. Oestradiol (10ng/ml) had no significant effect of PG output.

The amounts of $PGF_{2\alpha}$ and PGE_2 synthesised by homogenates of Day-7 endometrium significantly ($P < 0.05$) increased between 0 h and 12 h, and then remained high up to 24 h. The amounts of 6-keto-$PGF_{1\alpha}$ synthesised by homogenates of Day-7 endometrium showed a similar trend, but the differences were not statistically significant (Fig. 4). Progesterone (10 and 100 ng/ml) decreased the amounts of $PGF_{2\alpha}$ and PGE_2 synthesised by homogenates of Day-7 endometrium, and these dif-

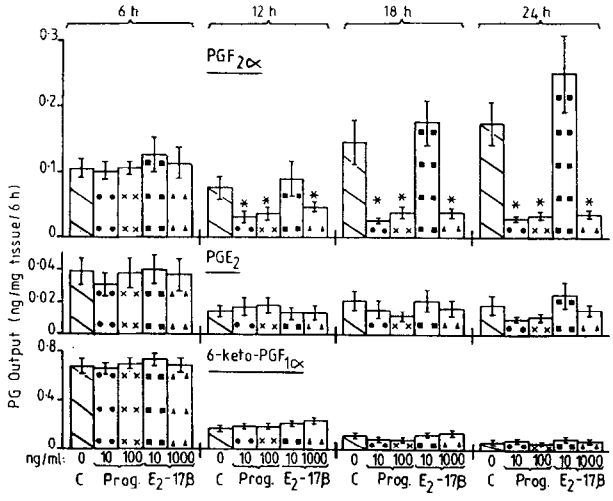

Fig. 2 Mean (± s.e.m., $n = 6$) outputs of prostaglandin (PG) $F_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ from Day-7 guinea-pig endometrium cultured for 24 h, with sampling every 6 h, in the absence (C) and presence of progesterone (Prog.; 10 and 100 ng/ml) and oestradiol-17β (E_2-17β; 10 and 1000 ng/ml). * Significantly ($P < 0.05$) lower than the control (C) value for the same PG at the same time.

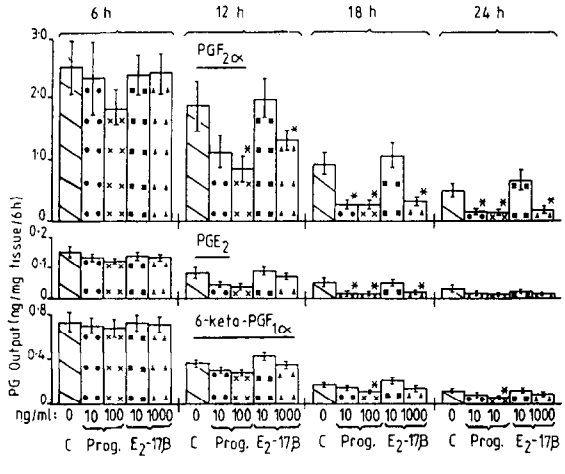

Fig. 3 Mean (± s.e.m., $n = 5$) outputs of prostaglandin (PG) $F_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ from Day-15 guinea-pig endometrium cultured for 24 h, with sampling every 6 h, in the absence (C) and presence of progesterone (Prog.; 10 and 100 ng/ml) and oestradiol-17β (E_2-17β; 10 and 1000 ng/ml). * Significantly ($P < 0.05$) lower than the control (C) value for the same PG at the same time.

ferences were statistically significant ($P < 0.05$) for $PGF_{2\alpha}$ after 12, 18 and 24 h, and for PGE_2 after 12 h and (10 ng/ml only) 18 h. Progesterone had no significant effect on the amounts of 6-keto-$PGF_{1\alpha}$ synthesised by homogenates of Day-7 endometrium. Oestradiol (10 ng/ml) increased the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesised by Day-7 endometrium, and this increase was statistically significant ($P < 0.05$) for $PGF_{2\alpha}$ and PGE_2 after 18 h of culture. Oestradiol (1000 ng/ml) had no such stimulatory effect, and tended to decrease PG synthesis by homogenates of Day-7 endometrium (Fig. 4).

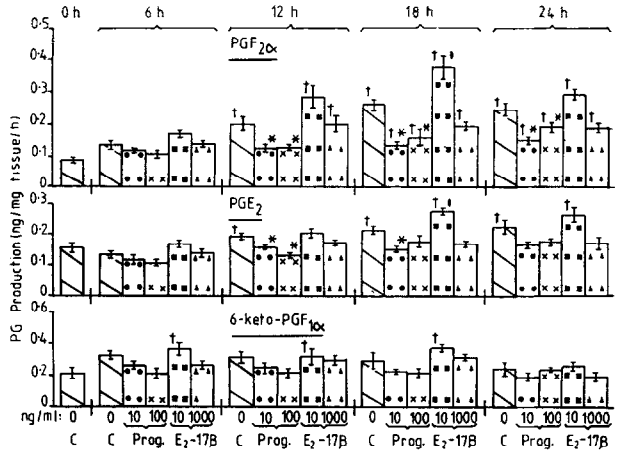

Fig. 4 Mean (± s.e.m., $n = 5$) amounts of prostaglandin (PG) $F_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesised by homogenates of Day-7 guinea-pig endometrium before culture (0 h), and at intervals of 6 h during 24 h of culture in the absence (C) and presence of progesterone (Prog.; 10 and 100 ng/ml) and oestradiol-17β (E_2-17β; 10 and 1000 ng/ml). † Significantly ($P < 0.05$) higher than the control (C) value at 0 h for the same PG. * Significantly ($P < 0.05$) lower than the control (C) value for the same PG at the same time. Significantly ($P < 0.05$) higher than the control (C) value for the same PG at the same time.

The amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesised by homogenates of Day-15 endometrium significantly ($P < 0.05$) increased between 0 h and 6 h, remained elevated up to 12 h, and then started to decline, although the amounts synthesised were still significantly ($P < 0.05$) higher at 24 h than at 0 h (Fig. 5). Progesterone (10 and 100 ng/ml) and oestradiol (1000 ng/ml) significantly ($P < 0.05$) reduced the amounts of $PGF_{2\alpha}$, PGE_2 and, to a lesser extent, 6-keto-$PGF_{1\alpha}$ synthesised by homogenates of Day-15 endometrium particularly after 12, 18 and 24 h of culture (Fig. 5).

Experiment 3: Effects of progesterone and oestradiol on endometrial protein synthesis

There was no significant difference between the amounts of [^{3}H] leucine incorporated into total cellular proteins by Day-7 and Day-15 guinea-pig endometrium cultured for 24 h. The amounts of [^{3}H] leucine incorporated into secreted proteins were significantly ($P < 0.05$) higher by Day-15 than by Day-7 endometrium (Fig. 6). These results agree with previous findings. Progesterone and oestradiol had no significant effect on the amounts of [^{3}H] leucine incorporated into total cellular proteins by Day-7 and Day-15 endometrium. However, progesterone (10 and 100 ng/ml) and oestradiol (1000 ng/ml) had a small but significant ($P < 0.05$) inhibitory effect on the amounts of [^{3}H] leucine in-

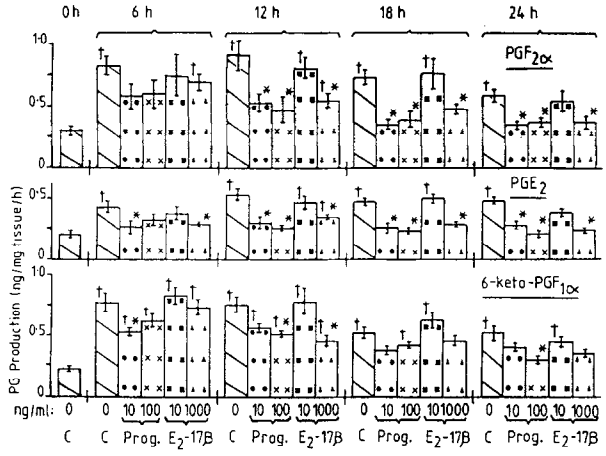

Fig. 5 Mean (± s.e.m., $n = 5$) amounts of prostaglandin (PG) $F_{2\alpha}$, PGE_2 and 6-keto-$PG_{1\alpha}$ synthesised by homogenates of Day-15 guinea-pig endometrium before culture (0 h), and at intervals of 6 h during 24 h of culture in the absence (C) and presence of progesterone (Prog; 10 and 100 ng/ml) and oestradiol-17β (E_2-17β; 10 and 1000 ng/ml).
† Significantly ($P < 0.05$) higher than the control (C) value at 0 h for the same PG.
* Significantly ($P < 0.05$) lower than the control (C) value for the same PG at the same time.

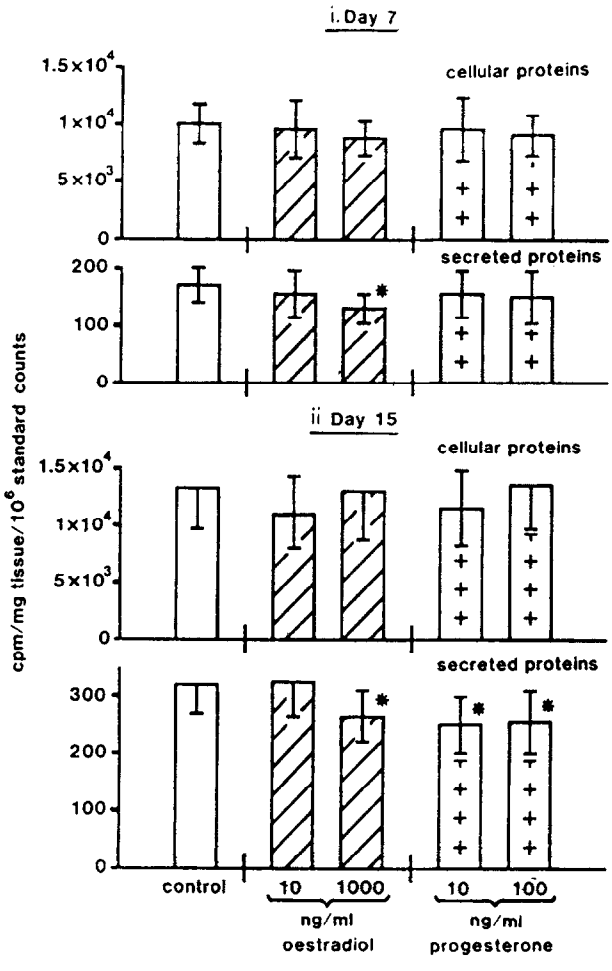

Fig. 6 Mean (± s.e.m., $n = 5$) amounts of [^{3}H] leucine incorporated into cellular and secreted proteins by (i) Day-7 and (ii) Day-15 guinea-pig endometrium cultured for 24 h in the absence (control) and presence of oestradiol (10 and 1000 ng/ml) and progesterone (10 and 100 ng/ml).
* Significantly ($P < 0.05$) lower than the control value for the same type of protein on the same day (by the paired t test).

corporated into secreted proteins by Day-15 endometrium. Oestradiol (1000 ng/ml) also significantly ($P < 0.05$) reduced the amounts of [^{3}H] leucine incorporated into secreted proteins by Day-7 endometrium.

DISCUSSION

The outputs of $PGF_{2\alpha}$ and PGE_2 but not of 6-keto-$PGF_{1\alpha}$ from guinea-pig endometrium in culture were higher on Day 15 than on Day 7 of the oestrous cycle. The presence of indomethacin in the culture medium inhibited the outputs of all three PGs, showing that PG synthesis by guinea-pig endometrium occurred during tissue culture. During the first 6-hour period of culture, the outputs of $PGF_{2\alpha}$ and PGE_2 were 10.8- and 2.4- fold higher from Day-15 than from Day-7 endometrium. These results show that the preferential stimulation of endometrial $PGF_{2\alpha}$ synthesis which occurs after Day 11 of the cycle, by oestradiol acting on a progesterone-primed uterus (26), is maintained in tissue culture in the absence of ovarian steroids. However, the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesised by and released from Day-7 and Day-15 endometrium decline during 24 h of culture, except for $PGF_{2\alpha}$ output from Day-7 endometrium which, after an initial decline, increases up to 24 h. These findings are in agreement with a previous study (20). Progesterone (10 and 100 ng/ml) and a high concentration of oestradiol (1000 ng/ml) had a pronounced inhibitory effect on the output of $PGF_{2\alpha}$ from Day-7 and Day-15 guinea-pig endometrium in culture. They also tended to reduce the output of PGE_2, but had little or no effect on the outputs of 6-keto-$PGF_{1\alpha}$ from Day-7 and Day-15 endometrium. These results are in agreement with a previous study in which Day-7 and Day-15 guinea-pig endometrium was cultured for 3 days with sampling every 24 h (9).

PGH synthase exhibits self-catalysed breakdown during the synthesis of PGs (27). Consequently, the total amount of PGs synthesised by endometrial homogenates is indicative of the concentration of PGH synthase, especially as the metabolism of PGs in the absence of exogenous nicotinamide-adenine dinucleotide (NAD^+) by the guinea-pig uterus is negligible (< 5%; 28). Initially the concentration of PGH synthase was 1.6-fold higher in Day-15 endometrium than in Day-7 endometrium. During culture, the concentration of PGH synthase in Day-7 and Day-15 endometrium increased 2-fold and

3-fold, respectively. Progesterone (10 and 100 ng/ml) and oestradiol (1000 ng/ml) reduced this increase in concentration of PGH synthase, particularly in Day-15 endometrium. This reduction is probably due to an inhibition of the synthesis of fresh enzyme, especially as protein synthesis inhibitors have a similar inhibitory effect (20). These findings agree with a previous study in which the in vivo treatment of ovariectomised guinea-pigs with progesterone attenuated the stimulatory effect of in vivo treatment with oestradiol on PGH synthase concentrations in the endometrium (24). However, in the present study, the inhibitory effect of progesterone and of a high concentration of oestradiol on PGH concentrations was not reflected by a decrease in the synthesis of total cellular proteins. Kelly and Smith (15) reported that progesterone had no effect on PGH synthase concentrations in human, proliferative endometrium cultured for 48 h in the presence of the steroid hormone. However, this study on human tissue needs repeating using secretory tissue in which PG synthesis is much higher (equivalent to Day-15 tissue in the guinea-pig), and examining the tissue every 6 h, as in the present study. Oestradiol (10 ng/ml) significantly increased the PGH synthase concentration in Day-7 guinea-pig endometrium after 18 h of culture. However, this stimulation did not result in an increase in endometrial PG output, agreeing with previous studies in the guinea-pig (24, 26) which showed that the concentration of PGH synthase is not the rate-limiting step in endometrial PG synthesis.

The synthesis of total secreted proteins by Day-15 guinea-pig endometrium was reduced by a small, but statistically significant, extent by progesterone (10 and 100 ng/ml) and oestradiol (1000 ng/ml). A high concentration of oestradiol also reduced the synthesis of secreted proteins by Day-7 guinea-pig endometrium. Figure 7 shows the relationship between the inhibition of $PGF_{2\alpha}$ synthesis and the inhibition of secreted protein synthesis by Day-15 guinea-pig endometrium over a 24-hour period in culture. Puromycin and cycloheximide are very effective inhibitors of $PGF_{2\alpha}$ synthesis and secreted protein synthesis, while actinomycin D has an intermediate effect on both parameters (from 20). Progesterone and high concentrations of oestradiol have a weak inhibitory effect on $PGF_{2\alpha}$ synthesis and secreted protein synthesis. Figure 7 suggests that there is a strong correlation between the inhibition of secreted protein synthesis and the inhibition of $PGF_{2\alpha}$ production on Day 15 of the cycle. Since, in the guinea-pig endometrium, increased $PGF_{2\alpha}$ synthesis towards the end of the cycle is dependent upon increased protein synthesis (20, 28, 29), the present study suggests that the inhibition of endometrial $PGF_{2\alpha}$ synthesis by progesterone and a high concentration of oestradiol is due to these steroids inhibiting the synthesis of a protein (20) which is necessary for the stimulation of endometrial $PGF_{2\alpha}$ production.

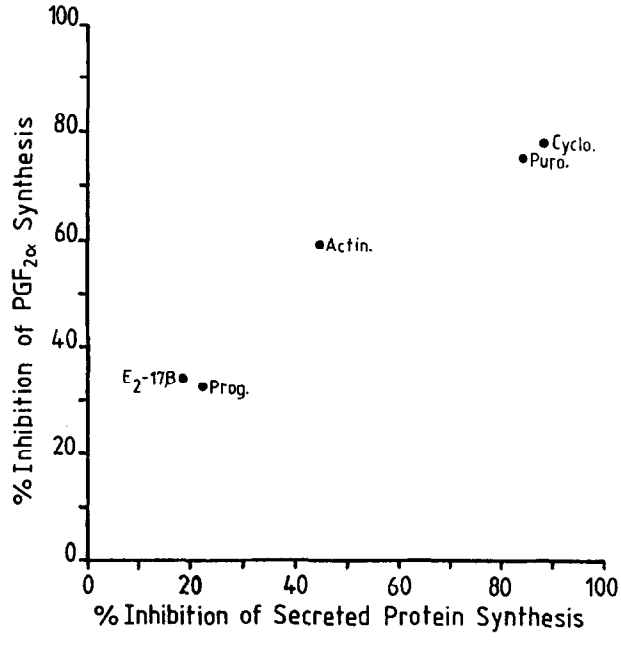

Fig. 7 Relationship between the % inhibition of $PGF_{2\alpha}$ synthesis (as reflected by $PG_{2\alpha}$ output) and % inhibition of secreted protein synthesis by Day-15 guinea-pig endometrium cultured for 24 h in the presence of cycloheximide (Cyclo.; 10 μg/ml), puromycin (Puro.; 50 μg/ml). actinomycin D (Actin.; 50 μg/ml), progesterone (Prog.; 10 ng/ml) and oestradiol-17β (E_2-17β; 1000 ng/ml). (Data for cycloheximide, puromycin and actinomycin D are taken from 20).

From studies on separated, glandular cells obtained from human endometrium, Smith and Kelly (30, 31) have suggested that progesterone stimulates the synthesis of a protein which inhibits PG synthesis. Uteroglobin, a progesterone-dependent protein, inhibits phospholipase A_2 (the enzyme which releases arachidonic acid from phospholipids for PG synthesis) since it is similar in structure to lipocortin (32, 33). A uteroglobin-like protein has been detected in human endometrium and it has been suggested that this protein inhibits PG production in human endometrium (34). However, the concentration of this uteroglobin-like protein in the endometrium is highest from the mid-secretory phase to the end of the cycle when endometrial $PGF_{2\alpha}$ levels are also raised (35, 36), although the temporary decline in endometrial $PGF_{2\alpha}$ levels in the late secretory phase (37) may be due to the production of uteroglobin. Consequently, it seems unlikely that the inhibitory effect of progesterone on endometrial $PGF_{2\alpha}$ synthesis is due solely to the production of uteroglobin. The present study in the guinea-pig indicates that progesterone reduces endometrial $PGF_{v\alpha}$ synthesis also by inhibiting the synthesis of a protein involved in the stimulation of $PGF_{2\alpha}$ production. Nevertheless, the relative con-

tribution of these two mechanisms by which progesterone inhibits $PGF_{2\alpha}$ synthesis in the endometrium may vary among the species.

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