Br. J. Pharmac. (1975), 55, 229-232

EFFECTS OF OESTRADIOL AND PROGESTERONE ON THE *in vitro* PRODUCTION OF PROSTAGLANDIN $F_{2\alpha}$ BY THE GUINEA-PIG UTERUS

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1 The effects of oestradiol and progesterone on the production of prostaglandin $F_{2\alpha}$ by the guinea-pig uterus *in vitro* have been studied.

2 Ovariectomized guinea-pigs were treated with oestradiol benzoate, progesterone, or a combination of these hormones. The uteri were homogenized and incubated and the prostaglandin $F_{2\alpha}$ produced was measured by radioimmunoassay or combined gas chromatography-mass spectrometry (g.c.-m.s.). Oestradiol treatment or progesterone followed by oestradiol caused a significant increase in prostaglandin $F_{2\alpha}$ synthesis compared with control values, whereas progesterone treatment caused no significant increase.

3 The uteri from guinea-pigs on day 7 of the oestrous cycle were split into four parts, and each part homogenized and incubated either alone or in the presence of oestradiol-17β, progesterone, or a combination of these hormones. Prostaglandin $F_{2\alpha}$ was measured by g.c.-m.s. Oestradiol was found to increase prostaglandin $F_{2\alpha}$ synthesis by the uterus whereas progesterone had no effect. However, progesterone did inhibit the response to oestradiol.

4 Oestradiol treatment of the guinea-pig uterus causes an increase in capacity to produce prostaglandin $F_{2\alpha}$ *in vitro*. The role of progesterone is difficult to interpret.

Introduction

Production of prostaglandin $F_{2\alpha}$ by the guinea-pig uterus *in vitro* increases at the end of the oestrous cycle (Poyser, 1972). Prostaglandin $F_{2\alpha}$ and oestradiol levels in utero-ovarian venous plasma also increase at this time, whereas progesterone levels fall (Blatchley, Donovan, Horton & Poyser, 1972; Joshi, Watson & Labhsetwar, 1973). These observations suggest that the synthesis and output of prostaglandin $F_{2\alpha}$ by the guinea-pig uterus may be controlled by the ovarian steroid hormones.

We, therefore, decided to measure the *in vitro* production of prostaglandin $F_{2\alpha}$ by the uteri of ovariectomized guinea-pigs after treatment with progesterone and oestradiol benzoate. We also measured the *in vitro* production of prostaglandin $F_{2\alpha}$ by the day 7 guinea-pig uterus with oestradiol and progesterone present in the incubate. These results have been reported briefly (Naylor, 1975).

Methods

The effect of in vivo *treatment with ovarian hormones*

Twenty virgin female guinea-pigs, which had normal oestrous cycles, were ovariectomized during the luteal phase of the cycle. The guinea-pigs were anaesthetized by injection of pentobarbitone sodium (30 mg/kg), and a dorsal incision was made on either side of the backbone. The fallopian tubes were ligatured in two places and severed between the ligatures, enabling the ovaries to be removed.

Four to eight weeks after surgery, the guinea-pigs were divided randomly into four groups and injected subcutaneously daily with 0.5 ml arachis oil containing steroids as follows: Group I—0.5 ml vehicle only for 10 days (control); Group II—0.5 ml vehicle for 7 days followed by oestradiol benzoate (10 μg daily for 3 days) in arachis oil; Group III—0.5 ml vehicle containing progesterone (2.5 mg daily) for 10 days; Group IV—0.5 ml vehicle containing progesterone (2.5 mg daily) for 7 days followed by 0.5 ml vehicle containing oestradiol benzoate (10 μg daily) plus progesterone (2.5 mg daily) for 3 days. Treatment for all groups thus lasted for 10 days.

On the 11th day after the start of treatment, all guinea-pigs were killed by stunning, then incising the neck. The uteri were removed, weighed, and homogenized in 15 ml Tyrode solution with a Fison's glass homogenizer. The homogenate was bubbled with O_2 and incubated at 37° C for 90

minutes. After incubation, each homogenate was adjusted to pH 4 with HCl and extracted immediately with solvent as described previously (Poyser, 1972).

After ethyl acetate extraction, most samples were assayed by radio-immunoassay (R.I.A.) (Blatchley & Poyser, 1974), though the antibody was solid-phased as described for ovarian steroid hormones by Bolton, Dighe & Hunter (1975). Some samples, however, were assayed by combined gas chromatography-mass spectrometry (g.c.-m.s.) with a Finnegan 3000D mass spectrometer. To these samples was added 1 μg deuterated (d_4) prostaglandin $F_{2\alpha}$ before extraction. After extraction the prostaglandins were prepared as the methyl ester-trimethylsilyl ether (Me-TMS), (Thompson, Los & Horton, 1970; Blatchley, *et al.*, 1972) and assayed by single ion monitoring of the peaks at 423 (M-(90 + 71) for the non-deuterated (d_0) prostaglandin $F_{2\alpha}$ and 427 (d_4-prostaglandin $F_{2\alpha}$) as described by Hensby & Naylor, (1974).

Comparable results were obtained by R.I.A. and g.c.-m.s.

The effect of in vitro *treatment with ovarian hormones*

Six virgin female guinea-pigs which had normal oestrous cycles were killed on day 7 of the oestrous cycle (oestrus = day 1) by stunning, then incising the neck. The uteri were removed and each uterus was divided into four pieces. The portions from each uterus were weighed, then homogenized separately in 15 ml Krebs solution, to which had been added 0.3 ml ethanol containing steroids as follows: Group I–0.3 ml ethanol only (control); Group II–oestradiol-17β to give a final concentration of 100 μg/ml incubate; Group III–progesterone to give a final concentration of 500 μg/ml of incubate; Group IV–oestradiol-17β (100 μg/ml) and progesterone (500 μg/ml). The procedure was designed so that one portion of each uterus was included in each group.

After homogenization, the homogenates were bubbled with 95% O_2 and 5% CO_2 and incubated at 37°C for 90 minutes. Following solvent extraction, the samples were further purified by silicic acid column chromatography (Poyser, 1972), and the prostaglandin fractions assayed by g.c.-m.s. as in the previous experiment.

All results were analysed by Student's *t* test. Oestradiol and progesterone were purchased from Koch-Light Laboratories Ltd., and oestradiol benzoate from Sigma Chemical Company.

Results

The effect of in vivo *treatment with ovarian hormones*

The results are shown in Table 1. Treatment with the two hormones caused the uteri to increase in weight compared with control values, the greatest increase being produced after treatment with oestradiol benzoate alone. Progesterone treatment caused a smaller increase in uterine weight and, in addition, was found to inhibit partially the increase in uterine weight in response to oestrogen treatment. These are well known effects of the ovarian steroid hormones.

Due to this variation in uterine size after treatment with the two hormones, the amounts of prostaglandin $F_{2\alpha}$ produced by the uteri have been expressed both as ng/uterus and as ng/100 mg tissue. Total prostaglandin $F_{2\alpha}$ production/uterus was greatest in animals treated with either oestradiol benzoate, or progesterone followed by oestrogen. There was no significant difference between these two treatments although both produced significantly more prostaglandin $F_{2\alpha}$ ($P < 0.01$) than animals treated with only arachis

Table 1. The *in vitro* production of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by the uterus of the ovariectomized guinea-pig after treatment with oestradiol and progesterone.

Treatment (No. of animals)	*Average weight of uteri (g) ± s.e. mean*	*Total $PGF_{2\alpha}$ production (ng/uterus) ± s.e. mean*	*ng $PGF_{2\alpha}$/100 mg tissue ± s.e. mean*
Control (4)	0.26 ± 0.02	250.8 ± 23.4[a]	97.6 ± 8.7[d]
Oestradiol benzoate (6)	1.00 ± 0.08	2009.3 ± 212.9[b]	205.8 ± 27.1[e]
Progesterone (6)	0.58 ± 0.04	641.7 ± 90.7[c]	109.0 ± 12.6[d]
Progesterone plus oestradiol benzoate (4)	0.71 ± 0.05	1478.3 ± 142.0[b]	208.3 ± 8.6[e]

Values in the same vertical column with the same superscript are not significantly different.

oil. Progesterone treatment alone also caused a significant ($P < 0.01$) increase over control values, but this production was significantly lower than after oestrogen or progesterone plus oestrogen treatment ($P < 0.01$).

When expressed in terms of ng prostaglandin $F_{2\alpha}$ production/100 mg tissue, the results clearly show that treatment with oestradiol benzoate, or progesterone followed by oestradiol benzoate caused a significant ($P < 0.05$) twofold increase in prostaglandin $F_{2\alpha}$ synthesis compared with uteri from animals treated with either arachis oil or progesterone. There was no significant difference between production levels after oestrogen, or progesterone plus oestrogen treatment. Similarly there was no significant difference between control levels, and levels after progesterone treatment.

The effect of in vitro *treatment with ovarian hormones*

The results are shown in Table 2. The control values are in good agreement with values obtained previously (Poyser, 1972; Hensby & Naylor, 1974). Oestradiol treatment causes a significant ($P < 0.01$) increase in production of prostaglandin $F_{2\alpha}$ by the day 7 guinea-pig uterus compared with control values. Treatment with progesterone alone did not cause any significant change in production of prostaglandin $F_{2\alpha}$, and values were significantly lower ($P < 0.01$) than after oestrogen treatment alone. However, progesterone abolished the increase in prostaglandin $F_{2\alpha}$ production caused by oestrogen, as the results of the combined treatment showed. Production of prostaglandin $F_{2\alpha}$ after this combined treatment was significantly lower ($P < 0.05$) than control values. It was also lower than prostaglandin $F_{2\alpha}$ production after progesterone treatment alone, though this difference was not significant.

Table 2. The *in vitro* production of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by the day 7 guinea-pig uterus in the presence of oestradiol and progesterone.

Treatment (No. of replicates = 6)	*ng $PGF_{2\alpha}$/100 mg tissue ± s.e. mean*
Control	52.1 ± 3.4^{f}
Oestradiol	90.1 ± 5.2^{g}
Progesterone	$55.0 \pm 7.9^{f,h}$
Oestradiol plus progesterone	33.7 ± 5.8^{h}

Values with the same superscript are not significantly different.

Discussion

In the guinea-pig, the increase in oestradiol secretion from the ovary towards the end of the oestrous cycle (Joshi *et al.*, 1973) may be the stimulus for increased prostaglandin $F_{2\alpha}$ production and release from the uterus at this time (Poyser, 1972; Blatchley *et al.*, 1972). Bland & Donovan (1968, 1970) have shown that injections of oestradiol benzoate on days 3-11 of the oestrous cycle, or a single injection on day 3 led to regression of the corpora lutea of guinea-pigs, an effect abolished by hysterectomy. Oestradiol benzoate injections early in the cycle also gave rise to increased prostaglandin $F_{2\alpha}$ levels in utero-ovarian venous blood (Blatchley, Donovan, Poyser, Horton, Thompson & Los, 1971; Blatchley *et al.*, 1972) though not in hysterectomized animals, indicating that the prostaglandin $F_{2\alpha}$ comes from the uterus.

Treatment of guinea-pigs with progesterone from the day of oestrus resulted in a shortening of the oestrous cycle (Woody, First & Pope, 1967; Ginther, 1969a), but other workers have shown that progesterone given immediately after this period has no effect on the life-span of the corpus luteum in intact animals (Bland & Donovan, 1968, 1970; Choudary & Greenwald, 1968; Ginther, 1969b). Similarly, 5 mg implants of progesterone in intact guinea-pigs had no effect on luteolysis, although ovulation was completely inhibited (Deanesly, 1968).

The present studies involving the treatment of ovariectomized guinea-pigs with oestrogen and progesterone indicate that the capacity of the uterus to synthesize different amounts of prostaglandin $F_{2\alpha}$ per unit weight of tissue, *in vitro*, is controlled by oestrogen, and progesterone is not a prerequisite for increased synthesis. However, progesterone does appear to be required for the synthesis and release of prostaglandin $F_{2\alpha}$ *in vivo* as shown by Blatchley & Poyser (1974). It is possible, therefore, that the rate-limiting step in the release of prostaglandin $F_{2\alpha}$ from the uterus of the guinea-pig is the availability of arachidonic acid, which consequently may be governed by progesterone.

It cannot be overlooked that decreased metabolism of prostaglandin $F_{2\alpha}$ by the uterus could account for the results obtained. However, metabolism of prostaglandin $F_{2\alpha}$ by the guinea-pig uterus is only 5 to 10% (F.M. Maule Walker & N.L. Poyser, unpublished observations). Therefore it would appear that complete inhibition of metabolism could not account for the increase in observed levels of prostaglandin $F_{2\alpha}$ and the results are better interpreted as due to an increase in synthesis of prostaglandin $F_{2\alpha}$.

A study by Barcikowski, Carlson, Wilson & McCracken, (1974) showed that prostaglandin $F_{2\alpha}$ output by the sheep uterus increased between 60 and 90 min after the start of infusion of oestradiol. Progesterone levels were high at that time. Our present studies have shown that a large dose of oestradiol *in vitro* also caused an increase in the capacity of the guinea-pig uterus to synthesize prostaglandin $F_{2\alpha}$ after a similar time interval. The effect of oestradiol was surprisingly abolished by the presence of progesterone in the incubate. Similar inhibition by progesterone of prostaglandin F synthesis has been demonstrated by Cane & Villee (1975) using human endometrial slices cultured *in vitro*. At present it is difficult to interpret this inhibitory action of progesterone.

The authors gratefully acknowledge the encouragement of Professor E.W. Horton, whose grant from the Medical Research Council made this work possible. Authentic prostaglandins were kindly supplied by Dr J.E. Pike of the Upjohn Company, Kalamazoo, Michigan, U.S.A. B.N. is an M.R.C. research student and N.L.P. is an M.R.C. research fellow.

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(Received June 11, 1975. Revised July 17, 1975)