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The Effect of Estrogen and Progesterone on Uterine Prostaglandin Biosynthesis in the Ovariectomized Rat

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ABSTRACT

The role of 17β-estradiol and progesterone in the *in vivo* synthesis of prostaglandins by the ovariectomized rat uterus was investigated. Seven days after ovariectomy, rats were treated with progesterone (2 mg × 2 days) then given a single injection of estradiol. Prostaglandin E (PGE) and F (PGF) were determined by RIA in uterine tissue and uterine vein plasma (UVP) at 0, 0.5, 1, 2, 4, 8, 12 and 24 h after the administration of 17β-estradiol (10 μg). PGF and PGE content (ng/uterus) remains constant from the time of estradiol administration to the latest time period studied. PGF and PGE concentrations (ng/100 mg uterine wt) show a gradual decline during the course of estrogen action which becomes significant only 12 and 24 h after estradiol administration and is correlated with the increase in uterine weight. Following progesterone administration, there is a significant increase in both uterine PGE content and concentration from ovariectomized levels. These results indicate that levels measured in UVP represent a true *de novo* synthesis of prostaglandins and could not be accounted for by the release of stored material into the uterine vein. PGF levels in UVP rose from control values of 2.42 ± 0.53 ng/ml to a maximum of 23.30 ± 4.96 ng/ml at 12 h and by 24 h had returned to basal levels. A comparison with animals not treated with progesterone revealed low levels of PGF in UVP at all time periods studied (0.5–12 h). PGE levels in UVP after estradiol administration to progesterone-treated and untreated rats were similar, although at 12 h, levels in progesterone-treated rats were significantly greater than the untreated group. Determinations of estradiol in peripheral plasma by RIA revealed a sharp peak 1 h after administration followed by a rapid decline to control values by 12 h.

Using the optimal conditions for prostaglandin biosynthesis as determined in the first part of this experiment (i.e.: 2 days of progesterone pretreatment and uterine cannulation 12 h after estradiol administration), the effect of estradiol doses between 0.001 and 100 μg was investigated. Maximum UVP levels of PGE and PGF were obtained with doses greater than 1.0 μg estradiol. The simultaneous administration of progesterone (2, 10 or 50 mg) with estradiol (10 μg) significantly reduced PGF concentration in UVP, whereas only 50 mg progesterone was effective in significantly reducing PGE levels. Fifty mg of progesterone alone was without effect on PGE and PGF levels in UVP.

It is concluded that estrogens are the predominant ovarian steroids involved in the regulation of uterine prostaglandin biosynthesis in the rat; however, important roles for progesterone in the expression of estradiol stimulated activity are indicated.

INTRODUCTION

Prostaglandins have been identified as the uterine luteolysin in the sheep (McCracken et al., 1972) and the guinea-pig (Poyser, 1974) with strong indications as the uterine luteolysin in laboratory rodents (Pharriss et al., 1972; Hilliard, 1973) and their uterine production has been correlated with increasing estrogen and/or decreasing progesterone at the end of the sheep estrous cycle (Caldwell et al., 1972; Wilson et al., 1972b; Bland et al., 1971) and at the termination of pregnancy in the rat (Labhsetwar and Watson, 1974; Williams et al., 1974) and rabbit (Challis et al., 1973). Similarly the administration of exogenous estrogen to sheep (Caldwell et al., 1972; Barcikowski et al., 1974), guinea pigs (Blatchley et al., 1971), hamsters (Saksena and Harper, 1972), mice (Saksena and Lau, 1973), monkeys (Demers et al., 1974) or rats (Ryan et al., 1974) has resulted in increased uterine prostaglandin synthesis, however the role of progesterone is not yet clearly defined. In some cases endogenous progesterone was present (Blatchley et al., 1971; Saksena and Harper, 1972; Barcikowski et al., 1974) and it is thus difficult to establish the relative roles of estrogen and progesterone

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in prostaglandin production. Results in ovariectomized ewes indicate that progesterone administered alone decreases the production of the F series of prostaglandins by the uterine endometrium (Wilson et al., 1972a) although a prior period of progesterone influence appears to be necessary before estrogen can be effective (Barcikowski et al., 1974). In contrast, progesterone or estrogen administration to rats have been found to have no effect on prostaglandin release by uteri *in vitro* (Harney et al., 1974).

The present study was designed to determine the effect of estrogen and progesterone, alone or in combination, on uterine prostaglandin biosynthesis. The ovariectomized rat was chosen as a model to exclude the effect of endogenous ovarian steroids. The initial study determined the content and concentration of PGF and PGE in uterine tissue in order to ascertain whether prostaglandin levels in UVP in subsequent studies could be due to the release of stored prostaglandins following estradiol administration or actually represented *de novo* synthesis. The levels of prostaglandins in UVP were then determined to study the following: a) the role of estradiol on uterine prostaglandin biosynthesis; b) the effect of progesterone pretreatment of the effect of estradiol; c) the effect of progesterone administration together with estradiol; and d) an estradiol dose response curve.

MATERIALS AND METHODS

Mature female rats of the Sprague-Dawley strain (150–200 grams) were received from the Charles River Breeding Laboratories and maintained under standard animal housing conditions with a 14:10 light:dark cycle and food and water *ad libitum*. Within one week of arrival, animals were bilaterally ovariectomized under ether anesthesia and one week later animals were treated under one of the following regimens.

(1) The Effect of Estradiol on the Uterine Production of Prostaglandin F (PGF) and Prostaglandin E (PGE): Role of Progesterone Pretreatment

Two mg of progesterone (Nutritional Biochemicals) was administered sc in 0.2 ml of arachis oil on two consecutive mornings and on the third morning 10 μg estradiol-17β (Sigma) was administered sc (0.1 ml arachis oil). Uterine vein blood was collected by direct cannulation of the uterine vein (described below) at 0, 0.5, 1, 2, 4, 8, 12 or 24 h following estradiol administration. Uteri were rapidly frozen on dry ice following cannulation for tissue analysis of PGE and PGF. Similarly, estradiol administration (10 μg) without progesterone pretreatment was performed with uterine vein cannulations at 0, 0.5, 1, 2, 4, 8 or 12 h after administration. Cannulations at 24 h were omitted in the latter group since preliminary results indicated prostaglandin levels had declined to control levels by 12 h.

It was determined from part (1) that progesterone pretreatment gave the most effective and prolonged synthesis of prostaglandins and that a maximum PGF concentration was seen at 12 h following estradiol administration, therefore these conditions were used in experiments (2) and (3). Injection volumes, vehicles and routes of administration remained the same unless otherwise indicated.

(2) Effect of Varying Estradiol Dosage on Uterine Prostaglandin Synthesis

Progesterone (2 mg) was administered to ovariectomized rats on two consecutive mornings and on the morning of the third day, 0.001, 0.01, 0.1, 1.0, 10 or 100 μg estradiol was injected and uterine vein blood was collected 12 h after the estradiol administration.

(3) Effect of Concomitant Progesterone Administration on the Estradiol Stimulated Production of Uterine Prostaglandins

Progesterone (2 mg) was administered on two consecutive mornings and on the morning of the third day animals received estradiol (10 μg) concomitantly with either a) arachis oil, b) 2 mg progesterone, c) 10 mg progesterone or d) 50 mg progesterone. In addition, one group of animals received 50 mg of progesterone alone. Uterine veins were cannulated 12 h after injection. In order to obtain the large amounts of progesterone necessary for this study, a commercial preparation of progesterone (Proluton; Schering) of 50 mg/ml was used for all progesterone injections other than the pretreatment injections.

Uterine Vein Cannulations

Cannulations were performed on heparinized animals under Nembutal anesthesia using a method developed by Shaikh and Shaikh (personal communication), which has been published for the hamster (Shaikh and Saksena, 1973). The anterior end of the uterine vein was ligated and a 23 gauge needle attached to catheter tubing was inserted into the uterine vein at the most posterior position available. Blood collections were for 15 min into centrifuge tubes immersed in ice water, after which peripheral blood was collected from the abdominal artery. The uterus was then rapidly removed, trimmed of adherent tissues, blotted, frozen on dry ice and weighed. Blood was immediately centrifuged and plasma stored at −20°C until assayed. Uteri were stored at −20°C until they were homogenized prior to extraction and assay.

Radioimmunoassays

Prostaglandins. For PGF and PGE determinations, plasma was extracted (Skarnes and Harper, 1972) and PGF and PGE separated on silicic acid columns (Caldwell et al., 1971). Frozen uteri were homogenized in 2 mls cold acetate buffer (0.6M; pH 4.5) containing 0.6 percent sodium sulphite. Homogeniza-

tions were done in all glass homogenizers with three series of ten strokes each and 40 seconds of cooling between each series. Entire homogenates were then extracted and chromatographed in the same manner as plasma samples. The radioimmunoassay of PGF was performed using the method of Stylos et al. (1972) and PGE as described by Stylos et al. (1974). Since PGF and PGE assays were performed in the same laboratories as were the original validation studies, readers are referred to these original reports for details. It is not possible with the F antiserum to distinguish between PGF_{1a} and PGF_{2a}, since PGF_{1a} cross-reacts greater than 75 percent, therefore results are expressed as PGF equivalents. Although data for PGE is expressed as PGE equivalents, there is only a 22 percent cross-reaction of PGE_2 with the PGE_1 antiserum. Therefore interpretation of the PGE data must keep this reservation in mind. The assay sensitivity was 50 pg for PGF and 25 pg for PGE. The interassay coefficient of variation for both the PGF and PGE assay was 13.8 percent.

17-β-estradiol. Peripheral plasma was extracted and estradiol was separated on Celite columns and assayed by the method as presented by Labhsetwar and Watson (1973). The assay sensitivity was 5 pg.

All radioimmunoassay results were corrected for 100 percent recovery by the addition of tracer amounts of labelled standards to plasma and uterine homogenates prior to extraction.

Statistics. Statistical analyses were calculated by Duncan's Multiple Range Test for correlated and heteroscedastic means (Duncan, 1957).

RESULTS

(1) The Effects of Estradiol on the Uterine Production of PGF and PGE: Role of Progesterone Pretreatment

Uterine PGF and PGE content and concentration. Uterine content of PGF increased slightly, but not significantly, from levels in ovariectomized animals following progesterone administration and remained constant from the time of estradiol injection until the last measurements were made, 24 h later. Uterine PGE content was significantly increased ($P<0.05$) from ovariectomized levels following progesterone treatment but, thereafter, remained constant.

The concentration of PGF in the uterus underwent a gradual decline following estradiol administration but this decrease was not significant until 12 and 24 h after estradiol administration ($P<0.05$). The pattern of PGE concentration in the uterus was identical to that of PGF, with one exception, that a significant increase ($P<0.05$) from the ovariectomized concentration was seen after progesterone administration (Table 1).

PGF in UVP. Treatment of ovariectomized

TABLE 1. Uterine PGF and PGE content and concentration following estradiol administration to the progesterone pretreated ovariectomized rat.

Treatment*	Uterine content (ng/uterus)		Uterine concentration (ng/100 mg uterine wet wt)	
	PGF	PGE	PGF	PGE
OVX	28.4 ± 4.4** (10)	2.46 ± 0.39 (10)	20.39 ± 2.37 (10)	1.72 ± 0.18 (10)
OVX-P	38.2 ± 6.3 (10)	5.04 ± 0.69 (10)	26.67 ± 2.99 (10)	3.56 ± 0.44 (10)
OVX-P-E-1 hr	38.2 ± 5.2 (11)	5.59 ± 0.93 (11)	25.22 ± 2.50 (11)	3.70 ± 0.46 (11)
OVX-P-E- 2 hr	43.2 ± 4.9 (14)	5.82 ± 0.56 (13)	24.24 ± 2.93 (14)	3.36 ± 0.33 (13)
OVX-P-E-4 hr	37.7 ± 3.8 (11)	5.10 ± 0.47 (11)	21.30 ± 2.66 (11)	2.80 ± 0.21 (11)
OVX-P-E-8 hr	35.0 ± 6.8 (7)	5.12 ± 1.19 (7)	18.66 ± 3.72 (7)	2.54 ± 0.68 (7)
OVX-P-E-12 hr	40.4 ± 6.1 (10)	4.49 ± 0.81 (10)	17.50 ± 2.68 (10)	1.89 ± 0.28 (10)
OVX-P-E-24 hr	29.0 ± 5.1 (9)	4.29 ± 0.97 (9)	12.66 ± 2.30 (9)	1.78 ± 0.36 (9)

*OVX = Ovariectomized 7 days; P = 2 mg progesterone X 2 days; E = 10 μg Estradiol.

**Mean ± S.E.M. (N).

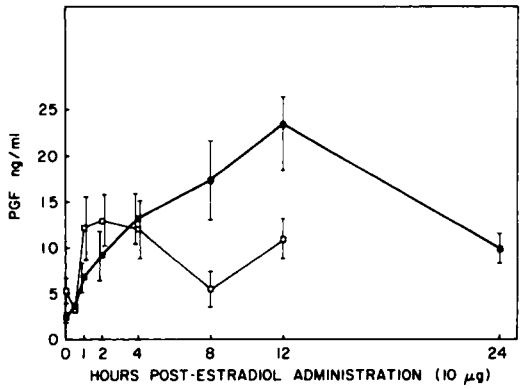

FIG. 1. PGF concentration in uterine vein plasma at intervals over a 24 h period following a single injection of estradiol (10 μg). Closed squares (■) indicate animals which were treated with 2 mg progesterone for two days prior to estradiol administration and open squares (□) indicate animals which were not pretreated. Individual points and brackets represent means and standard errors. The number of individual PGF determinations per group in the progesterone treated series was 10, 5, 11, 10, 9, 10, 12 and 12 at 0, 0.5, 1, 2, 4, 8, 12 and 24 h after estradiol, respectively. In the animals not treated with progesterone, 9, 7, 7, 9, 8, 4 and 9 determinations per group at 0, 0.5, 1, 2, 4, 8 and 12 h after estradiol, respectively.

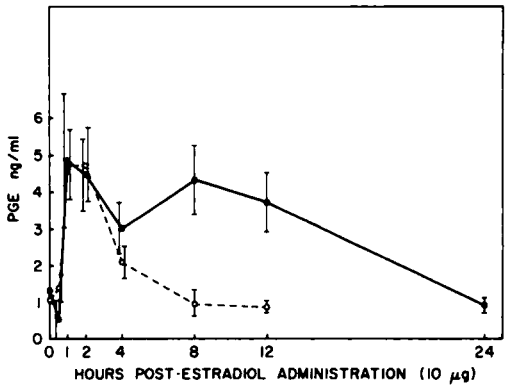

FIG. 2. PGE concentration in uterine vein plasma at intervals over a 24 h period following a single injection of estradiol (10 μg). Closed circles (●) indicate animals which were treated with 2 mg progesterone for two days prior to estradiol administration and open circles (○) indicate animals which were not pretreated. Individual points and brackets represent means and standard errors. The number of individual PGE determinations per group in the progesterone treated series was 10, 4, 10, 10, 9, 10, 12 and 12 at 0, 0.5, 1, 2, 4, 8, 12 and 24 h after estradiol, respectively. In the animals not treated with progesterone, 9, 7, 7, 8, 8, 4 and 9 at 0, 0.5, 1, 2, 4, 8 and 12 h after estradiol, respectively.

rats with 2 mg progesterone for 2 days prior to estradiol administration (10 μg) resulted in a significant elevation of PGF concentration above 0 time levels in uterine venous plasma at 4, 8 and 12 h following estradiol administration (Fig. 1). Maximal levels were observed at 12 h with a return to levels not significantly different from controls at 24 h.

Without progesterone pretreatment increases in PGF concentration were seen at 1, 2 and 4 h but were not significantly different from 0 time controls. Values at 8 and 12 h in the progesterone treated group were significantly elevated in comparison to the same time periods in the groups without progesterone treatment.

PGE in UVP. A significant elevation in uterine venous PGE concentration was observed at 1 and 2 h following estradiol administration to either progesterone-treated or untreated animals (Fig. 2). These elevations were followed by a decline at 4 h to levels not significantly different from untreated values in both groups. In the progesterone-treated animals a significant elevation was again observed at 8 h and both 8 and 12 h levels in this group were significantly greater than animals which had not received progesterone at the same time intervals.

Uterine weight and peripheral estradiol. Estradiol administration without progesterone resulted in significantly greater uterine weights at 4 and 8 h in comparison to the progesterone treated animals at the same time period (Table 2). Peripheral plasma estradiol concentration was sharply increased with a peak at 1 h following the administration of 10 μg estradiol. Levels were still elevated at 2 and 4 h but were significantly less than peak levels. Control values were reached at 12 h. Since identical estradiol levels were observed in both progesterone-treated and untreated animals, the data presented in Table 2 are a combination of both groups.

(2) Effect of Varying Estradiol Dosage on Uterine Prostaglandin Production

PGF. PGF concentration in uterine venous plasma was not affected 12 h following the administration of 0.001 or 0.01 μg estradiol, however a slight, but not significant, increase was noted with 0.1 μg (Fig. 3). Administration of estradiol in doses of 1.0, 10 or 100 μg

TABLE 2. Mean peripheral plasma estradiol concentration and uterine weight following estradiol injection.

Hours after estradiol administration[a]	Peripheral estradiol[c] pg/ml	Uterine weights	
		With progesterone pretreatment (mg)	Without progesterone pretreatment (mg)
0	102.6 ± 7.9[b] (20)	142.8 ± 10.2 (10)	152.4 ± 17.3 (13)
0.5	1069.8 ± 174.9 (9)	171.3 ± 25.2 (5)	159.9 ± 6.5 (8)
1	1122.9 ± 153.5 (15)	155.3 ± 11.8 (12)	170.3 ± 10.2 (9)
2	586.0 ± 37.5 (22)	180.3 ± 8.9 (15)	196.0 ± 12.4 (9)
4	255.0 ± 14.0 (16)	187.2 ± 10.4 (12)	239.6 ± 20.5 (9)
8	159.4 ± 27.5 (10)	216.2 ± 20.2 (10)	273.2 ± 28.1 (5)
12	102.3 ± 9.1 (12)	256.7 ± 18.0 (13)	254.1 ± 12.1 (9)
24	105.8 ± 37.6 (5)	259.7 ± 14.9 (12)	... ...

[a]Represents the time when cannulations were begun; peripheral blood and weights were obtained 15 min later.

[b]Mean ± S.E.M. (N).

[c]Following sc administration of 10 μg 17β-estradiol to both progesterone pretreated and untreated ovariectomized rats.

resulted in significant elevations in PGF concentration, although there was an increase in mean PGF concentration with each dose, they were not significantly different from each other.

PGE. Statistical comparisons for uterine venous PGE concentrations were identical for those presented for PGF concentrations. The increase in mean values noted from 1 to 10 to 100 μg estradiol for PGF was not seen with PGE levels.

Uterine weight and peripheral estradiol. Uterine weights were significantly elevated above untreated values for all administered doses of estradiol. No significant differences were observed after 0.1, 1.0 and 10 μg of estradiol but 100 μg resulted in a significant increase in uterine weight above all lower doses. The peripheral plasma estradiol concentration 12 h following the administration of 10 μg estradiol was not significantly different from control values (Table 2), however, following 100 μg estradiol peripheral concentrations at 12 h were twice that (203.8 ± 28.1 pg/ml) of animals administered 10 μg.

(3) Effect of Concomitant Progesterone Administration on the Estradiol Stimulated Production of Uterine Prostaglandins (Fig. 4)

PGF. The simultaneous administration of progesterone (2, 10 or 50 mg) with estradiol (10 μg) resulted in a significant reduction in uterine venous PGF concentrations. Although a stepwise decrease was observed with increasing doses of progesterone, the values were not significantly different from each other. Administration of 50 mg progesterone alone did not result in any change in PGF concentrations from control values.

PGE. The simultaneous administration of 50 mg progesterone with estradiol (10 μg) resulted in a significant decrease in the elevated PGE concentration observed with estradiol alone. Two or 10 mg progesterone did not cause any change in the effectiveness of estradiol administration and 50 mg alone was without effect on uterine venous PGE concentrations.

Uterine weight. None of the doses of progesterone administered with estradiol produced

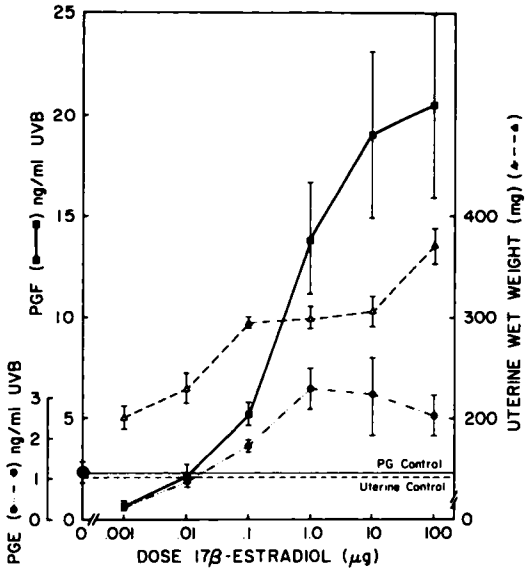

FIG. 3. Effect of increasing doses of estradiol on concentration of PGF (■) and PGE (●) in uterine vein plasma and on uterine wet weight (△). Individual points represent means and standard errors. Lines designated PG control and uterine control are the means of both PGF and PGE concentration and of uterine wet weight, respectively, in untreated controls. There are a minimum of seven determinations for each point on the graph.

any significant lowering of uterine weight but 50 mg or progesterone alone caused a significant increase of uterine weight above untreated controls, although significantly lower than all groups treated with estradiol.

DISCUSSION

The current opinion is that prostaglandins are synthesized and rapidly released from tissues without appreciable storage in the tissue itself (Bito, 1975). In those rats pretreated with progesterone, followed by estradiol administration, the uterine content (ng/uterus) of both PGF and PGE remains constant throughout the 24 h period following estradiol administration. The concentrations (ng/100 mg uterine wt) of PGF and PGE show a gradual decline starting at the time of estradiol administration, which correlates with the increase in uterine weight over this same period. Since the increase in uterine weight for the first 6 h and more following estrogen administration is, in large part, water imbibition (Astwood, 1938), the decrease in concentration represents a dilution of prostaglandins and not a release into the uterine vein. Additionally, the entire content of PGF of any one uterine horn is not sufficient to account for more than about 1 ml of the maximum levels in UVP seen at 12 h after estradiol administration (>20 ng/ml). As further evidence of *de novo* synthesis, indomethacin, an inhibitor of prostaglandin synthetase, results in undetectable levels of both PGF and PGE in UVP following estradiol administration, indicating a direct effect on synthesis and not release (Castracane and Jordan, in preparation). The same conclusion from indomethacin treatment and estradiol infusion in the sheep has been made by Barcikowski et al. (1974).

While it is possible that changes in prostaglandin concentration in the venous effluent of a tissue may be a reflection of an alteration in prostaglandin metabolism by that tissue, this seems to be unlikely in the present study. If, indeed, alterations in metabolism were to account for the 10-fold increase in PGF seen from ovariectomized to maximal concentrations at 12 h after estradiol, then some corresponding alteration in tissue content might be expected but was not seen. Little work seems to have been done in the area of uterine prostaglandin metabolism and preliminary studies indicate that the human uterus does not effectively metabolize prostaglandins (Nakano et al., 1970; Kierse et al., 1975). Similarly, the distribution of prostaglandin metabolizing enzymes in the swine, while very active in several tissues are only minimally active in the uterus (Larsson and Anggard, 1970; Anggard, 1971). Consequently, the remaining studies, based on prostaglandin levels in UVP, have been interpreted as *de novo* uterine synthesis of prostaglandins.

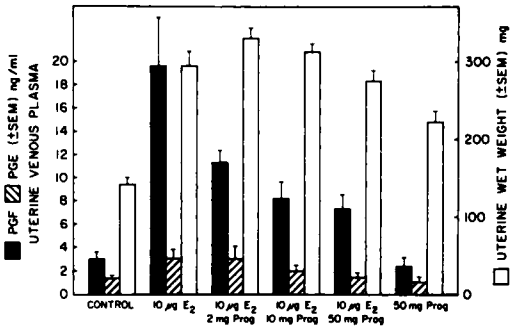

FIG. 4. The effect of concomitant administration of estradiol and progesterone, or of estradiol or progesterone alone, on uterine vein plasma concentration of PGF and PGE and on uterine wet weight. There are a minimum of seven determinations for each mean.

These studies in uterine vein plasma confirm and extend earlier studies (Caldwell et al., 1972; Barcikowski et al., 1974; Blatchley et al., 1971; Saksena and Harper, 1972; Saksena and Lau, 1973; Demers et al., 1974; and Ryan et al., 1974) indicating the effectiveness of estradiol in stimulating uterine PGF or PGE synthesis. In addition, this study has demonstrated an important role for progesterone in estradiol stimulated production of uterine prostaglandins in the ovariectomized rat. Without progesterone pretreatment, estradiol produces a small, but nonsignificant elevation in PGF concentration in the uterine vein. Despite ovariectomy, it must be recalled that adrenal progesterone is secreted in appreciable amounts (Resko, 1969; Fajer et al., 1971; Shaikh and Shaikh, 1975) so that this animal cannot be considered completely free of progesterone. It is possible that following adrenalectomy and ovariectomy, estradiol administration without progesterone pretreatment might have been less effective than it was in the present study following ovariectomy alone. Progesterone pretreatment promotes a much greater and more prolonged response in uterine PGF production following a single injection of estradiol (Fig. 1). With regard to PGE production, both progesterone-treated and untreated ovariectomized rats demonstrated identical increases for the first four hours following estradiol administration, but again, a more prolonged increase is seen in the progesterone-treated animals (Fig. 2). It has also been shown that progesterone is required for estradiol stimulation of PGF production by the ovine uterus (Barcikowski et al., 1974). Warren et al. (1973) observed that the luteolytic effect of estradiol in the ewe, an effect presumed to be mediated via increased uterine prostaglandin production (Lewis and Warren, 1974), is facilitated by the prior administration of progesterone. This is clearly shown in the present study, as well as in that of Barcikowski et al. (1974) and suggests a priming effect of progesterone on estradiol stimulated uterine prostaglandin synthesis. Similarly, prior treatment with progesterone greatly enhances the effect of estrogen on uterine prostaglandin production in the ovariectomized guinea pig (Blatchley and Poyser, 1974).

In the sheep, infusion of physiological levels of estradiol into the uterine arterial supply for six hours during the late luteal phase (Day 14), a period of constantly elevated blood progesterone concentration, results in PGF production which reaches a maximum at two hours after the initiation of infusion and then declines, although remaining above control levels for 10 h. The same estradiol infusion at Days 6 or 10 of the ovarian cycle did not result in an increase in the basal release of PGF (Barcikowski et al., 1974). Caldwell et al. (1972) administered a single large injection of estradiol to the ovariectomized ewe after several days of progesterone injections and observed increased peripheral levels of PGF, with a peak at 12 h and a return to basal levels by 24 h, which was very similar to results obtained in this study in the rat. The six-hour period of intra-arterial estradiol-17β infusion increased PGF production within two hours but later starts to decline while estradiol infusion is still in progress (Barcikowski et al., 1974). However, following a single injection of estradiol in the ovariectomized ewe, PGF levels in peripheral blood remain elevated, long after circulating levels have presumably declined (Caldwell et al., 1972). This difference suggests that a constant or prolonged exposure to estradiol may actually depress uterine prostaglandin synthesis and may be the reason for the decline in PGF production in the presence of infused estradiol. A second injection of estradiol (10 μl) was administered to rats 12 h after the first injection, a time of maximal PGF levels, the uterine vein cannulated 12 h after the second injection and levels (3.89 ± 0.22) found to be similar to untreated controls (2.42 ± 0.53). This suggests that uterine PGF synthesis becomes refractory to estradiol stimulation, although the time after the priming dose of progesterone may have been sufficiently long to lose its effectiveness for the second estradiol injection. An alternative possibility to explain the more rapid decline in the infusion study may be related to the constant background of progesterone in that study (Barcikowski et al., 1974). Thus in the sheep (Caldwell et al., 1972) and in the rat, a single injection of estradiol may cause prolonged PGF synthesis, either because estrogen was given as a single injection or because of the lack of progesterone in these animals or both. Since this study has shown that progesterone administered concomitantly with estradiol will depress uterine PGF production, a depressant effect of progesterone could explain the more transient stimulation of PGF in the study of Barcikowski et al. (1974).

There is no apparent correlation between uterine weight and prostaglandin synthesis since uterine weights are comparable 12 h following

estradiol in both progesterone-treated and untreated groups but only the treated group shows an increased PGF production at that time. Similarly, PGE synthesis is greatest soon after estradiol administration and is not correlated with the maximal increase in uterine weight. In a companion study to this one, the use of inhibitors of prostaglandin synthesis is without effect on uterine weight increases at doses which effectively inhibit prostaglandin synthesis (Castracane and Jordan, in preparation.

After a single subcutaneous injection of estradiol-17β, estradiol levels in the peripheral plasma reached a peak at one hour and dropped sharply by two hours, reaching ovariectomized levels by 12 h. The continued increase in uterine PGF production, with a maximum at 12 h following estradiol, indicates that a constant blood level is not required for stimulation, although peripheral levels obtained in this study are much higher than peripheral levels reached during the estrous cycle (Shaikh and Shaikh, 1975). It is well established that in the rat uterus there are receptor proteins capable of binding and retaining estradiol (Noteboom and Gorski, 1965; Toft and Gorski, 1966). The time course of estradiol-stimulated PGF production occurs in parallel with the time course of increased protein synthesis, suggesting that these events may be related. However, the pattern of PGE production, with a maximum at one hour, suggests that increased PGE secretion may be independent of the estradiol-induced protein synthesis (Aizawa and Mueller, 1961). In addition, preliminary results from our laboratory show that several agents known to interfere with this classical estrogen-receptor initiated sequence of events are without effect on estradiol-stimulated uterine prostaglandin synthesis and include the anti-estrogen MER-25, Actinomycin D and cycloheximide (Castracane and Jordan, in preparation).

It seems that progesterone mediates some biochemical event essential for the production of PGF by the uterus prior to estrogenic stimulation, although such a role for progesterone seems less important in the production of PGE. More commonly, the prior exposure to estradiol is essential to or enhances the action of progesterone in many biological systems (Courrier, 1950). Barcikowski et al. (1974) have suggested that the role of progesterone in the period prior to estradiol administration may serve to allow the necessary fatty acid precursors to accumulate in the endometrium. This is supported by the observation, in the ewe, of lipid droplets in the endometrium following progesterone administration and the decrease in concentration of droplets following estradiol administration (Brinsfield and Hawk, 1973). If the same dosage of progesterone (2 mg) used for pretreatment is administered concomitantly with estradiol, there is a significant reduction in uterine PGF production which suggests that the facilitory action of progesterone occurs prior to estradiol administration and not during the period of estrogen action. The inhibitory effect of progesterone on PGE production is only seen when the highest dose (50 mg) of progesterone is used. Fifty mg of progesterone alone is without effect on uterine production of either PGF or PGE, consistent with the study of Wilson et al. (1972a) of the ovine uterus. A possibility exists, not considered by the experimental design, that the peak of prostaglandin production is shifted by the concomitant administration of progesterone with estradiol and thus would not be observed at the time of sampling. This possibility remains to be investigated, although studies in the ovariectomized monkey (Demers et al., 1974) and *in vitro* studies with human endometrium (Cane and Villee, 1975) indicate a suppressive action of progesterone on uterine prostaglandin synthesis.

It can be seen from the estradiol dose response section of this study (Fig. 3) that 1 μg of estradiol is as effective as 10 or 100 μg in stimulating PGF or PGE production. Since these doses are equally effective, it would probably have been advantageous to have used 1 μg estradiol in conjunction with the concomitant progesterone administration since that dosage would have resulted in a more physiological ratio of these steroids. Other studies in this laboratory (Castracane and Shaikh, 1976) indicate PGF concentration to be in a range of about 4–6 ng/ml during pseudopregnancy, more in line with the levels obtained following 0.1 μg estradiol. The levels of uterine vein PGF in cycling rats (Saksena et al., 1973), although higher than pseudopregnant rats, are still only half of that obtained under optimal conditions in this study.

The present study has demonstrated the importance of both estrogen and progesterone in the control of uterine prostaglandin synthesis. Excellent correlations between uterine vein prostaglandin concentration and peripheral levels of ovarian steroids can be seen in cycling,

pregnant and pseudopregnant rats and hamsters (Saksena et al., 1973; Shaikh and Saksena, 1973; Shaikh et al., 1973; Labhsetwar and Watson, 1974) and support the findings in this study. Further studies are in progress to study the effect of inhibitors of prostaglandin synthesis in this system and also investigations into the mechanism of estrogen action on uterine prostaglandin synthesis.

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RECOMMENDED REVIEWS

Barcikowski, B., Carlson, J., Wilson, L. and McCracken, J. A. (1974). The effect of endogenous and exogenous estradiol-17β on the release of prostaglandin $F_{2\alpha}$ from the ovine uterus. Endocrinology 95, 1340-1349.

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