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Evidence for the Involvement of Prostaglandins Throughout the Decidual Cell Reaction in the Rat[1]

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ABSTRACT

Previous studies in which prostaglandin (PG) production was inhibited for a limited time by the s.c. administration of indomethacin have suggested that PGs are involved in the initiation of decidualization as well as the growth and differentiation of decidual cells. To reduce PG production during decidualization, in the present study indomethacin was infused from Alzet osmotic minipumps into the uterine lumen of ovariectomized rats with uteri sensitized for decidualization. To determine the effect of route of indomethacin administration on decidualization, rats received a single s.c. injection of indomethacin or its vehicle, and unilateral intrauterine infusion of indomethacin or its vehicle, in a factorial experiment. The inhibitory effects on decidualization, as assessed 5 days later by uterine weights, were greatest when both treatments were combined. Prostaglandins E and F concentrations 24 and 48 h after the insertion of the pumps were lower in the indomethacin-infused horns, suggesting that the indomethacin reduced uterine PG production. By contrast, subcutaneously administered indomethacin reduced uterine PG concentrations at 24 h but not at 48 h. Prostaglandin E_2 and $PGF_{2\alpha}$ alone or combined, infused with indomethacin into the uterine lumen of rats treated subcutaneously with indomethacin, overrode the inhibitory effects of indomethacin. The dose-response relationships between these PGs and decidualization did not differ. These data suggest that PGs are required during the growth and differentiation of decidual cells from endometrial stromal cells.

INTRODUCTION

The differentiation of endometrial stromal cells to decidual cells occurs in rodents in response to blastocysts or aritificial stimuli. Decidualization can be obtained in response to many different types of artificial stimuli, but only when these are applied during a limited period of pregnancy or pseudopregnancy, or when the uterus has been sensitized by the administration of an appropriate regimen of hormone injections to the animals (Psychoyos, 1973; Finn and Porter, 1975). Decidualization, whether in response to a blastocyst or an artificial stimulus, is always preceded by an increase in endometrial vascular permeability (Psychoyos, 1973).

There is now considerable evidence suggesting that prostaglandins (PGs) have a role in implantation and decidualization (see review by Kennedy, 1983). Based on experiments in which PGs were infused into the uterine lumen of rats, Kennedy and Lukash (1982) suggested that PGs were involved not only in the initiation of decidualization but also throughout the differentiation of decidual cells. However, in those experiments, endogenous PG production was inhibited for a limited time by the subcutaneous administration of indomethacin, an inhibitor of PG biosynthesis (Vane, 1971). Thus it is possible that the responses to the infused PGs may, at least in part, have resulted from interactions between the exogenous PGs and endogenous ones produced after the inhibitory effect of indomethacin had passed. In the experiments to be reported here, we have examined the effect of infusing indomethacin into the uterine lumen on decidualization.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats were obtained from Charles River Inc. (St. Constant, Quebec) and were

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housed under temperature- and light-controlled conditions (lights on from 0500 to 1900 h) with free access to food and water.

Preparation of Animals

Rats weighing 200–225 g were ovariectomized under ether anesthesia and allowed at least 4 days to recover from the operation. To obtain rats with uteri sensitized for the decidual cell reaction, estradiol and progesterone were given to the rats in the sequence described by Kennedy and Lukash (1982) except that, on the afternoon of the day before the intrauterine treatment, 0.15 μg estradiol plus 2 mg progesterone were administered s.c. instead of 0.05 μg estradiol plus 2 mg progesterone. The protocol is illustrated in Fig. 1. Preliminary experiments indicated that with this modification the timing and duration of uterine sensitization was more equivalent to that found in pseudopregnancy.

Intrauterine Infusions

Alzet osmotic minipumps, model 2001 (pumping rate 1 μl/h) were used for the unilateral infusion of compounds into the uterine lumen. Stock solutions of indomethacin (50 mM) and PGE_2 (100 mg/ml) in ethanol were diluted with phosphate-buffered saline containing gelatin (PBSG) (Kennedy, 1979) to give the required concentrations. Prostaglandin $F_{2\alpha}$-tris (hydroxymethyl) aminomethane ($PGF_{2\alpha}$-THAM) salt was dissolved in PBSG to give the required concentration in acid equivalents. Within an experiment, all solutions for infusion had the same concentration of ethanol, which never exceeded 2%.

The empty pumps were incubated overnight at room temperature 0.9% NaCl and were filled with the freshly prepared solutions just prior to being inserted in the animals.

The technique for inserting the pumps has been described (Kennedy and Lukash, 1982). In summary, one uterine horn of each animal was infused from the uterotubal end toward the cervical end; the contralateral noninfused horn served as a control, which provided an opportunity to detect possible systemic effects of the infused compounds.

Subcutaneous Indomethacin

To inhibit PG synthesis in response to the inevitable trauma associated with insertion of the pumps in the uterus, indomethacin was given to some animals as 2-mg doses in 0.4 ml sesame oil s.c. 2 h prior to the procedure. Control animals received an equivalent volume of sesame oil.

Decidualization

The extent of uterine decidualization was evaluated 5 days after the insertion of the pumps by weighing separately the infused and noninfused horns (Finn and Keen, 1963; Yochim and De Feo, 1973).

Uterine PG Levels

The extraction procedures and radioimmunoassays used to determine the concentrations of PGs of the E and F series in the infused and noninfused uterine horns have been described previously (Kennedy, 1979). The anitserum for the PGE assay did not differentiate between PGs of the E series; cross-reactivity with PGA_2 was approximately 10%, whereas that with the F and B type PGs and 6-oxo-$PGF_{1\alpha}$ was <3%. The antiserum for the PGF assay did not differentiate between F series PGs; cross-reactivity with the E series PGs and 6-oxo-$PGF_{1\alpha}$ was less than 1%. The intraassay coefficients of variation for the PGE and PGF assays were 6% and 12%, respectively.

Data Analysis

The significance of treatment effects was determined by analysis of variance, with the variance being partitioned on a between- and within-animal basis. When significant interactions were found, Duncan's New Multiple Range Test (Steel and Torrie, 1960) was used for between-animal group comparisons, and paired *t*-tests for within-animal comparisons. Data were logarithmically transformed prior to statistical analysis in order to remove, or at least reduce, heterogeneity of variance, as determined by Bartlett's test (Snedecor, 1956).

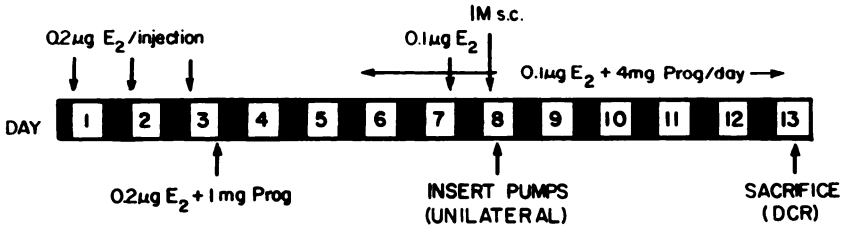

FIG. 1. Schematic representation of the sequence of hormone administration to ovariectomized rats. *Dark areas*, lights off; *open areas*, lights on. Alzet osmotic minipumps were installed on Day 8 to infuse uterine horns unilaterally. The rats were killed 5 days later to determine the extent of the decidual cell reaction (*DCR*). Indomethacin (*IM*) or its vehicle was given approximately 2 h prior to pump installation. E_2, 17β-estradiol; *Prog*, progesterone. From Day 6 on, the steroid hormones were administered s.c. once daily at approximately 0800 h, except for Day 7 when they were given in 2 divided doses at 0800 and 1630 h; the second injection was supplemented with 0.1 μg E_2.

RESULTS

Mode of Indomethacin Administration and Decidualization

In a factorial experiment, rats with uteri sensitized for the decidual cell reaction were randomized to 4 groups and treated with or without indomethacin subcutaneously and with or without a unilateral intrauterine infusion of indomethacin. The animals were killed 5 days after the insertion of the pumps and decidualization was assessed by uterine weights. The data (Fig. 2) were analyzed as a 3-factor mixed-model analysis of variance as follows: *Factor 1*, indomethacin s.c. (vehicle vs. 2 mg indomethacin); *Factor 2*, indomethacin intrauterine (vehicle vs. 0.5 mM indomethacin); and *Factor 3*, infusion (infused horn vs. contralateral noninfused horn.)

Factors 1 and 2 were between-animal comparisons, and Factor 3 a within-animal comparison. Analysis of variance indicated significant 2-way interactions between the effects of intrauterine-infused indomethacin (Factor 2) and infusion (Factor 3) ($P<0.001$) and between the effects of subcutaneously administered indomethacin (Factor 1) and infusion (Factor 3) ($P<0.005$). These interactions were examined by additional analyses of variance and Duncan's New Multiple Range Tests. For noninfused horns, neither mode of indomethacin administration significantly affected uterine weights. By contrast, for infused horns, the indomethacin treatments were associated with significantly reduced weights, with weights being lowest in those receiving both modes of indomethacin administration.

Mode of Indomethacin Administration and Uterine PG Concentrations

To determine the effects of route of indomethacin administration on uterine PG synthesis, as indicated indirectly by uterine PG concentrations, rats with uteri sensitized for the decidual cell reaction were randomized to 8 groups in a factorial experiment. The rats were treated with or without indomethacin subcutaneously, with or without a unilateral intrauterine infusion of indomethacin, and were killed 24 or 48 h after pump insertion. Uterine weights and uterine PGE and PGF concentrations are presented in Table 1. The data were analyzed as a 4-factor mixed-model analysis of variance as follows: *Factor 1*, indomethacin s.c. (vehicle vs. 2 mg indomethacin); *Factor 2*, indomethacin intrauterine (vehicle vs. 0.5 mM indomethacin); *Factor 3*, time (killing at 24 h vs. 48 h); and *Factor 4*, infusion (infused horn vs. contralateral noninfused horn).

Factors 1, 2, and 3 were between-animal comparisons, and Factor 4 a within-animal comparison. Significant ($P<0.01$) 2-way interactions between the effects of infusion (Factor

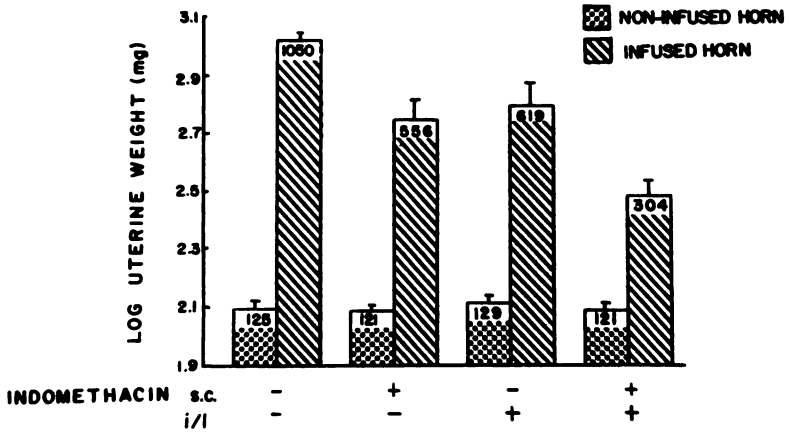

FIG. 2. Effect of mode of administration of indomethacin on wet weight of uterine horns. *Indomethacin s.c.*, 2 mg indomethacin in 0.4 ml sesame oil approximately 2 h before pump insertion on Day 8; controls received 0.4 ml sesame oil. *Indomethacin i/l*, unilateral intrauterine infusion (1 μl/h) of 0.5 mM indomethacin in 1% ethanol in phosphate-buffered saline containing gelatin; controls received the vehicle. Each bar represents the mean + SEM of the logarithmically transformed data from 12 rats. The geometric mean is given at the top of each bar.

TABLE 1. Effects of mode of indomethacin administration on uterine weights and uterine PGE and PGF concentrations 24 and 48 h after beginning unilateral intrauterine infusions in rats (mean ± SEM after log transformation[a]; $n = 6$; geometric mean given in parentheses).

Time of killing	Subcutaneous treatment[b]	Intrauterine treatment[c]	Uterine weight (mg)		Uterine PGE concentration (pg/mg)		Uterine PGF concentration (pg/mg)	
			Infused horn	Noninfused horn	Infused horn	Noninfused horn	Infused horn	Noninfused horn
24 h	Vehicle	Vehicle	2.324 ± 0.053 (211)	2.020 ± 0.020 (105)	0.663 ± 0.028 (4.61)	0.354 ± 0.124 (2.26)	0.378 ± 0.093 (2.39)	0.445 ± 0.132 (2.79)
		Indomethacin	2.208 ± 0.053 (161)	1.981 ± 0.018 (95.7)	0.519 ± 0.117 (3.30)	0.362 ± 0.080 (2.30)	0.304 ± 0.100 (2.02)	0.570 ± 0.149 (3.72)
	Indomethacin	Vehicle	2.015 ± 0.037 (103)	1.962 ± 0.024 (91.6)	0.166 ± 0.084 (1.46)	0.215 ± 0.061 (1.64)	−0.116 ± 0.203 (0.765)	−0.519 ± 0.181 (0.303)
		Indomethacin	2.005 ± 0.044 (101)	2.027 ± 0.038 (106)	0.201 ± 0.078 (1.59)	0.024 ± 0.073 (1.06)	−0.181 ± 0.116 (0.658)	−0.467 ± 0.243 (0.341)
48 h	Vehicle	Vehicle	2.346 ± 0.068 (222)	1.985 ± 0.039 (96.5)	1.028 ± 0.104 (10.7)	0.705 ± 0.115 (5.07)	0.766 ± 0.129 (5.83)	1.207 ± 0.172 (16.1)
		Indomethacin	2.163 ± 0.078 (146)	1.938 ± 0.038 (86.7)	0.764 ± 0.114 (5.81)	0.873 ± 0.056 (7.47)	0.525 ± 0.115 (3.35)	1.353 ± 0.085 (22.6)
	Indomethacin	Vehicle	2.079 ± 0.044 (120)	1.974 ± 0.013 (94.3)	0.973 ± 0.113 (9.39)	0.653 ± 0.112 (4.50)	0.833 ± 0.184 (6.81)	1.061 ± 0.181 (11.5)
		Indomethacin	2.028 ± 0.031 (107)	1.972 ± 0.026 (93.7)	0.490 ± 0.140 (3.09)	0.759 ± 0.129 (5.74)	0.639 ± 0.036 (4.35)	1.278 ± 0.060 (19.0)

[a] For uterine PGF concentrations, the transformation was log ($x \pm 0.1$).

[b] 0.4 ml sesame oil with or without 2 mg indomethacin approximately 2 h prior to pump insertion.

[c] 1% ethanol in phosphate-buffered saline containing gelatin, with or without 0.5 mM indomethacin, at 1 μl/h.

4) and of subcutaneously administered indomethacin (Factor 1) and between infusion (Factor 4) and intrauterine-administered indomethacin (Factor 2) were revealed by analysis of variance of uterine weights. For infused horns, analysis of variance indicated that uterine weights were lower in animals receiving indomethacin subcutaneously ($P<0.001$) and intraluminally ($P<0.02$). Weights of noninfused horns were not affected by any of the treatments. Time of killing of the animals had no significant ($P>0.05$) effect.

For uterine PGE concentrations, analysis of variance of the data indicated a significant ($P<0.01$) 3-way interaction between the effects of time, subcutaneously administered indomethacin, and infusion. For both infused and noninfused horns, indomethacin administration subcutaneously was associated with reduced PGE concentrations in animals killed at 24 h, but not at 48 h. Prostaglandin E concentrations were higher in both infused and noninfused horns at 48 h compared to 24 h. For infused horns, the intrauterine infusion of indomethacin was associated with reduced PGE concentrations.

Uterine PGF concentrations were below the sensitivity of the assay (0.2–0.4 pg/mg tissue, depending on uterine weight) for a large proportion of animals treated with indomethacin s.c. and killed at 24 h. For statistical analysis, these samples were considered to have zero concentration of PGF and the analysis was performed on log $(x + 0.1)$ data. A significant ($P<0.01$) 3-way interaction between the effects of time, subcutaneously administered indomethacin, and infusion was revealed by analysis of variance. For noninfused horns, indomethacin administered subcutaneously was associated with lower uterine PGF concentrations in animals killed at 24 h, but not in those killed at 48 h. This same effect was seen in infused horns, but the reduction at 24 h was not as great in these horns as in the noninfused horns. Uterine PGF concentrations were greater at 48 h than at 24 h in both the infused and noninfused horns. Intraluminally administered indomethacin was associated with decreased uterine PGF concentrations in infused, but not in noninfused, horns.

Effect of Intrauterine Infusion of PGs

To determine if the infusion of PGs into the uterine lumen could override the inhibitory effect of indomethacin when administered by both subcutaneous and intrauterine routes, rats with sensitized uteri were treated with indomethacin subcutaneously and received a unilateral intrauterine infusion of PGE_2 (1 μg/h), $PGF_{2\alpha}$ (1 μg/h), combined PGE_2 + $PGF_{2\alpha}$, or the vehicle (0.5 mM indomethacin in PBSG). The animals were killed 5 days later and the weights of uterine horns were recorded (Fig. 3). The data were analyzed as a 3-factor mixed-model analysis of variance as follows: *Factor 1*, PGE_2 (vehicle vs. 1 μg/h PGE_2); *Factor 2*, $PGF_{2\alpha}$ (vehicle vs. 1 μg/h $PGF_{2\alpha}$); and *Factor 3*, infusion (infused horn vs. contralateral noninfused horn).

Factors 1 and 2 were between-animal comparisons, and Factor 3 a within-animal comparison. The analysis indicated a significant ($P<0.005$) interaction between the effects of PGE_2, $PGF_{2\alpha}$, and infusion. For noninfused horns, there were no significant differences between treatment groups. For infused horns, both PGE_2 and $PGF_{2\alpha}$ increased the weights of the uterine horns compared to those receiving the vehicle; there was no significant difference in weights between those infused with PGE_2, $PGF_{2\alpha}$, or PGE_2 + $PGF_{2\alpha}$.

Dose-Response Relationship

Rats with sensitized uteri received a unilateral intrauterine infusion of either the vehicle (0.5 mM indomethacin in PBSG) or 0.01, 0.1, or 1 μg PGE_2 or $PGF_{2\alpha}$. Prior to insertion of the minipumps, the animals received indomethacin subcutaneously. Decidualization was assessed 5 days later by determining the weights of the infused and noninfused uterine horns (Fig. 4). For animals receiving a PG infusion, the data were statistically analyzed as a 3-factor, mixed-model analysis of variance, as follows: *Factor 1*, PG (PGE_2 vs. $PGF_{2\alpha}$); *Factor 2*, rate of infusion (0.01 μg/h vs. 0.1 μg/h vs. 1 μg/h); and *Factor 3*, infusion (infused horn vs. contralateral noninfused horn).

Factors 1 and 2 were between-animal comparisons, and Factor 3 a within-animal comparison. For animals receiving a PG infusion, analysis of variance indicated a significant ($P<0.005$) 3-way interaction between the effects of PG, rate of infusion of PG, and infusion. For noninfused horns, weights did not differ between groups, nor from those of animals receiving the vehicle. For infused horns, weights were dependent on the rate of PG infusion, being greatest for animals receiving the highest rate. The lowest rate of infusion

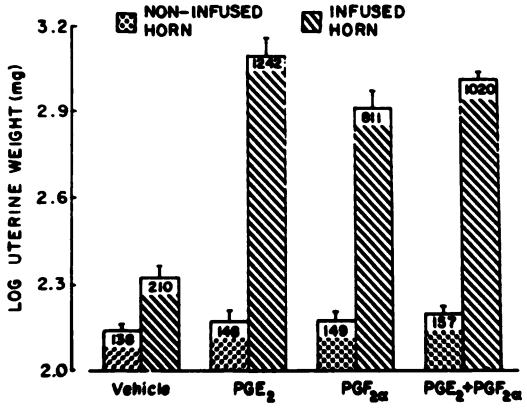

FIG. 3. Effects of unilateral intrauterine infusion of vehicle (0.5 mM indomethacin in 2% ethanol in PBSG, 1 μl/h), prostaglandin E_2 (PGE_2, 1 μg/h), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 1 μg/h), or combined PGE_2 and $PGF_{2\alpha}$ for 5 days on wet weights of uterine horns. All animals received indomethacin s.c. prior to pump insertion. Each bar represents the mean + SEM of the logarithmically transformed data from 6 rats. The geometric mean is given at the top of each bar.

(0.01 μg/h) resulted in uterine horns that were heavier than those receiving the vehicle. There was no statistical difference between the effects of PGE_2 or $PGF_{2\alpha}$.

DISCUSSION

Combined administration of indomethacin both subcutaneously before a deciduogenic stimulus and intraluminally into the uterine lumen resulted in a substantially reduced decidual cell reaction, as indicated by the weights of uterine horns. Taken together with the data on the effects of these indomethacin treatments on uterine PG concentrations (assumed to be an indirect indicator of uterine PG production) 24 and 48 h after the application of a deciduogenic stimulus, these results suggest that PGs are involved throughout the decidual cell reaction. Indomethacin administered subcutaneously effectively reduced uterine PG concentrations at 24 h but not at 48 h, suggesting that the effect of the indomethacin on uterine PG production was of relatively short duration. By contrast, the intrauterine infusion of indomethacin reduced uterine PG concentrations at both 24 and 48 h. The magnitude of this effect was relatively small, probably reflecting the distribution of indomethacin in the uterus after infusion. Since in indomethacin-infused horns uterine PG concentrations were higher at 48 h than at 24 h, it seems that indomethacin was unable to inhibit PG synthesis throughout the uterus. Because indomethacin was presumably at higher concentrations in the endometrium than elsewhere in the uterus, the lower uterine PG concentrations probably reflect decreased production, particularly in the endometrium. An alternative explanation cannot be ruled out; if the infused indomethacin was not distributed along the length of the uterus but was confined to areas adjacent to the pump, the lower PG levels may represent decreased PG production in these areas.

It is possible that the decidualization observed in animals receiving the indomethacin subcutaneously only was due to uterine PG synthesis that occurred after 24 h; weights of uterine horns at 24 and 48 h (Table 1) and at 5 days (Fig. 1) are in accord with this interpretation. Likewise, the decidualization in rats receiving only the intrauterine infusion of indomethacin may have been due to stimulation of PG synthesis during pump insertion, although PGs from areas of the uterus where indomethacin did not reach levels sufficient to inhibit PG synthesis may also have contributed. The combined treatments were presumably more effective because PG synthesis was inhibited to a greater extent throughout the process of decidualizaton. These data therefore supplement those of Tobert (1976) and Kennedy and Lukash (1982), and taken together indicate

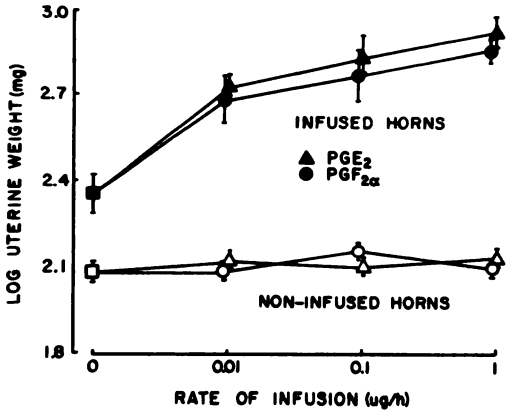

FIG. 4. Uterine wet weights after the unilateral intrauterine infusion of vehicle (0.5 mM indomethacin in 2% ethanol in PBSG, 1 μl/h), prostaglandin E_2 (PGE_2), or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) for 5 days at varying rates. All animals received indomethacin s.c. prior to pump insertion. Each point represents the mean ± SEM of the logarithmically transformed data from 6 rats.

that PGs are involved in the initiation, differentiation, and growth of decidual cells.

The present investigation indicates that in studies designed to determine the role of PGs in decidualization, endogenous uterine PG production is most appropriately inhibited by administering indomethacin both subcutaneously and into the uterine lumen. Under these conditions, both PGE_2 and $PGF_{2\alpha}$ were able to override the inhibitory effect of indomethacin. Moreover, the dose-response relationships between these PGs and decidualization did not differ. Based upon considerations of which PGs are involved in decidualization and their possible modes of action, these results are perplexing. Arguing by analogy with the early inflammatory response and based upon the ability of PGs to increase endometrial vascular permeability when injected into the uterine lumen, Kennedy (1983) has suggested that it is PGs of the E series that are involved in the early stages of the decidual cell reaction. Indeed, under these experimental conditions, $PGF_{2\alpha}$ by itself was found to be without effect and, when combined with PGE_2, inhibited the response to PGE_2 (Kennedy, 1979). However, this inhibitory effect was not observed in the study of Kennedy and Lukash (1982) when the PGs were infused. The possibility that $PGF_{2\alpha}$ is converted to PGE_2, or vice versa, before having an effect on the endometrium seems unlikely because of the similar dose-response relationships. In terms of mode of action of the PGs, the results are perplexing because, although endometrial PGE binding sites have been detected (Kennedy et al., 1983a,b), those for $PGF_{2\alpha}$ have not (Martel et al., 1985). If indeed there are no endometrial $PGF_{2\alpha}$ receptors, how does this PG have its effects on the endometrium?

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