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PROSTAGLANDIN $F_{2\alpha}$ OUTPUT BY HUMAN ENDOMETRIUM UNDER SUPERFUSION AND ORGAN CULTURE CONDITIONS

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Summary—Specimens of proliferative and secretory human endometrium were incubated under organ culture or superfusion conditions and the levels of $PGF_{2\alpha}$ in the medium were measured by radioimmunoassay. Basal rates of $PGF_{2\alpha}$ output during short-term superfusions and long-term (1–2 day) batch incubations, performed on the same tissue specimens, were similar. Basal output of $PGF_{2\alpha}$ by proliferative endometrium (230–280 ng/mg protein × d) was significantly higher than that of secretory tissue under both experimental conditions. Estradiol (10^{-8} M) increased $PGF_{2\alpha}$ output significantly (4-fold) only in secretory endometrium under organ culture conditions; Progesterone (10^{-7} M) decreased it significantly (to $\frac{1}{2}$–$\frac{1}{4}$ of the basal level) in both types of endometria during long-term incubations and in proliferative endometrium during superfusion.

Glands isolated from proliferative and secretory endometrium produced $PGF_{2\alpha}$ during superfusion at a rate comparable to that of endometrial tissue under similar conditions. $PGF_{2\alpha}$ output by glands isolated from secretory endometrium increased significantly (3-fold) when estradiol was added to the superfusion medium.

INTRODUCTION

Human endometrium in organ culture releases $PGF_{2\alpha}$ to the incubation medium at daily rates that can be increased by estradiol (E_2) and lowered by progesterone (P) [1]. Liggins *et al.* have also demonstrated significant $PGF_{2\alpha}$ output by human endometrial tissue during superfusions carried out for a few hours [2]. Under these conditions, rapid changes in $PGF_{2\alpha}$ output can be detected and potential problems derived from degradation of prostaglandins reentering the tissue during prolonged incubations can be avoided. However, the correspondence between rates of output of prostaglandins under organ culture and superfusion conditions or the responsiveness of the endometrium to hormones and drugs in these different experimental situations have not been described.

The purpose of the present study was to develop a superfusion method to measure prostaglandin output and to compare results obtained with the same tissue specimens under superfusion or organ culture conditions, measuring rates of $PGF_{2\alpha}$ output of proliferative and secretory endometrium or isolated endometrial glands, in the absence or presence of exogenous E_2 or P.

EXPERIMENTAL

Specimens of endometrium were obtained from patients undergoing dilatation and curettage or hysterectomy. The tissues were trimmed and minced under a laminar flow hood in medium containing an antibiotic–antimycotic mixture, as described elsewhere [3]. A small portion of each specimen was fixed in formalin for histologic dating according to Noyes *et al.* [4].

Endometrial tissue in organ culture

Tissue fragments were placed on lens paper resting upon stainless steel grids distributed among several polystyrene culture dishes (6 cm dia, Falcon Plastics, Los Angeles, CA) which contained 3.5 ml of culture medium (Ham's F10 supplemented with 10 μg/ml of insulin, 1.2 mg/ml of $NaHCO_3$, 4 mg/ml of glucose, 1% of antibiotic–antimycotic mixture obtained from Grand Island Biological Co., Grand Island, NY, and 10% charcoal-stripped calf serum). The tissue (4–15 mg per dish) only touched the surface of the medium in order to facilitate oxygenation. Dishes were kept for 24 h in an incubator at 37°C, with a humidified atmosphere of 95% air–5% CO_2. At the end of this period, tissue in some of the dishes was transferred to superfusion chambers. The medium in the other dishes was replaced with fresh control medium or changed to culture medium containing 10^{-8} M E_2 or 10^{-7} M P and incubations were continued for 2 days, renewing the medium daily. Collected medium was centrifuged and the supernatant was stored frozen for $PGF_{2\alpha}$ radioimmunoassay, as described previously [3]. Measurements were made in duplicate at 2 dilutions of the sample. Usually, 2 dishes were analyzed separately for each experimental condition. At the end of the incubation, the tissue was recovered, washed and homogenized for protein assay [5].

Isolation of endometrial glands

Endometrial tissue was trimmed and digested for 1 h at 37°C with 0.25% type I collagenase (Worth-

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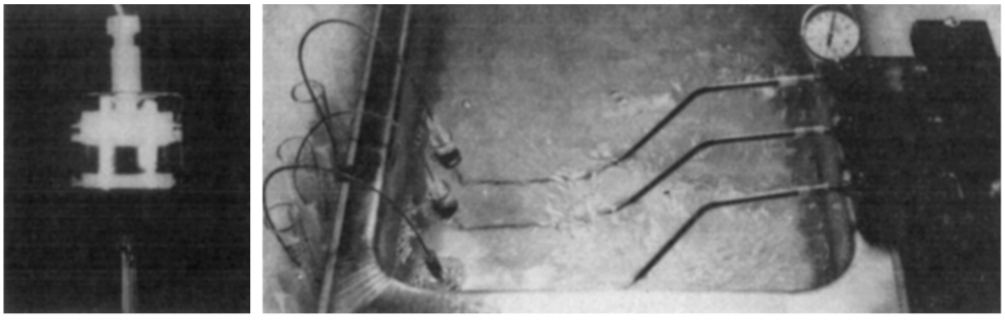

Fig. 1. Photograph of the superfusion apparatus and a close-up of the superfusion chamber.

ington Biochemical Co., Freehold, NJ), dissolved in Ham's F10 medium, supplemented with 10% calf serum. Glands were isolated by filtration through a 38 μm stainless steel sieve as previously described [3] and washed with the same medium supplemented with 10% charcoal-stripped calf serum. They were resuspended in this medium and left in the incubator at 37°C overnight in 12 × 75 mm clear plastic tubes (Falcon No. 2058). The preincubated glands were collected by centrifugation, washed in culture medium and transferred to the superfusion chamber.

Superfusion procedures

Continuous flow incubations were carried out in the apparatus shown in Fig. 1. The chamber consists of a section of a $\frac{1}{2}''$ glass chromatography column used for gel filtration to which a coarse sintered glass disk was annealed. The thick-walled capillary glass tube attached to the chamber leads to a glass syringe containing the superfusion medium. The chamber outlet consists of a Teflon chromatography column bed support (Laboratory Data Control, Riviera Beach, FL) attached to a Teflon capillary tubing that carries the superfusate to collecting tubes immersed in a chilling bath. Most of the chamber and the glass tubing connecting it to the syringe are immersed in a water bath maintained at 37°C. A Sage pump (Orion Research, Cambridge, MA) was used to drive the piston of the syringe. Tissue fragments (about 20–40 mg) or isolated glands (1.6–7.1 mg), preincubated in culture medium as described above, were loaded into the chamber partially filled with superfusion medium flowing at a slow rate. The medium used for superfusions had the same composition as the medium used for incubation of endometrial fragments under organ culture conditions. Three superfusions (control, E_2 and P) were usually carried out in parallel for 8–10 h at a flow rate of 3 ml/h, collecting 20 min fractions. These fractions were kept frozen and some of them were thawed to measure $PGF_{2\alpha}$ levels by radioimmunoassay after appropriate dilutions. Output rates during the period of collection of the samples analyzed were expressed as pg $PGF_{2\alpha} \times$ mg protein$^{-1} \times$ min^{-1} and were plotted as functions of time. Cumulative output curves, shown in Fig. 2, were constructed disregarding the first 2 h of superfusion, considered to be a "settling period" [2, 6, 7]. Areas under the output curve were calculated as sums of trapezoids, i.e.

$$\text{Area} = \frac{1}{2} \sum_{i=1}^{n} (OR_i + OR_{i-1})\,(t_{i-1})$$

where OR_i is the output at time t_i. Output rates were calculated from the slope of the cumulative output curves in the time interval of linearity common to the three parallel superfusions (Fig. 2). This procedure corrects for early overproduction due to the stimulation by handling of the specimens at the beginning of the superfusion and for the declining output of $PGF_{2\alpha}$ at longer times of superfusion.

RESULTS

Table 1 shows rates of output of $PGF_{2\alpha}$ by 5 specimens of proliferative and five specimens of secretory tissue incubated under organ culture conditions in medium supplemented with 10% charcoal-stripped calf serum without added steroids (basal output) or

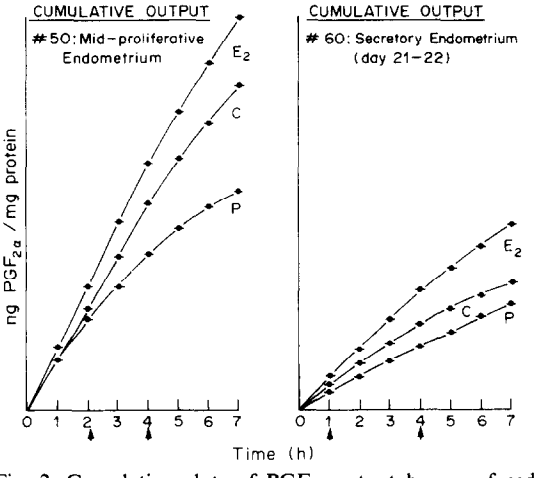

Fig. 2. Cumulative plots of $PGF_{2\alpha}$ output by superfused fragments of proliferative and secretory endometria constructed as described in the text. Starting times correspond to the beginning of sample collections after a 2 h "settling" period of parallel superfusions with medium alone (C), medium with 10^{-8} M estradiol (E_2) or with 10^{-7} M progesterone (P). Rates of $PGF_{2\alpha}$ output were estimated from the slopes within the intervals delimited by the arrows.

Table 1. Effects of estradiol (E_2) and progesterone (P) on $PGF_{2\alpha}$ output by human endometrium in organ culture

Spec. no.	Histologic dating	$PGF_{2\alpha}$ output [ng/(mg protein × d)]									
		Day 1					Day 2				
		Basal rate	+E_2 (10^{-8} M)		+P (10^{-7} M)		Basal rate	+E_2 (10^{-8} M)		+P (10^{-7} M)	
			Rate	% Control	Rate	% Control		Rate	% Control	Rate	% Control
	Proliferative										
50	Mid	430	780	180	370	86	210	490	230	89	42
52	Mid	180	200	110	60	33	90	100	110	15	16
57	Mid	250	320	130	97	39	110	150	140	40	36
53	Late	210	240	110	91	43	160	160	100	39	24
56	Late	100	150	150	84	84	160	220	140	36	22
Average		230	340	140	140	57	150	220	140	44	28
(±SEM)		(±55)	(±113)	(±13)	(±58)	(±7)	(±21)	(±69)	(±23)	(±12)	(±5)
	Secretory										
55	day 15–16	26	110	420	18	69	24	250	1040	12	50
61	day 19	38	150	390	22	58	24	250	1040	10	42
15	day 20	110	350	320	—	—	77	360	470	—	—
60	day 21–22	66	410	620	61	92	73	360	490	62	85
49	day 28–29	120	290	240	110	90	68	240	350	50	73
Average		72	260	400	53	77	53	290	680	33	62
(±SEM)		(±19)	(±57)	(±63)	(±21)	(±13)	(±12)	(±28)	(±149)	(±13)	(±10)

with added E_2 (10^{-8} M) or P (10^{-7} M). Rates of output are expressed as ng $PGF_{2\alpha}$/(mg protein × d) or as percentages of basal output (100 × output in the presence of E_2 or P/basal output) during the first and second day of incubation after addition of E_2 or P.

Table 2 presents results on $PGF_{2\alpha}$ output obtained by superfusing the specimens listed in Table 1 with the same supplemented media used for the incubations under organ culture conditions. Rates determined during a 2–6 h period of superfusion are expressed as rates of output per day per mg tissue protein or as percentages of basal output in medium without added steroids.

Average rates of $PGF_{2\alpha}$ output by proliferative and secretory endometrium under organ culture conditions during the first day of incubation and under superfusion conditions are summarized in Table 3, indicating statistical significance of differences among $PGF_{2\alpha}$ output according to phase of the cycle, presence or absence of E_2 or P, and method of measurement (batch incubation vs superfusion).

The following conclusions can be drawn from these data:

(1) Equivalent rates of $PGF_{2\alpha}$ output were estimated using the superfusion or the organ culture methods, under basal conditions or with added P. However, endometrial responses to E_2 were significantly stronger in organ culture.

(2) Rates of $PGF_{2\alpha}$ output by endometrium during the first and second day in organ culture were not significantly different. However, a larger effect of P was noted during the second day in culture ($P < 0.05$).

(3) Basal $PGF_{2\alpha}$ output by proliferative endometrium was about 3 times greater than the output by secretory tissue.

(4) Estradiol significantly increased (about 4-fold) $PGF_{2\alpha}$ output by secretory endometrium in organ culture. However, no such responses were noted in proliferative endometrium in organ culture or in superfused proliferative or secretory tissue.

Table 2. Effects of E_2 and P on $PGF_{2\alpha}$ output by superfused human endometrium

Specimen no.	Histologic dating	$PGF_{2\alpha}$ output [ng/(mg protein × d)]				
		Basal rate	+E_2 (10^{-8} M)		+P (10^{-7} M)	
			Rate	% Control	Rate	% Control
	Proliferative					
50	Mid	410	470	110	260	62
52	Mid	340	230	68	60	18
57	Mid	170	200	120	65	38
53	Late	210	220	100	120	57
56	Late	250	160	64	97	39
Average		280	260	92	120	43
(±SEM)		(±44)	(±55)	(±11)	(±37)	(±8)
	Secretory					
55	day 15–16	78	100	130	76	97
61	day 19	43	76	180	48	110
15	day 20	120	150	130	—	—
60	day 21–22	170	240	140	120	70
49	day 28–29	110	100	91	61	55
Average		100	130	130	76	83
(±SEM)		(±21)	(±29)	(±14)	(±15)	(±12)

Table 3. Comparison of effects of E_2 and P on $PGF_{2\alpha}$ output by proliferative and secretory endometrium under organ culture and superfusion conditions

Condition	Tissue	Organ culture (day 1)		Superfusion
Basal	Proliferative	230	(NS)	280
ng $PGF_{2\alpha}$ / mg prot. × day		($P < 0.05$)		($P < 0.01$)
	Secretory	72	(NS)	100
$+E_2$ (10^{-8} M) (% of control)	Proliferative	(NS)* 140%	($P < 0.05$)	92% (NS)*
		($P < 0.005$)		($P < 0.05$)
	Secretory	($P < 0.01$)* 400%	($P < 0.02$)	130% (NS)*
+P (10^{-7} M) (% of control)	Proliferative	($P < 0.01$)* 57%	(NS)	43% ($P < 0.01$)*
		(NS)		($P < 0.05$)
	Secretory	($P < 0.05$)* 77%	(NS)	83% (NS)*

The values for statistical significance shown in this Table (P levels, NS: not significant) were obtained by applying 2-tailed Student's t-tests to data linked by the corresponding dotted lines. Significance levels carrying an asterisk (*) correspond to comparisons of the adjoining data to basal $PGF_{2\alpha}$ output.

(5) Progesterone significantly lowered basal $PGF_{2\alpha}$ output by both proliferative and secretory endometrium in organ culture. During superfusion, P affected $PGF_{2\alpha}$ output only in proliferative endometrium.

Table 4 presents results obtained by superfusing glands isolated from specimens of proliferative and secretory endometrium. These data indicate that isolated glands produce $PGF_{2\alpha}$ at rates comparable on average to the output by fragments of endometrium under similar superfusion conditions. Responses to E_2 were also highest in glands derived from secretory endometrium.

DISCUSSION

The similarities in the basal rates of $PGF_{2\alpha}$ output determined in parallel by short-term (2–3 h) superfusion or prolonged incubation (1–2 days) of fragments of the same endometrial specimens indicate constancy in prostaglandin production. This conclusion is supported by the constancy of the $PGF_{2\alpha}$ output by endometrial fragments during the 2 consecutive days in organ culture.

The feasibility of estimating rates of prostaglandin output by minced endometrium and even isolated endometrial glands under the reported superfusion conditions is of particular interest for kinetic studies of physiologic compounds and drugs affecting prostaglandin synthesis or metabolism. The demonstration that isolated glands produce $PGF_{2\alpha}$ and can respond to E_2 emphasize their importance as a source of endometrial prostaglandins. As reported by Schatz *et al.* [3, 8], monolayer cultures of epithelial cells derived from glands isolated from either proliferative or secretory endometrium also produce $PGF_{2\alpha}$ and respond markedly to the stimulatory effect of E_2 added to the culture medium.

In agreement with results obtained in a larger series of experiments involving incubation of endometrium under organ culture conditions [8] and with data reported by Tsang and Ooi [9], $PGF_{2\alpha}$ output was found in the present study to be larger in proliferative than in secretory endometrium. This conclusion was reached not only on the basis of data from organ culture but also from results of superfusion of fragments of endometrium and isolated endometrial glands. Liggins *et al.* also reported a fall in *in vitro* basal $PGF_{2\alpha}$ output from late proliferative to secre-

Table 4. $PGF_{2\alpha}$ output by superfused glands isolated from proliferative and secretory endometrium

Specimen no.	Histologic dating	$PGF_{2\alpha}$ output (ng/mg prot. × d)		
		Basal rate	$+E_2$ (10^{-8} M)	
			Rate	% Control
	Proliferative			
26	Mid	350	500	140
29	Mid	14	20	140
34	Mid	230	330	140
Average		200	280	140
	Secretory			
31	day 18	75	85	110
27	day 21	14	43	310
24	late	15	83	550
Average		35	70	320

tory endometrium, as measured by superfusion [2]. The higher output by proliferative tissue may result from exposure of the endometrium to estrogenic stimulation *in vivo* during the follicular phase of the cycle; the lower output by secretory endometrium may be due to the inhibitory effect of endogenous progesterone which counteracts the estrogenic action during the luteal phase, as shown by *in vitro* data published by Abel and Baird [1] and confirmed by us [8]. The weak response to exogenous estrogens of specimens of proliferative endometrium in organ culture could be the result of a protracted stimulation by endogenous E_2 *in vivo*. In contrast, secretory endometrium in organ culture shows a marked response to E_2. The lack of response of secretory tissue to E_2 during short-time superfusions may be due to insufficient time for the hormone to exert its effect on $PGF_{2\alpha}$ production.

The elevation in $PGF_{2\alpha}$ levels in endometrium [2, 10, 11] or in uterine washes [12] during the luteal phase of the cycle cannot be simply explained by a concerted action of E_2 and P since $PGF_{2\alpha}$ output by secretory endometrium *in vitro* is not significantly stimulated by mixtures of the 2 hormones [8]. Premenstrual decline in the extent of metabolism of $PGF_{2\alpha}$ has been suggested to account for the increases in prostaglandin output [13] but more direct experiments are needed to arrive at a satisfactory description of the mechanisms regulating endometrial prostaglandin output *in vivo*. This question is of great importance in view of the postulated roles for prostaglandins on implantation, onset of menstruation and dysmenorrhea.

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REFERENCES

1. Abel M. H. and Baird D. T.: The effect of 17β-estradiol and progesterone on prostaglandin production by human endometrium in organ culture. *Endocrinology* **106** (1980) 1599–1606.
2. Liggins G. C., Campos G. A., Roberts C. M. and Skinner S. J.: Production rates of prostaglandins F, 6-keto $PGF_{1\alpha}$ and thromboxane E_2 by perfused human endometrium. *Prostaglandins* **19** (1980) 461–477.
3. Schatz F. and Gurpide E.: Effects of estradiol on prostaglandin $F_{2\alpha}$ levels in primary monolayer cultures of epithelial cells from human proliferative endometrium. *Endocrinology* **113** (1983) 1274–1279.
4. Noyes R. W., Hertig A. T. and Rock J.: Dating the endometrial biopsy. *Fert. Steril.* **1** (1950) 3–25.
5. Lowry O. H., Rosenbrough N. J., Farr A. L. and Randal P. J.: Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
6. Mitchell M. D. and Flint P. F.: Prostaglandin production by intra-uterine tissues from periparturient sheep: use of a superfusion technique. *J. Endocr.* **76** (1978) 111–121.
7. Poyser N. L. and Brydon L. J.: Prostaglandin release from the guinea pig uterus superfused *in vitro*. Effect of stage of estrous cycle, progesterone, estradiol, oxytocin and A 23187. *Prostaglandins* **25** (1983) 443–456.
8. Schatz F., Markiewicz L., Barg P. and Gurpide, E.: Hormonal influences on the *in vitro* output of prostaglandin $F_{2\alpha}$ by proliferative and secretory human endometrium and endometrial cell cultures. In *Medical Management of Endometriosis* (Edited by J.-P. Raynaud, T. Oyasoo and L. Martini). Raven Press, New York (1984) pp. 97–106.
9. Tsang B. K. and Ooi T. C.: Prostaglandin secretion by human endometrium *in vitro*. *Am. J. Obstet. Gynec.* **142** (1982) 626–633.
10. Singh E. J., Baccarini I. M. and Zuspan F.: Levels of prostaglandins $F_{2\alpha}$ and E_2 in human endometrium during the menstrual cycle. *Am. J. Obstet. Gynec.* **121** (1975) 1003–1006.
11. Downie J., Poyser N. L. and Wunderlich M.: Levels of prostaglandins in human endometrium during the normal menstrual cycle. *J. Physiol.* **236** (1974) 465–472.
12. Demers L. M., Halpert D. R., Jones D. E. D. and Fontana J.: Prostaglandin F levels in endometrial jet wash specimens during the normal menstrual cycle. *Prostaglandins* **10** (1975) 1057–1065.
13. Casey M. L., Hemsell D. L., MacDonald P. C. and Johnston J. M.: NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase activity in human endometrium. *Prostaglandins* **19** (1980) 115–122.