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ESTRADIOL ENHANCES PROSTAGLANDIN SYNTHASE ACTIVITY IN EPITHELIAL BUT NOT IN STROMAL CELLS OF HUMAN ENDOMETRIUM

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Summary—Exogenous estradiol (E_2) has been shown to elevate $PGF_{2\alpha}$ output by explants of human secretory endometrium and in monolayer cultures of glandular epithelial, but not of stromal cells isolated from endometrium. In this study, $PGF_{2\alpha}$ output was measured in each of these cultures in the presence of E_2 and the calcium ionophore A23187, added singly or in combination. The ionophore, known to liberate arachidonic acid (AA) by stimulating phospholipase activity, produced a calcium-dependent increase in $PGF_{2\alpha}$ output in the cultures of epithelial cells, whereas greater than additive effects were obtained with mixtures of E_2 and A23187. In contrast, $PGF_{2\alpha}$ levels were not elevated by A23187 in the stromal cell cultures even in medium supplemented with $CaCl_2$ or when E_2 was added. A calcium-dependent increase in $PGF_{2\alpha}$ output was also observed in fragments of secretory endometrium incubated with A23187. Effects on $PGF_{2\alpha}$ output by endometrial fragments incubated with E_2 and A23187 were essentially additive and intermediate between those of the two component cells types.

Arachidonic acid produced similar increases in $PGF_{2\alpha}$ output in the epithelial and stromal cell cultures but only in the epithelial cell cultures was there greater utilization of AA in the presence of E_2. When mixtures of E_2 and AA were added to the cultures of epithelial cells the increase in $PGF_{2\alpha}$ output was 2.5-fold greater than the sum of the increases elicited by E_2 or AA alone. In contrast, no enhancement of the AA effect by E_2 was observed in the stromal cell cultures.

Extrapolation of these results from cell cultures to intact tissue suggests that the epithelium and not the stroma is the primary target for the effects of E_2 on $PGF_{2\alpha}$ output by secretory endometrium. The synergistic actions of E_2 and either AA, the obligatory precursor of $PGF_{2\alpha}$, or A23187, an enhancer of AA release from phospholipid stores, point to a stimulatory effect of E_2 on prostaglandin synthase activity.

INTRODUCTION

In women, endometrial prostaglandin (PG) $F_{2\alpha}$ has been implicated in the initiation of menstruation [1] and may be involved in implantation of the embryo by affecting endometrial vascular permeability, as has been shown for nonprimates [2]. Concentrations of $PGF_{2\alpha}$ in endometrial tissue [3] or in jet washes of the uterine lumen [4] vary cyclically during the menstrual cycle, showing a characteristic rise during the early to mid-luteal phases. Estrogens have been shown to affect $PGF_{2\alpha}$ output of *in vitro* endometrial preparations. Thus, $PGF_{2\alpha}$ output is elevated by the addition of estrogens to explants of secretory [5–10], but not proliferative [5–8, 10] endometrium, whereas they elicit an increase in levels of $PGF_{2\alpha}$ when added to cultures of epithelial cells derived from endometrium during both phases of the cycle [6, 8, 11]. Evidence that these estrogen-initiated effects are mediated by specific receptors came from the observation that the non-steroidal antiestrogens tamoxifen and 4-hydroxy tamoxifen suppress the effects of estradiol (E_2) on $PGF_{2\alpha}$ output in cultures of secretory endometrial explants and monolayers of epithelial cells, showing inhibitory potencies that corresponded to their reported affinities for estrogen receptors [12].

Recent studies in this laboratory have indicated that in contrast to the responsiveness of epithelial cells, E_2 does not affect $PGF_{2\alpha}$ levels in primary monolayer cultures of the other predominant cell type in human endometrium, the stroma [13]. Arachidonic acid (AA), the obligatory substrate for PG synthase leading to $PGF_{2\alpha}$ synthesis, and the calcium ionophore A23187, which liberates AA from membrane lipids by stimulating phospholipase activity [14–16], have been shown to increase $PGF_{2\alpha}$ output by explants of human endometrium [7]. We have tested the effects of both agents to determine (a) whether the stimulatory effects of E_2 on $PGF_{2\alpha}$ output by secretory endometrium and endometrial epithelial cells reflect an increase in the PG synthase-mediated conversion of AA; (b) whether the contrasting refractoriness of the stromal cells to E_2 derives from a lack of effect of the hormone on phospholipase-mediated release of AA from intracellular stores, or from impaired sensitivity of PG synthase to estrogens.

MATERIALS AND METHODS

Materials

Stock solutions of E_2 (Steraloids, Wilton, NH) and A23187 (Calbiochem., La Jolla, CA) were prepared in ethanol. In each experiment in which AA (Sigma

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Chemical Co., St Louis, MO) was used, the contents of a sealed ampule were dissolved in absolute ethanol to obtain 20 mg/ml solutions, then diluted with 0.2% Na_2CO_3 to 1 mg/ml.

Tissue

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 Minimum Essential Medium containing 1% of an antibiotic–antimycotic mixture (GIBCO, Grand Island, NY). A small portion of each specimen was fixed in formalin for histologic dating according to the criteria described by Noyes *et al.* [17].

Incubation of endometrial fragments

Tissue fragments were placed on lens paper resting on stainless steel grids and distributed among 6-cm dia. polystyrene culture dishes (Falcon Plastics, Los Angeles, CA), which contained 3.5 ml Ham's F-10 medium supplemented with 10% charcoal-treated calf serum (Flow Laboratories, McLean, VA), 10 μg/ml porcine insulin (Nordisk-USA, Bethesda, MD), 1.2 mg/ml $NaHCO_3$, 4 mg/ml glucose, and 1% of the antibiotic–antimycotic mixture supplied by Grand Island Biochemical Co. (Grand Island, NY). The tissue (4–15 mg/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, the medium was replaced with fresh control medium or changed to culture medium containing E_2, A23187 or E_2 + A23187 or 0.1% of ethanol as the vehicle control. After 24 h incubation the medium was collected and centrifuged, and the supernatant was stored frozen for $PGF_{2\alpha}$ assay. Tissue recovered at the end of the incubation was washed, homogenized, and assayed for protein by the method described by Lowry *et al.* [18].

Preparation and incubation of epithelial and stromal cell cultures

Endometrial tissue was digested for 1.5 h at 37°C with 0.25% Type I collagenase (Worthington Biochemical Co., Freehold, NJ) dissolved in culture medium (Ham's F-10 containing 10 μg/ml porcine insulin and 1% antibiotic–antimycotic mixture, supplemented with 10% charcoal-treated calf serum.

Glands and stroma were separated by filtration through a 38-μm stainless steel sieve (Newark Wire Cloth Co., Newark, NJ) according to a method previously developed in our laboratory [19].

To facilitate comparisons of $PGF_{2\alpha}$ output by cultures of epithelial (mostly glandular with possible contamination by luminal cells) and stromal cells isolated from the same specimen, stromal and epithelial cells were exposed to test compounds in parallel.

Glands were allowed to attach and form monolayer sheets of cells for 24–72 h; the $PGF_{2\alpha}$ testing period was initiated by replacing the medium with culture medium supplemented with 10% charcoal-treated calf serum containing either E_2, AA, A23187, E_2 + AA, E_2 + A23187, or 0.1% ethanol, the vehicle used to add E_2 or A23187. The dishes were returned to the incubator for 64 h. Then the medium was collected and frozen until analyzed for $PGF_{2\alpha}$ content. The cells were harvested with trypsin EDTA and assayed for protein content using the method of Lowry *et al.* [18].

Measurement of $PGF_{2\alpha}$

Prostaglandin $F_{2\alpha}$ was measured in unextracted culture medium by RIA, using rabbit anti-$PGF_{2\alpha}$ antiserum (Pasteur Institute, Paris) and a method involving overnight incubation at 5°C followed by separation of the bound and free compound by dextran-coated charcoal [20]. Standards were prepared in culture medium containing 10% charcoal-treated calf serum. We previously reported no significant differences in $PGF_{2\alpha}$ levels measured directly in the medium or after extraction and chromatography [11].

RESULTS

Effects of E_2 and AA on $PGF_{2\alpha}$ output by epithelial and stromal cells

Figure 1 illustrates the effects of E_2 (10^{-8} M), AA (4 and 20 μg/ml), and combinations of E_2 + AA at these concentrations on $PGF_{2\alpha}$ output during parallel 64-h incubations of cultures of epithelial and stromal cells derived from a specimen of mid-proliferative endometrium. As previously shown, 10^{-8} M E_2 elevated $PGF_{2\alpha}$ levels in the cultures of epithelial but not of stromal cells [13]. Consistent with effects described in endometrial fragments [7], incubation with AA (4 or 20 μg/ml) evoked large increases in $PGF_{2\alpha}$ output by cultures of both cell types. In the epithelial cell cultures, $PGF_{2\alpha}$ output in the presence of E_2 or AA was much higher than the sum of the outputs elicited by E_2 or AA alone, but no E_2 effects were noted in stromal cell cultures exposed to E_2 + AA.

This pattern of $PGF_{2\alpha}$ output under basal conditions and in response to addition of E_2, AA or E_2 + AA is representative of the results of several experiments which are summarized in Table 1. Basal output of $PGF_{2\alpha}$ is similar in cultures of epithelial and stromal cells; addition of AA evoked similar dose–response increases in cultures of epithelial and stromal cells. Clearly, superadditive effects were noted in epithelial cell cultures incubated with E_2 and AA. For example, mean elevations of $PGF_{2\alpha}$ output of about 5-fold were observed after addition

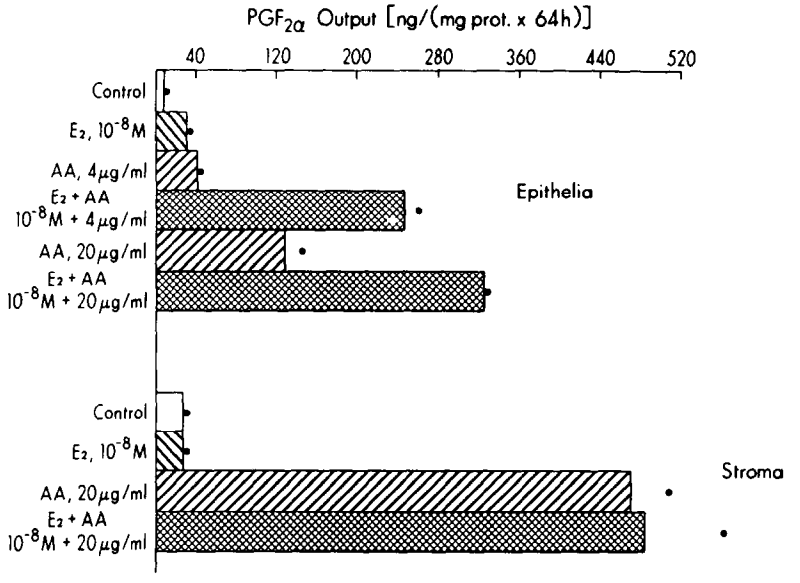

Fig. 1. Effects of E_2 and AA on $PGF_{2\alpha}$ output by cultures of epithelial and stromal cells isolated from a specimen of mid-proliferative endometrium.

of either 10^{-8} M E_2 or 4 μg/ml AA and about 27-fold when the cells were incubated with both compounds.* A similar synergistic effect is apparent with 10^{-8} M E_2 and 20 μg/ml AA. In the stromal cell cultures in contrast, $PGF_{2\alpha}$ output was elevated above controls to the same extent in incubations with AA or E_2 + AA.

The enhanced conversion of AA to $PGF_{2\alpha}$ in the presence of E_2, observed for the epithelial cell cultures, suggests that the effects of E_2 involves increased activity of PG synthase. The similarities between the epithelial and stromal cells in basal output of $PGF_{2\alpha}$ and in utilization of AA indicate that the stromal cells also possess a functional PG synthase that is, however, fundamentally different from that of the epithelial cells in its responsiveness to estrogens.

*If the effects of a test compound and E_2 on $PGF_{2\alpha}$ output are additive, it follows that

Increase due to mixture = Increase due to test compound + Increase due to E_2

or

Output with mixture − Control output =

(Output with test compound − Control output) + (Output with E_2 − Control output)

or, multiplying by $\frac{100}{\text{Control output}}$,

% control with mixture = % control with test compound + % control with E_2 − 100.

Effects of A23187 on $PGF_{2\alpha}$ output by epithelial and stromal cells

Figure 2 presents data on $PGF_{2\alpha}$ output by parallel cultures of epithelial and stromal cells derived from a

Table 1. Effects of E_2 and arachidonic acid on $PGF_{2\alpha}$ output by endometrial cells*

Cell type	Basal output (ng $PGF_{2\alpha}$) mg prot. × 64 h		% Controls				
			+ E_2 (10^{-8} M)	+ Arachidonic acid 4 μg/ml	+ Arachidonic acid 20 μg/ml	+ E_2 (10^{-8} M) + arachidonic acid 4 μg/ml	+ E_2 (10^{-8} M) + arachidonic acid 20 μg/ml
Epithel.	Mean†	9.4	510	550	1900	2700	6200
	SE	±3.0	±130	±100	±180	±830	±1900
	n	5	5	5	3	5	3
Stromal	Mean†	11.0	110	320	1300	330	1300
	SE	±4.1	±2.0	±100	±280	±150	±310
	n	5	5	2	3	2	3

*Averages from results of duplicate dishes. The experiments were carried out in medium containing 10% charcoal-treated calf serum.

†Cultures of epithelial and stromal cells derived from a specimen of early proliferative endometrium, 3 from mid-proliferative endometrium and 1 from late proliferative endometrium, and from additional cultures of stromal cells derived from another specimen of secretory endometrium.

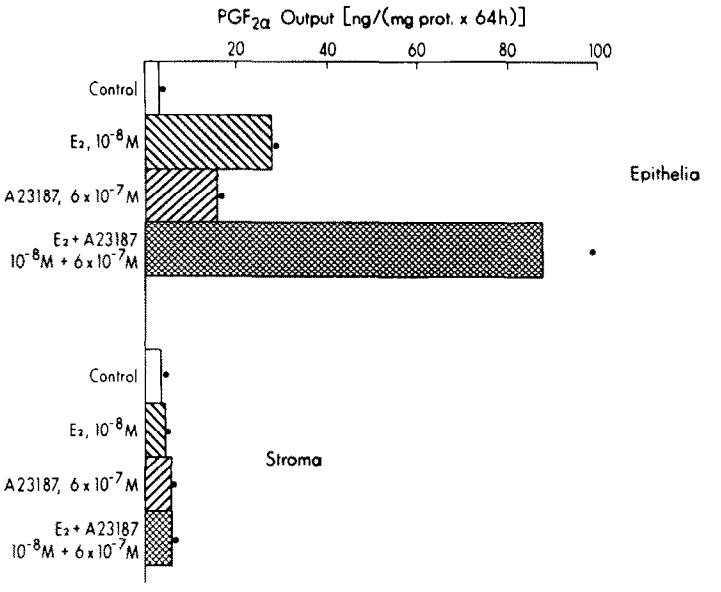

Fig. 2. Effects of E_2 and A23187 on $PGF_{2\alpha}$ output by cultures of epithelial and stromal cells derived from a specimen of early proliferative endometrium.

specimen of early proliferative endometrium during a 64-h period of incubation with E_2 (10^{-8} M), A23187 (6×10^{-7} M) or mixtures of E_2 and A23187 in medium supplemented with 1.5 mM $CaCl_2$. Estradiol and the ionophore each elevated $PGF_{2\alpha}$ levels in the cultures of epithelial but not of stromal cells. Interestingly, in the cultures of epithelial cells, mixtures of E_2 and A23187 produced an increase in $PGF_{2\alpha}$ output about twice as great as the sum of that produced by each compound alone. As expected from the lack of response of the stromal cells to E_2 or A23187 alone, $PGF_{2\alpha}$ levels were not elevated above control levels when the cells were incubated together with E_2 + A23187.

This differential response to the various experimental treatments by epithelial and stromal cells was confirmed in a series of experiments that are summarized in Table 2. Thus, for the epithelial cells, 10^{-8} M E_2 elicited an average increase in $PGF_{2\alpha}$ levels of about 5.8-fold, irrespective of supplementing the culture medium with 1.5 mM $CaCl_2$, and A23187 resulted in $PGF_{2\alpha}$ levels which were on average 3.6-fold higher than those of controls in Ca^{2+}-supplemented medium. The combination of both agonists elevated $PGF_{2\alpha}$ to levels about 18-fold greater than controls. For the group of stromal cell cultures, addition of A23187 or E_2 + A23187 evoked only about 40% increase in $PGF_{2\alpha}$ output.

Table 2. Effects of E_2, A23187 and $CaCl_2$ on $PGF_{2\alpha}$ output by endometrial cells*

Cell type		Control output (ng $PGF_{2\alpha}$) / mg prot. × 64 h	% Controls			
			+ E_2 (10^{-8} M)	+ E_2 (10^{-8} M) + Ca^{2+} (1.5 mM)	+ A23187 (6×10^{-7} M) + Ca^{2+} (1.5 mM)	+ E_2 (10^{-8} M) + A23187 (6×10^{-7} M) + Ca^{2+} (1.5 mM)
Epithel.	Mean†	7.4	570	580	360	1800
	SE	±3.0	±150	±170	±38	±360
	n	5	4	3	5	5
Stromal	Mean†	9.2	110	120	140	150
	SE	±4.3	±3.0	—	±18	±16
	n	5	4	1	5	4

*Averages from results of duplicate dishes. The experiments were carried out in medium containing 10% charcoal-treated calf serum.

†Cultures of epithelial and stromal cells derived from 2 specimens of early proliferative endometrium, 2 from mid-proliferative endometrium and one from late proliferative endometrium, and from additional cultures of stromal cells derived from another specimen of secretory endometrium.

Since A23187 is a known stimulator of phospholipase activity [14–16], the greater than additive effects obtained by incubating epithelial cells with E_2 and A23187 is consistent with the results obtained with mixtures of E_2 and AA described above, and suggests that E_2 increases the conversion to $PGF_{2\alpha}$ of AA liberated by the ionophore.

Effects of E_2, A23187 and $CaCl_2$ on $PGF_{2\alpha}$ output by endometrial fragments

Table 3 shows $PGF_{2\alpha}$ levels measured in the culture medium of tissue fragments from four specimens of secretory endometrium after 24 h of incubation with exogenous E_2 (10^{-8} M), A23187 (10^{-6} M) and $CaCl_2$ (1.5 mM), added singly and in combination. As expected [7], the calcium ionophore, A23187, was an effective agonist, producing average increases in $PGF_{2\alpha}$ output of 3.5-fold in the normal culture medium and 6.2-fold in medium to which $CaCl_2$ had been added. While $PGF_{2\alpha}$ output was elevated to some extent above controls in $CaCl_2$-supplemented culture medium (1.7-fold), E_2 increased $PGF_{2\alpha}$ output approximately 4.5-fold whether or not $CaCl_2$ was added to the medium. Incubations of the endometrial fragments with E_2 + A23187 resulted in rates of $PGF_{2\alpha}$ output that was somewhat more than additive in the Ca-supplemented medium. Thus, for the 3 specimens shown in Table 3 in which the effects of E_2, A23187 and E_2 + A23187 were examined using medium supplemented with $CaCl_2$, $PGF_{2\alpha}$ levels 12.0-fold ±0.67 (Mean ±SE) greater than the controls were measured in the incubations with E_2 + A23187, whereas the expected additive effect calculated from separate incubations of E_2 and A23187 would be only 9.9-fold.

DISCUSSION

When previous results obtained in this laboratory from cell cultures are extrapolated to intact tissue, they suggest that although both epithelial and stromal cells contribute significantly to endometrial $PGF_{2\alpha}$ output, only the former are primary targets for the reported *in vitro* stimulatory effects of E_2 on $PGF_{2\alpha}$ output by secretory endometrium [5–10]. Basal output of $PGF_{2\alpha}$ is similar in primary cultures of glandular epithelial and stromal cells, whereas addition of E_2 elicited increases in $PGF_{2\alpha}$ output by cultures of epithelial, but not stromal cells [13].

The differential responsiveness of endometrial epithelial and stromal cells to estrogens is further demonstrated in these studies by examining the effects of E_2 in the presence of exogenous AA, precursor of $PGF_{2\alpha}$ through the action of PG synthase. Consistent with the similarities in basal output of $PGF_{2\alpha}$, added AA produced marked and comparable elevations in $PGF_{2\alpha}$ output by cultures of both cell types. In the epithelial cell cultures, substantially greater than additive effects were obtained with mixtures of E_2 and AA, suggesting that enhancement of $PGF_{2\alpha}$ production by E_2 reflects an increase in PG synthase activity. The contrasting refractoriness of the stromal cells to E_2, even in the presence of $PGF_{2\alpha}$-elevating concentration of AA, indicates a failure of E_2 to activate PG synthase in these cells, either directly or via estrogen receptor-mediated mechanisms.

Phospholipase-catalyzed liberation of AA from membrane stores has been thought to represent the rate-limiting step in the synthesis of prostaglandins and thromboxanes, given the small pool of free AA present in mammalian cells [14, 21], as well as the large number of systems in which increased production of these compounds is associated with increased phospholipase activity [22–26]. However, the E_2-enhanced conversion of exogenous AA to $PGF_{2\alpha}$ adds human endometrial epithelial cells to a growing list of examples in which an increase in PG synthase activity accompanies increased production of prostanoids, thereby suggesting a regulatory role for this

Table 3. Effects of E_2, A23187 and $CaCl_2$ on $PGF_{2\alpha}$ output by secretory endometrium*

Exp. no.	Endometrial histology	Control output (ng $PGF_{2\alpha}$) mg prot. × 24 h	% Controls: +Ca^{2+} (1.5 mM)	+E_2 (10^{-8} M)	+E_2 (10^{-8} M) +Ca^{2+} (1.5 mM)	+A23187 (10^{-6} M)	+A23187 (10^{-6} M) +Ca^{2+} (1.5 mM)	+E_2(10^{-8} M) +A23187 +Ca^{2+} (1.5 mM)
178	Secretory Day 21	130	220	400	400	400	710	1300
180	Secretory Day 21	88	160	470	570	400	550	1100
90	Secretary Day 23	58	—	830	—	410	590	1100
128	Secretory Day 26	86	130	120	430	200	620	1100
	Mean ± SE	91 ±14.8	170 ±26.5	460 ±14.6	470 ±52.4	350 ±50.9	620 ±34.0	1200 ±50.00

*Averages from results of duplicate dishes. The experiments were carried out in medium containing 10% charcoal-treated calf serum.

enzyme complex in their production. For instance, E_2 increases both production of prostacyclin [27] and amounts of immunodetectable PG synthase [28] when added to cultures of rat aortic smooth muscle cells, whereas E_2 administered to guinea-pigs [29] or rats [30] stimulates an increase in the conversion of AA to $PGF_{2\alpha}$ by uterine microsomes. In addition, peptides such as platelet derived growth factor [31] and bradykinin and thrombin [32] have been found to increase the conversion of AA to prostanoids in Swiss 3T3 cells.

Evidence obtained by using a well-documented stimulator of phospholipase activity, the calcium ionophore A23187, also supports the thesis that E_2 elevates $PGF_{2\alpha}$ output by increasing PG synthase activity in the epithelial cells. Results presented here confirm the reported stimulation of $PGF_{2\alpha}$ output by A23187 in fragments of secretory endometrium [7], and show that adding $CaCl_2$ to the medium increases the effectiveness of the ionophore, as has been reported in some systems [33]. Moreover, specimens of secretory endometrium incubated with a mixture of E_2 and A23187 in Ca^{2+}-supplemented medium produced increases in $PGF_{2\alpha}$ output somewhat greater than the sum of that elicited by each compound alone. The ionophore also evoked a Ca^{2+}-dependent increase in $PGF_{2\alpha}$ output in the epithelial cell cultures. However, a superadditive effect of greater magnitude than that obtained with the endometrial fragments and consistent with that observed with E_2 and AA, was seen with epithelial cells incubated with E_2 together with A23187.

The cultures of stromal cells behaved differently than the epithelial cells, showing no significant elevation in $PGF_{2\alpha}$ output in the presence of E_2, or A23187 or the combination of the two. The differential responsiveness of these endometrial cells to A23187 and AA has an interesting parallel in endothelial and smooth muscle cells of calf aorta since both of these cells convert exogenous AA to prostacyclin, but A23187 increases prostacyclin output only in the endothelial cells [34]. Whether in those cells that are refractory to A23187 the AA substrate for PG synthase cells is liberated primarily by the action of a phospholipase C that is resistant to the ionophore [16], or by calcium-independent lysosomal phospholipases [35] would seem to be testable questions.

Since epithelial and stromal cells are the predominant components of human endometrium, the intermediate response of intact tissue seems consistent with the divergent effects that E_2 and A23187 exert on $PGF_{2\alpha}$ output by the cultures of the 2 types of cells. However, $PGF_{2\alpha}$ output by endometrium may reflect more than the algebraic sum of the output of its component cells, given the complex interactions that exist between epithelial and stromal cells in other systems [36], as well as the intriguing relationship between blood platelets and adjoining endothelial cells in which an endoperoxide intermediate produced by one cell type is transformed to prostacyclin by the other [37]. Future experiments dealing with E_2 effects on $PGF_{2\alpha}$ output in cocultures of epithelial and stromal cells would therefore appear justified.

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