Platelet-activating factor may act as an endogenous pulse generator for sheep of luteolytic PGF$_{2\alpha}$ release

O. CHAMI, A. MEGEVAND, T. OTT, F. BAZER, AND C. O’NEILL

1Human Reproduction Unit, Department of Physiology, University of Sydney, Royal North Shore Hospital of Sydney, St. Leonards, NSW 2065, Australia; and 2Center for Animal Biotechnology, Institute of Biosciences and Technology, Texas A & M University, College Station, Texas 77843-2471

Platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) is an ether phospholipid that is involved in a number of pathophysiological pathways (for reviews see Refs. 13, 34). PAF is produced by the ovine uterus, mimics the actions of OT in inducing PGF$_{2\alpha}$ release (11), and is present in the uterus when the pulsatile release of PGF$_{2\alpha}$ occurs (11). Therefore, this study tested the hypothesis that uterine PAF is an endogenous uterine pulse generator for pulsatile release of PGF$_{2\alpha}$. This hypothesis is supported by evidence that 1) the production and action of PAF on the uterus is steroid hormone dependent; 2) the actions of PAF are potentiated by OT and inhibited by interferon-$\gamma$ (IFN-$\gamma$), the endogenous antiluteolytic factor for pregnancy recognition (36); and 3) the PAF-induced

SOME BIOLOGICAL MEDIATORS and hormones are released in a pulsatile manner to enhance the response of target cells. Whereas continuous exposure of target cells to ligand may downregulate responses (tachyphylaxis), the pulsatile exposure of cells to ligand may prevent tachyphylaxis and thus enhance or maintain cellular responses; for example, the pulsatile release of insulin from pancreatic islet cells, induction and maintenance of gonadotrophin release from the anterior pituitary by pulsatile release of gonadotrophin-releasing hormone (GnRH) from the hypothalamus, and pulsatile release of uterine prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) in ruminants to induce luteolysis (the structural demise and cessation of progesterone production by the ovarian corpus luteum). On the other hand, constant exposure to ligand causes downregulation of cellular responses; for example, continuous infusion of GnRH prevents gonadotrophin release (58). Although several inputs into the hypothalamus coordinate the pulsatility, immortalized hypothalamic cell lines release GnRH with an inter-pulse interval similar to that in vivo, which suggests a pulse-generator endogenous to GnRH neurons (58). The nature of the putative endogenous pulse-generator remains ill defined.

The pulsatile release of luteolytic PGF$_{2\alpha}$ in sheep is generally coordinate with pulsatile release of oxytocin (OT) from the corpus luteum (CL) and posterior pituitary and of PGF$_{2\alpha}$ from the uterus (24). These two events may provide for a coupled positive feedback loop (20), although unequivocal proof for the existence of such a loop has not been provided. Although constant infusion of OT in ruminants prevents pulsatile release of PGF$_{2\alpha}$ and luteolysis (21), exhaustion of 75% of ovarian OT by use of norepinephrine (29) and inhibition of OT receptors (OTR) with an antagonist (30) did not disrupt luteolysis. Furthermore, pulsatile release of PGF$_{2\alpha}$ from the uterus occurs spontaneously even in ovariectomized (54) and hypothalamic-pituitary stalk-sectioned ewes (16, 35). Although the pulses of PGF are of smaller amplitude, their persistence in these ewes questions the role of OT. The spontaneous pulsatile release of PGF$_{2\alpha}$ requires steroid hormone treatment of ewes and may result from an endogenous pulse generator (54).

Platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) is an ether phospholipid that is involved in a number of pathophysiological pathways (for reviews see Refs. 13, 34). PAF is produced by the ovine uterus, mimics the actions of OT in inducing PGF$_{2\alpha}$ release (11), and is present in the uterus when the pulsatile release of PGF$_{2\alpha}$ occurs (11). Therefore, this study tested the hypothesis that uterine PAF is an endogenous uterine pulse generator for pulsatile release of PGF$_{2\alpha}$. This hypothesis is supported by evidence that 1) the production and action of PAF on the uterus is steroid hormone dependent; 2) the actions of PAF are potentiated by OT and inhibited by interferon-$\gamma$ (IFN-$\gamma$), the endogenous antiluteolytic factor for pregnancy recognition (36); and 3) the PAF-induced

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pulsatile release of PGF occurred, even during chronic exposure of the uterus to PAF.

**MATERIALS AND METHODS**

Animals. Merino ewes were penned indoors and exposed to a 12:12-h light-dark cycle. All ewes were anesthetized by pentobarbital sodium induction with halothane-O₂-N₂O maintenance and ovarioctomized with aseptic techniques. They were allowed to recover for ≈6 wk before beginning treatment. All experiments were performed according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (43) and were approved by the Institutional Animal Care and Ethics Committee.

Hormone replacement of ovarioctomized ewes with progesterone (P) and estradiol-17β (E) (Sigma Chemical, St. Louis, MO) was previously described (11, 23) and creates blood hormone levels similar to those achieved in the natural cycle (23). All days referred to below are the equivalent of that day in the luteal phase or from the insertion of the E implant on day 1. Hormones were dissolved in vegetable oil and administered intramuscularly except luteal phase E, which was administered intravaginally. To test the effect of individual hormones, the schedule of injections was modified so that the P injections were replaced with oil injections given to E-only-treated ewes, or E replaced with oil and E Silastic implants replaced with empty implants given to P-only animals. In experiments testing the effects of PAF infusion into the uteri on the venous stable PGF₂α metabolite (PGFM), control animals received oil injection in place of the P and E injection, while another control group was left untreated. Untreated ewes were ovarioctomized and left for ≈6 wk. Ovarioctomized but untreated ewes were used as the controls for experiments measuring PAF content and PAF:acetylhydrolase activity in the uterus. There were at least three animals per treatment per day for each experiment.

PAF and recombinant ovine IFN-τ (rIFN-τ) were infused directly into the uterine lumen via uterine catheters. Catheters (1 m, 1.4 mm OD and 0.63 mm ID, Silastic, Critchley Products, Sydney, NSW, Australia) were inserted surgically into the lumen just above the uterotubal junction site on day 8 (see Table 1). Catheters were exteriorized through a sublumbar stab incision, stored in an ethanol-soaked plastic bag, and held in place by a body stocking.

When repeated blood samples were required, an indwelling catheter was placed into the jugular vein on day 8 and blood samples (5 ml) were withdrawn at the intervals indicated in each experiment and placed into heparinized tubes on ice. After centrifugation, the plasma was recovered and stored at −20°C until assayed for PGFM.

uterine flushings and endometrial samples. On the equivalent of days 14, 15, and 16 of the luteal phase, endometrial explants and luminal fluids were collected from ewes in each steroid replacement group. For endometrial tissue collection, ewes were anesthetized by pentobarbital sodium induction and euthanized with an overdose of saturated KCl. For ewes in which uterine POAF:acetylhydrolase were measured, each uterine horn was immediately removed without excess mesentery, blotted free of all blood, and placed on ice. The lumen of each was flushed with 3 ml of ice-cold sterile Tyrode solution (in mM: 137 NaCl, 2.68 KCl, 11.9 NaHCO₃, 1 MgCl₂-6H₂O, 0.41 NaH₂PO₄-2H₂O, 0.5 glucose, 5 HEPES, and 0.5% Na azide, pH 7.4) just below the uterotubal junction and was collected at the cervical end. The uterine flushings were divided into 0.5-ml aliquots and stored at −20°C. The luminal fluid protein concentration was measured using a Bio-Rad Total Protein Assay kit (Bio-Rad Laboratories, Hercules, CA).

Individual caruncles were dissected from the uterus and placed in ice-cold sterile saline (Baxter, NSW, Australia). Caruncles were thinly sliced to avoid contamination with myometrial tissue. The slices were washed in sterile saline, and 10 µg aliquots were cultured in 1 ml of Eagle's minimum essential medium (Sigma Chemical) containing 3 mg BSA/ml (CSL, Victoria, Australia) for 90 min at 37°C in 5% CO₂ in air. The medium supernatant was removed and frozen at −20°C until assayed for PAF.

Actions of PAF:acetylhydrolase. The PAF:acetylhydrolase activity was also measured in supernatants of caruncular endometrium homogenized in Tyrode solution and centrifuged (2,500 g, 4°C, 15 min). The protein concentration of the supernatants was measured using a Bio-Rad Total Protein Assay kit (Bio-Rad Laboratories). Samples were then stored at −20°C until assayed for PAF:acetylhydrolase activity.

PAF:acetylhydrolase converts PAF into its biologically inactive metabolite lyso-PAF. The effect of PAF:acetylhydrolase on PAF in uterine flushings and released from endometrial explants was measured by using human serum as the enzyme source. The serum was collected and heat inactivated for 20 min at 56°C. One-half of the serum was left untreated (PAF:acetylhydrolase active), and the remainder was acid-treated (pH 3.0) with 1 mM HCl (BDH Chemicals, Poole, UK) for 20 min at 37°C to inactivate the PAF:acetylhydrolase, and then pH 7.4 was restored by addition of 1 mM NaOH (BDH Chemicals, Poole, UK)(57). Untreated or acid-treated serum (10% vol/vol) was added to culture medium for 90 min at 37°C, after which time PAF was extracted from the medium and measured by RIA.

Treatments. The PAF (1-O-hexadecyl/octadecyl-2-acetyl-sn-glycero-3-phosphocholine; Sigma Chemical) was maintained as a stock solution in chloroform (1 mg/ml). Aliquots were evaporated to dryness under N₂ in siliconized glass test tubes and brought to solution in Ca²⁺- and Mg²⁺-free PBS supplemented with 2.5 mg BSA/ml (PBS-BSA). PAF was sterilized by filtration through a 0.22-μm filter (Millipore, Sydney, NSW, Australia), prepared as aliquants in sterile 5-ml plastic tubes (Falcon, Becton-Dickinson Lab, Sydney, NSW, Australia) and then stored at −20°C until used. Control infusions were PBS-BSA prepared, thawed, and stored in the same manner.

OT (Sigma Chemical) was prepared in sterile saline, stored, and thawed under the same conditions as the PAF solution. The OT was administered through the jugular cannulas.

rIFN-τ was produced from a synthetic gene in Pichia pastoris (Invitrogen, Carlsbad, CA) (56) and purified as described previously (47). The protein concentration of purified rIFN-τ was determined using the bichinchoninic acid (BCA) protein assay reagent (Pierce, Rockford, IL). The purity of rIFN-τ was determined using 2D SDS-PAGE and silver staining. Antiviral activity was determined by a cytopathic effect assay with Madin Darby bovine kidney cells challenged with vesicular stomatitis virus and compared with a calibrated laboratory standard human IFN (Roferon) (47). PAF:acetylhydrolase was determined using 1D SDS-PAGE and silver staining. Antiviral activity was determined by a cytopathic effect assay with Madin Darby bovine kidney cells challenged with vesicular stomatitis virus and compared with a calibrated laboratory standard human IFN (Roferon) (47). PAF:acetylhydrolase was determined using 1D SDS-PAGE and silver staining.

Assays. PAF:acetylhydrolase activity in luminal fluids and endometrial homogenates was assayed at three protein concentrations as previously described (39, 46). Briefly, 5 µmol/l [³H]PAF (5.25 GBq/mmol) was incubated with each sample for 15 min at 37°C in a shaking water bath. The reaction was stopped by adding 170 µg BSA and then ice-cold TCA (9.2% wt/vol) to facilitate protein precipitation. Aliquots of the supernatant were collected, and the free [³H]acetate was
PAF in uterine flushings from ovariectomized ewes given E, P, or P+E are shown in Fig. 1. To assess the potential turnover of PAF released from this source, PAF:acyl-hydrolase activity values in uterine flushings and endometrial homogenates (Fig. 2) on days 14–16 were compared with values for untreated ewes.

The amount of PAF released in vitro and detected in uterine flushings was variable; thus both the medians and means of the results are shown (Fig. 1). Ewes treated with E alone released more PAF on day 14 than on day 15 or 16, when the amount was greater for untreated ewes (P < 0.05). Endometrium from ewes given P only released more PAF (P < 0.05) than untreated ewes only on day 16, although there was a clear trend for high levels on day 15. Release of PAF from ewes treated with P+E was higher on day 16 than on days 14 and 15 and also higher than from untreated ewes (P < 0.05). As a further control, some animals were treated with oil as a vehicle instead of being left untreated.

The PAF response was measured as the stable metabolite PGFM by RIA. The 13,14-dihydro-keto[5,6,8,9,11,12,14(N)3H]PGF2α (186 Ci/mmol; Amersham Life Science, Little Chalfont, Buckinghamshire, UK) and porcine anti-sheep antibody C-0462 (Sigma Chemical) in PGFM-free serum was used to produce a dose-response curve and quality controls. Standards were performed in triplicate and samples in duplicate. To each tube sample or standard, PGFM buffer (142 mM NaCl; 76.5 mM Na2HPO4; 7.7 mM Na azide; 23–25 mM NaH2PO4 ·2H2O, pH 7.4), antibody (1/20,000 dilution), and 8 pmol/l [3H]PGF2α (278 Ci/mmol) were added, and the mixture was incubated for 80 min at 37°C and then for 12 h at 4°C. Bound and unbound radiolabels were separated using polyethylene glycol 6000 (20% wt/vol; BDH Laboratory Supplies, Poole, UK), and the [3H]PGF2α was counted on a Packard Tricarb model 1500 scintillation counter (Canberra Packard).

The sensitivity of the PGFM assay was 0.05 ng/ml. The interassay coefficients of variance were 13.62, 10.31, and 10.27%, respectively, for 0.2, 0.4, and 0.8 ng/ml. The intra-assay coefficients of variance were 8.13, 5.62, and 8.74%, respectively, at 0.2, 0.4, and 0.8 ng/ml.

Data analysis. All statistical analyses were performed using the statistical package SPSS for Windows (Release 6.10). For PAF in caruncular endometrial culture medium and uterine flushings, and for PAF:acyl-hydrolase activity in endometrial homogenates and uterine flushings, homogeneity of variance was assessed by Bartlett's test. Where the data were heterogeneous, medians were compared using Mann-Whitney tests and Kruskal-Wallis is one-way ANOVA. Repeated-measures ANOVA was used for analysis of PGFM responses.

RESULTS

The effect of steroid hormones on PAF release by the endometrial explants in vitro and the concentration of
specific activity (ewes). Ewes receiving E alone had significantly lower E. In both E and P treatment groups, the activity was higher (P < 0.05) for all hormone-treated ewes on flushings (Fig. 2A). PAF:acetylhydrolase specific activity decreased significantly (P < 0.05) after day 14, whereas activity in untreated ewes was maintained at a consistently high level throughout the study period.

The PAF:acetylhydrolase activity in endometrial explant tissues (Fig. 2B) was lower than that in uterine flushings. For both P- and E-treated ewes, there was no change in activity (P > 0.05) between days 14 and 16; however, there was an increase (P < 0.05) in PAF:acetylhydrolase activity between days 14 and 16 for P+E-treated ewes.

PAF released by some cell types binds to domain II of albumin, and in this configuration its degradation by PAF:acetylhydrolase is prevented (7). To determine whether the PAF measured in uterine flushings and the PAF released by endometrial explants were in this protected conformation, endometrial culture medium and uterine flushings were exposed to PAF:acetylhydrolase (Table 1). Endometrium-derived and uterine flushing PAF was hydrolyzed by PAF:acetylhydrolase and was not affected by acid-treated serum, because PAF:acetylhydrolase is acid labile, confirming the specificity of the reaction.

Control ewes having oil injections instead of steroids showed no change in plasma PGFM levels after intrauterine infusion of PAF (200 µg/horn) or PBS-BSA (P > 0.05). Ewes treated with P, E, or P+E also showed no change in PGFM after PBS-BSA infusion (P > 0.05). Because there were no differences (P > 0.05) in response among control groups, results were pooled to form the one control group shown in Fig. 3 and used in analysis. The infusion of PAF into the uterus on days 15 and 16 of ewes receiving either P or E alone induced a small but significant PGFM response (P < 0.05) compared with control ewes (Fig. 3, A and B, respectively), whereas a much larger increase (P < 0.001) in PGFM occurred in P+E-treated ewes. In P+E ewes the response was greater than for control ewes and ewes treated with E or P. The duration of the PGFM response to PAF on day 15 was ~15 min (Fig. 3A), whereas on day 16 the response lasted for up to 1 h (Fig. 3B).

Both PAF and OT were administered on days 15 and 16 to P+E-treated ewes. Both PAF and OT alone increased (P < 0.05) plasma PGFM within 10 min of administration.

Table 1. Susceptibility of PAF in endometrial explant-conditioned medium and uterine flushings to PAF:acetylhydrolase

<table>
<thead>
<tr>
<th>Hormone Regimen</th>
<th>Pooled Medium</th>
<th>Medium + 10% HT Serum</th>
<th>Medium + 10% HTAT Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>P + E</td>
<td>3.9</td>
<td>0.09</td>
<td>1.7</td>
</tr>
<tr>
<td>P</td>
<td>9.1</td>
<td>0.06</td>
<td>8.9</td>
</tr>
<tr>
<td>E</td>
<td>1.8</td>
<td>0</td>
<td>2.4</td>
</tr>
<tr>
<td>PAF in uterine flushings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P + E</td>
<td>5.3</td>
<td>0</td>
<td>5.8</td>
</tr>
<tr>
<td>P</td>
<td>3.6</td>
<td>0.3</td>
<td>2.7</td>
</tr>
<tr>
<td>E</td>
<td>4.6</td>
<td>0.09</td>
<td>4.9</td>
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Values of platelet-activating factor (PAF) are means of 2 assays expressed in ng/ml. P, progesterone; E, estradiol-17β; P + E, combined P and E treatment; HT, human serum; HTAT, acid-treated human serum.
When PAF was administered before OT, the increase in plasma PGFM in response to OT was six to seven times \((P < 0.05)\) greater than that induced by PAF or OT alone. To determine whether this synergistic effect resulted from a priming action of PAF on the uterus, PAF and OT were also administered simultaneously (Fig. 4A). This treatment resulted in a PGFM response equivalent to the sequential treatment, but peak values occurred 10 min earlier, probably due to the earlier time of administration of OT (Fig. 4B).

Ovine conceptus trophoderm secretes IFN-\(\tau\), which inhibits luteolytic pulses of PGF2\(\alpha\) during early pregnancy (36). The P+E-treated ewes given rolFN-\(\tau\) 24 h before PAF challenge on days 15 and 16 did not respond with an increase in plasma PGFM (Fig. 5A). Similarly, pretreatment with rolFN-\(\tau\) on day 14, followed by PAF and OT on days 15 and 16, resulted in a reduced PGFM response compared with ewes not treated with rolFN-\(\tau\) \((P < 0.05\), Fig. 5B). These results indicate that the effect of rolFN-\(\tau\) administration on day 14 continued to inhibit the effects of PAF and OT through day 16.

It is currently not known whether PAF is produced or released from the endometrium in a pulsatile manner. We therefore asked the question, does prolonged exposure to PAF cause chronic downregulation of the uterine response? Thus exposure to PAF over an 8-h period was performed by hourly infusion into P+E-treated ewes on days 14 and 15 (Figs. 6, A and B). On both days, a strong response to the initial PAF challenge was followed by complete tachyphylaxis by the third challenge. Uterine sensitivity to PAF was again detected at the sixth challenge, with responsiveness being 64 ± 4 and 57 ± 7% of the first response on days 14 and 15, respectively. Further challenges induced another refractory period. On days 14 and 15, the 6-h response to PAF was greater \((P < 0.01)\) than the 5-h and 7-h challenges \((P < 0.05)\).

**CONCLUSIONS**

Results of the present study are the first to demonstrate that PAF may be responsible for the pulsatile release of PGF2\(\alpha\) from the uterus. PAF release by the uterus was dependent on sex steroids, and rolFN-\(\tau\) prevented PAF-induced PGF2\(\alpha\) release from the uterus. The uteri from ovariectomized ewes treated with E+P hormone replacement similar to the luteal phase became spontaneously responsive to PAF at ~6-h intervals, which is similar to the frequency of PGF pulses for intact ewes during the luteolytic period. After PGF2\(\alpha\) release, the uterus was desensitized to PAF for ~6 h.

The synergistic interaction between PAF and OT in the generation of PGF pulses supports substantial evidence for a role of OT in luteolysis in sheep (19, 37). However, the persistence of PGF pulses in both ovariectomized (54) and hypothalamic-pituitary stalk-sec-
tioned (16, 35) ewes has raised doubts about an essential role for OT in the luteolytic process. The observation that PGF pulsatility occurred in steroid-replaced ovariec-tomized ewes is tempered by the observation that the amplitude of the PGF pulses was markedly reduced compared with intact ewes (54). Studies with intact ewes indicated that uterine PGF$_2\alpha$ levels often increase before the release of ovarian OT (40) and that inhibitors of PG synthesis (41, 48) or action (22, 44) can block OT release. Results of the present study indicate that PAF-induced low-amplitude PGF pulses synergistically enhanced subsequent effects of OT to stimulate PGF secretion. The synergism between PAF and OT was blocked by IFN-$\gamma$, regardless of whether PAF and OT were administered simultaneously or sequentially.

The presence of PAF in the uterus has been reported for rat (59), human (4), rabbit (9), and sheep (11), but this is the first report of its presence in uterine flushings. The high degree of variability in PAF release from endometrial explants and in uterine flushings was unexpected and may reflect the combined effects of episodic release of PAF and its relatively short half-life due to the presence of PAF:acetylhydrolase in luminal fluids and endometrial tissue. It was shown (6) that good agreement in quantitative measurement of PAF was achieved when bioassays and radioimmunoassay were compared. Thus the variability seen is unlikely to be due to assay artifacts. In many cell types, most PAF produced is retained by the cell and may act as a juxtracrine hormone (60); that is, it is presented to its receptor on the outer leaf of the plasma membrane of adjacent cells. In human endometrial cells, high concentrations of PAF are associated with stromal cells (4). The uterine endometrium possesses the enzymes necessary for PAF synthesis (31), and PAF accumulates in the endometrial stroma rather than in the epithelium (4). Synthesis of PAF in human endometrium is
dependent primarily on progesterone (4). If stromal cells accumulate PAF in ewes, intraluminal administration of PAF may not best mimic its normal sites of action. This may explain why relatively high concentrations of PAF were required to be administered into the lumen to elicit responses. Further studies of PAF’s site of release, action in the uterus, and the assessment of PAF levels in the endometrial tissue are required.

Angle et al. (9) showed that concentrations of PAF in the rabbit endometrium increased ~20-fold during the first half of the luteal phase. In pregnant rabbits, PAF content declined to postovulatory levels around the time for luteolysis, whereas levels of PAF in endometrium were high throughout pseudopregnancy. Although regulation of luteolysis in the rabbit is not well defined, PGF2α is considered to be the uterine-derived luteolysin (14). Thus high levels of PAF in the uterus of pseudopregnant females may be required for the generation of luteolytic PGF2α.

PAF biosynthesis may occur by two alternate pathways, “the re-modeling,” where phospholipase A2 converts 1-O-alkyl-2-arachidonyl-glycerophosphocholine into with the lyso-PAF with the release of arachidonic acid (55), and the alternative or “de-novo” pathway, which does not use phospholipase A2 (33). The precursor and final converting enzymes of the de novo and remodeling pathways have been reported in the rabbit uterus (32). Although the remodeling pathway has not been demonstrated in the ovine uterus, the spontaneous production of prostaglandins in the uterus in the ovariectomized ewe model infers that phospholipase A2 activity is present. It may be possible, therefore, that PAF present in the uterus is a by-product of PG production. The possibility for coordinate regulation of the synthesis of these lipid mediators requires further study.

In mice (46), PAF:acetylhydrolase activity in the endometrium and uterine lumen is high in the mid-luteal phase but falls rapidly around the time of luteolysis because of the effects of estrogen. In the present study, E-treated ewes had less PAF:acetylhydrolase activity than ewes treated with P or P+E treatment. This study does not demonstrate whether the change in activity is due to changes in the concentration of protein. Relatively low levels of PAF:acetylhydrolase activity would be advantageous, because PAF in the uterine flushings and released by the ovine endometrium is susceptible to hydrolysis by PAF:acetylhydrolase, whereas the PAF released by the embryo (8) or endothelial cells (7) is highly resistant to the actions of PAF:acetylhydrolase. PAF present on the surface of cells can act as a juxtacrine hormone, but it is also susceptible to PAF:acetylhydrolase (32). A method of demonstrating the actions of PAF on the endometrium independent of the effects of its metabolism by PAF: acetylhydrolase may be the use of a nonhydrolyzable analog, such as methyl-carbamyl PAF. Investigation of such a method is a priority for the future.

The effect of PAF on PGF secretion was greatest when the hormone replacement regimen mimicked the normal luteal phase, and it increased as the cycle progressed toward the time for luteolysis. The ovine PAF receptor has not been defined, but a heptahelical G protein-linked PAF receptor has been identified in several species (53). The mRNA for the human PAF receptor was localized in the uterus by in situ hybridization (10). It was not detected in the proliferative phase but was abundant in the late luteal phase in both stromal and epithelial cells. Infusion of the PAF receptor antagonist WEB 2086 in the uterus caused some release of PGFM (11). In this regard, its actions were more like a partial agonist than a true antagonist. Such partial agonism has not been reported for the actions of these agents on G protein-linked receptors. The apparent actions of WEB 2086 as a partial agonist have also been seen in mouse embryo implantation studies (45) and in a perfused rat heart model (25). On the basis of this pharmacological evidence, it is thought that another class of PAF receptor may also exist in the uterus (28).

Although the ovine PAF receptor has not been characterized, a functional ovine PAF receptor in endometrial explants is presumed to be responsible for PAF-induced activation of phospholipase C and mobilization of phosphatidylinositol phosphates (12). Similar responses to PAF have been reported for human endometrial explants (3), also including activation of phospholipase D (1, 2). PAF synthesis and PAF receptor expression are both influenced by the presence of steroids in some tissues. The human PAF receptor gene has two steroid-responsive elements and one estradiol-responsive region in the promoter region (52). Estradiol treatment enhanced PAF production and PAF-receptor mRNA expression in cultures of human endometrial cells (50). After ovariectomy, the PAF concentration in the uterus of rats significantly declined, and this could be restored by treatment with estradiol (42). Treatment of human endometrial cell cultures with estradiol enhanced PAF-induced PGE2 production (5) and PAF-induced phospholipase D activity (2). Therefore, the presence of steroids
in the uterus may stimulate both PAF production and PAF-receptor formation to sensitize the uterus to PAF. The results presented in Fig. 6 show that the PAF ligand may play an important role in pulsatile release of PGF by the uterus. Many G protein-linked heptahelical receptors undergo a cycle of agonist-induced desensitization, recycling, and re-sensitization (26, 27). Desensitization of the receptor involves functional uncoupling of the receptor by gonadotropin-releasing hormone-associated proteins, including the regulators of G protein signaling (RGS) proteins (17) and ligand-induced receptor sequestration into the intracellular compartment after endocytosis of clathrin-coated vesicles (26). PAF-induced sequestration of its receptor was ligand dependent, and recycling of the active form did not require the synthesis of a new receptor (26). In B cells, PAF-induced expression of RGS 1 resulted in downregulation of signal transduction (18), which may determine the duration of tachyphylaxis. This may provide a mechanism for periodic response to ligation of cells that are independent of frequency of release of the ligand.

Results of the present study suggest a new hypothesis for the generation of luteolytic pulses of PGF, by the ovine uterus; this is presented in Fig. 7, with the primary points of control indicated by the letters A-H. Luteal phase steroids (A) induce production of PAF, stimulate PAF-receptor mRNA production (51, 52) by the endometrium, and cause the uterus to become sensitive to PAF (B). The responsiveness of the uterus to PAF (at the receptor or postreceptor level) requires both E and P for low-amplitude PGF, pulses (C). The low-amplitude PGF pulses may act on the CL to induce OT release (D) (40), which then binds to uterine OTR (E) to induce high-amplitude pulses of PGF, (F) because of the sequential action of PAF and OT on the uterus. After a high-amplitude pulse, the uterus becomes relatively desensitized to PAF for ~6 h, followed by spontaneous resensitization and subsequent high-amplitude pulses of PGF that culminate in complete luteolysis (G). Thus, even if relatively constant levels of PAF production occur in the uterus, pulsatile release of PAF may occur because of the periodicity of responsiveness of the uterus to PAF.

IFN- completely blocks the release of PGFM in response to PAF (H) and inhibits the synergism between PAF and OT. IFN- also inhibits transcription of the genes for the estrogen receptor and OTR (38). McCracken et al. (37) showed that the production of subluteolytic pulses of PGF by the uterine endometrium is similar between cycic and pregnant ewes between days 12 and 14 and that they were unaffected by the presence of IFN-. Also, OT release has been shown (49) to be similar between cycic and pregnant ewes during this period. Perhaps pregnancy has the effect of uncoupling the PAF-induced luteolytic PAF pulses and OT release and action on the uterus. The ability of IFN- to block the synergism of these agents is consistent with this possibility. The hypothesis presented here is suited for testing in intact animals during natural luteolysis and pregnancy.

In conclusion, PAF fulfills many of the criteria of an endogenous initiator of PGF, release from the ovine uterus. The synergistic interaction between PAF and OT, compared with the modest PGF pulses produced by PAF alone, may account for the high amplitude of PGF responsible for luteolysis. The results also provide an explanation for the apparent coupling of uterine PGF, and ovarian OT pulses.

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Present address of T. Ott: Department of Animal and Veterinary Sciences, 216 Agricultural Sciences Building, University of Idaho, Moscow, ID 83844-2330.

Address for reprint requests and other correspondence: C. O’Neill, Human Reproduction Unit, Department of Physiology, Univ. of Sydney, Royal North Shore Hospital of Sydney, St. Leonards, NSW 2065, Australia (E-mail: chriso@med.usyd.edu.au).

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REFERENCES


