Effect of platelet-activating factor (PAF) on human spermatozoa–oocyte interactions

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The effect of exogenous platelet-activating factor (PAF) on human spermatozoa was examined by monitoring acrosomal status and hamster oocyte penetration. Induction of the acrosome response by PAF was dose, and time, dependent and required capacitated spermatozoa. Addition of 10^{-14} mol PAF l⁻¹ enhanced the acrosome reaction eightfold compared with controls treated with human serum albumin (HSA). Similar concentrations of lyso-PAF failed to induce acrosomal loss and preincubation of spermatozoa with CV3988, an inhibitor of PAF, prevented PAF induction of the acrosome reaction. PAF significantly increased the number of zona-free hamster oocytes penetrated compared with controls (9.8 ± 0.5 of 25 oocytes were penetrated by control spermatozoa compared with 23.3 ± 0.8 out of 25 oocytes penetrated after incubation of spermatozoa with 10^{-14} mol PAF l⁻¹; 93% of all oocytes were penetrated by at least one spermatozoon following incubation with PAF), and also increased the number of decondensed spermatozoa found per egg during the sperm penetration assay (from 1.7 ± 0.3 spermatozoa/egg with control spermatozoa to 3.3 ± 0.5 spermatozoa/egg with PAF-treated spermatozoa). PAF-induced increases in acrosome reaction and sperm penetration assay values were similar to effects obtained with human follicular fluid and were calcium dependent. Induction of the acrosome reaction by physiological concentrations of PAF appeared to be morphologically similar to the response induced by follicular fluid as assessed by electron microscopy.

Introduction

Members of a family of biologically active phospholipids, collectively known as platelet-activating factor (PAF; 1-O-alkyl-2-acetyl-sn-glycerol-3-phosphocholine), have been shown to modulate a number of physiological effects, including systemic vasodilation, pulmonary bronchiole constriction, platelet and neutrophil activation, initiation of cardiac ischaemia, tissue rejection and gastric ulcer formation (Braquet *et al.*, 1987).

Recently PAF has been linked with various reproductive processes. Several studies implicate PAF in events associated with follicular development and ovulation in the female: intra-ovarian instillation of PAF has been shown to induce ovulation, and the use of PAF antagonists blocks follicular rupture (Abisogun *et al.*, 1989); PAF has been found in human follicular fluid; and addition of exogenous PAF to human granulosa cell cultures modulates progesterone biosynthesis (Rabinovici and Angle, 1991). PAF also appears to mediate events directly associated with fertilization: addition of PAF increases the fertilization rate of mouse oocytes *in vitro*, and the use of PAF antagonists can block fertilization *in vivo* as well as *in vitro* (Harper *et al.*, 1989; Minhas *et al.*, 1989; Kuzan, 1990). It has been proposed that

PAF is the first embryonic signal of pregnancy (O'Neill, 1985a, b, c; Orozco *et al.*, 1986), and embryonic production of PAF may reflect embryo health and viability (O'Neil and Saunders, 1985; O'Neill *et al.*, 1985, 1987; O'Neill, 1987; Punjabi *et al.*, 1990). PAF also appears to be associated with implantation, as PAF concentrations in the uterus change during the implantation period (Angle *et al.*, 1988a); administration of PAF antagonists inhibits implantation (Acker *et al.*, 1988; Spinks and O'Neill, 1988), and injection of PAF into the uterus induces a decidual-like reaction in pseudopregnant rats (Acker *et al.*, 1989). PAF may also play a role in the maintenance of pregnancy (Geissler *et al.*, 1989). Finally, during the final stages of pregnancy, increasing concentrations of PAF are found in amniotic fluid and appear to be associated with the onset of parturition (for review, see Johnston *et al.*, 1990).

PAF also plays a role in male reproductive physiology and has been reported in spermatozoa from normal men, rabbits and mice (Kumar *et al.*, 1988; Minhas *et al.*, 1989; Kuzan *et al.*, 1990), although Collier *et al.* (1990) failed to detect PAF in human spermatozoa using a platelet-aggregation assay. It has also been reported that PAF increases the motility of spermatozoa from normal human donors (Ricker *et al.*, 1989). However, other investigators have been unable to observe an effect of PAF on the motility of spermatozoa from normal donors or subfertile patients either before or after overnight capacitation (Angle *et al.*, 1991). However, since PAF antagonists appear to be spermicidal (Harper *et al.*, 1989) and can inhibit fertilization, PAF

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may affect other aspects of sperm function without modulating sperm motility. These experiments were therefore designed to examine the role of PAF in spermatozoa–egg interactions by monitoring the effect of exogenous PAF on initiation of the acrosome reaction and on hamster oocyte penetration.

Materials and Methods

Semen collection and preparation

Semen was collected from normal donors routinely used as controls for clinical tests in the Andrology Laboratory at the University of California, San Francisco. Permission to use the donated spermatozoa for experimental purposes was obtained from each patient and from the Research Evaluation and Allocation Committee. Semen was washed twice with Biggers, Whitten and Whittingham (BWW) media supplemented with 0.6% human serum albumin (HSA; Fraction V, Sigma, St Louis, MO), pelleted, and overlaid with BWW to obtain a 'swim-up' population of motile spermatozoa. This population of spermatozoa was either used immediately (noncapacitated spermatozoa) or incubated overnight at 37°C; the spermatozoa were then used to assess the effect of PAF on the acrosome reaction or on spermatozoa–egg interaction in the zona-free hamster oocyte penetration assay.

Preparation of PAF

PAF was prepared by resuspending the original sample, from Avanti Polar Lipids (Birmingham, AL), in chloroform:methanol 2:1 and storing it at –20°C until phosphorus analysis was completed (Rouser *et al.*, 1966). After quantitation of the phosphorus content, an aliquot of the chloroform:methanol solution was removed, dried under a stream of nitrogen at 37°C, then resuspended in 0.25% HSA in 0.9% saline to give the required concentration of PAF. Lyso-PAF was treated in a similar manner.

Assessment of acrosome reaction

The effect of PAF or lyso-PAF on the spermatozoa acrosome was evaluated by staining with fluorescein isothiocyanate (FITC)-labelled *Pisum sativum* agglutinin (PSA) (Vector Labs, Inc., Burlingame, CA) as described by Cross *et al.* (1986). Briefly, washed, swim-up spermatozoa that had been capacitated overnight in BWW containing 0.6% HSA were incubated with various concentrations of PAF (10^{-9} – 10^{-16} mol l⁻¹), lyso-PAF (10^{-14} mol l⁻¹) or 0.25% HSA in physiological saline (PAF control) for 30 min. When the PAF inhibitor CV3988 was used, spermatozoa were incubated with the inhibitor (10^{-12} mol l⁻¹) for 30 min before addition of PAF (10^{-14} mol l⁻¹). Extraneous protein was removed by washing, and the spermatozoa fixed in 95% ethanol for 45 min at 4°C. A smear of spermatozoa was then made and combined with 30 µg FITC-labelled PSA ml⁻¹ for 2 min. Coverslips were mounted using 1.5% Dabco (1,4 diazobicyclo [2.2.2] octane; Sigma, St Louis, MO) in glycerol: PBS (9:1 v/v), final pH of 8.6. The presence or absence of an acrosome reaction was evaluated by counting at least 100

spermatozoa under a fluorescence microscope at ×400 magnification. Spermatozoa were incubated with equal volumes of 0.25% HSA in physiological saline as a negative control. Heat-inactivated human follicular fluid, final dilution 1:4, was also used in some experiments.

Spermatozoa penetration assay

The effect of PAF on penetration of zona-free hamster oocytes by spermatozoa was assessed using a modification of the methods described by Yanagimachi *et al.* (1976). Oocytes were retrieved from superovulated, sexually mature Golden Syrian hamsters and treated with 0.1% hyaluronidase (Sigma) to remove the cumulus and 0.1% trypsin (Sigma) to remove the zona pellucida. Spermatozoa were capacitated overnight in BWW containing 0.6% HSA, washed, then resuspended in 10^{-14} mol PAF l⁻¹ in 0.25% HSA or in 0.25% HSA alone for 30 min. At the end of the preincubation period, spermatozoa were washed, and 5–10 × 10⁶ motile spermatozoa were added to 25 oocytes in BWW with 0.6% HSA under oil and incubated for 3 h. At the end of the incubation period, the eggs were removed, mounted on slides and examined at ×400 with phase-contrast optics. The number of oocytes penetrated by at least one spermatozoa was recorded, as was the number of decondensed spermatozoa heads per oocyte.

Electron microscopy

PAF-treated, acrosome-reacted spermatozoa were fixed for electron microscopy in 2.5% glutaraldehyde in 0.1 mol sodium cacodylate l⁻¹ for 1–2 h at room temperature, stored overnight at 4°C, then embedded and prepared for transmission electron microscopy (TEM). Two-hundred spermatozoa were examined by TEM. The patterns described by Yudin *et al.* (1988) were used to determine which spermatozoa had acrosome reacted. The acrosomal status of the samples of spermatozoa was assessed using FITC-labelled PSA lectin before fixation, and the percentage of acrosome-reacted spermatozoa recorded by TEM correlated with the PSA assessment.

Determination of calcium effects

The role of calcium in PAF-mediated activation of spermatozoa function was assessed in capacitated, swim-up spermatozoa washed in BWW containing Ca²⁺ or in BWW that was nominally free of Ca²⁺ (Morales *et al.*, 1989), then incubated with PAF (10^{-14} mol l⁻¹) for 30 min. Subsequently, spermatozoa were co-incubated with hamster oocytes in BWW with or without Ca²⁺ for 3 h. The SPA was scored and the acrosomal status determined using spermatozoa that were left after removal of eggs for SPA.

Statistical analysis

One-way and repeated measures analysis of variance, and paired comparisons were used to evaluate concentration and time effects on the acrosome reaction. Student's *t* tests with Bonferroni adjustment of the *P* value were used to compare

Fig. 1. The effect of decreasing concentrations of platelet-activating factor (PAF) on the acrosomal status of human spermatozoa. PAF was resuspended in 0.25% human serum albumin (HSA) to give a final concentration of 10^{-11} to 10^{-16} mol l⁻¹. Each point represents the mean $\pm$ SEM for the number of acrosome-reacted spermatozoa per 100 cells counted from each of nine donors. Values with different letters are significantly different from each other ($P < 0.05$); values with the same letters were not significantly different.

treatment and control groups when one-way analysis of variance was done. An unpaired Student's *t* test was used to examine statistically the effect of PAF, lyso-PAF, CV3988 and follicular fluid on the acrosome reaction of spermatozoa penetration.

Results

Dose effect of PAF on acrosome reaction

Addition of PAF to human spermatozoa initiated a concentration-dependent change in the number of spermatozoa that acrosome-reacted during a 30 min incubation (Fig. 1). Addition of 10^{-11} mol PAF l⁻¹ did not increase the number of acrosome-reacted spermatozoa compared with that seen in spermatozoa treated with HSA alone. However, as the concentration of PAF decreased, the number of acrosome-reacted spermatozoa per 100 cells increased, reaching a peak value at 10^{-14} mol PAF l⁻¹, a dose similar to physiological concentrations of PAF. The number of acrosome-reacted spermatozoa then decreased as the PAF concentration further decreased, again approaching control values by 10^{-16} mol PAF l⁻¹. Physiological concentrations of 10^{-14} mol PAF l⁻¹ were used for all subsequent experiments.

Time course of PAF action

In an effort to determine the time required for PAF to initiate the acrosome response, 10^{-14} mol PAF l⁻¹ was added to washed spermatozoa that had been capacitated overnight in BWW with 0.6% HSA. An aliquot of spermatozoa was removed

Fig. 2. Time course for PAF induction of the acrosome response. PAF was resuspended in 0.25% HSA to give a final concentration of 10^{-14} mol l⁻¹ and incubated with spermatozoa from six donors. At various times an aliquot of the spermatozoa suspension was removed and the acrosomal status of 100 spermatozoa determined. Data points indicate mean $\pm$ SEM.

at 1, 2.5, 5, 10 and 30 min and the acrosomal status of the spermatozoa assessed. Repeated measures analysis of variance suggested a significant effect of time on the acrosome reaction ($P = 0.0028$). The number of acrosome-reacted spermatozoa increased three-fold within 2 min and fourfold within 5 min of addition of PAF (Fig. 2). The response continued to increase throughout the remainder of the 30 min incubation. Thirty minute incubations with PAF were therefore performed during all subsequent experiments.

Effect of PAF on noncapacitated spermatozoa

The previous studies were conducted using spermatozoa that had been capacitated overnight in BWW and 0.6% HSA. An aliquot of washed, swim-up spermatozoa from ten donors was exposed to PAF or 0.25% HSA for 30 min to determine whether the capacitation step was required for development of the PAF response. The remaining washed spermatozoa were tested with PAF on the following morning. Addition of 10^{-14} mol PAF l⁻¹ did not appear to enhance the number of reacted spermatozoa compared with controls when PAF was added to spermatozoa within 1 h of swim-up (1.2 ± 0.3 acrosome-reacted spermatozoa, mean $\pm$ SEM, treated with HSA compared with 1.3 ± 0.5 acrosome-reacted spermatozoa, mean $\pm$ SEM, treated with PAF within 1 h after swim-up; data not shown). Failure to induce an acrosome reaction in noncapacitated spermatozoa was also observed following the addition of human follicular fluid (1:4 final dilution) or progesterone (50 ng ml⁻¹ final concentration).

Fig. 3. The effect of human follicular fluid (FF; 1:4 final dilution), PAF (10^{-14} mol l^{-1}), lyso-PAF (10^{-14} mol l^{-1}) and CV3988 (10^{-12} mol l^{-1}) on the acrosomal status of capacitated spermatozoa. Spermatozoa were incubated with FF, PAF, or lyso-PAF for 30 min before assessment of the acrosome response or preincubated with CV3988 for 30 min before addition of PAF. The acrosomal status of 100 spermatozoa was assessed in the number of specimens indicated in parentheses above each bar. Bars represent means $\pm$ SEM. Significant differences ($P < 0.01$) between experimental and control values (t test for unpaired samples) are indicated by asterisks.

Effect of follicular fluid, lyso-PAF and CV3988 on capacitated spermatozoa

After overnight capacitation, addition of human follicular fluid or 10^{-14} mol PAF l^{-1} for 30 min significantly increased ($P < 0.0001$, Student's t test) the number of acrosome-reacted spermatozoa by eight times compared with HSA-treated controls (Fig. 3). The number of spermatozoa that acrosome-reacted following addition of PAF did not appear to be significantly different from the number of spermatozoa that acrosome-reacted following the addition of human follicular fluid, a known inducer of the acrosome reaction. Addition of lyso-PAF (10^{-14} mol l^{-1}), the PAF synthetic precursor and the PAF degradation product, did not appear to induce an acrosome response. In addition, preincubation of spermatozoa with 10^{-12} mol CV3988 l^{-1} , an inhibitor of PAF, appeared to block initiation of the acrosome reaction following addition of 10^{-14} mol PAF l^{-1} .

Effects of PAF on hamster oocyte penetration

Addition of PAF (10^{-14} mol l^{-1} for 30 min) to 25 human spermatozoa significantly enhanced the number of zona-free hamster oocytes penetrated by PAF-treated spermatozoa compared with controls ($P < 0.0001$, Student's t test) (Fig. 4a). Decondensed spermatozoa were observed in 93% of the hamster oocytes after pretreatment of the spermatozoa with PAF compared with 39% of oocytes with decondensed spermatozoa following incubation in HSA. Pretreatment of human

Fig. 4. (a) The effect of PAF (10^{-14} mol l^{-1}) on the number of zona-free hamster oocytes penetrated by human spermatozoa (** $P < 0.0001$; unpaired t test) and (b) on the number of decondensed sperm heads observed per oocyte (** $P = 0.003$; unpaired t test) during the sperm penetration assay (SPA). Twenty-five hamster oocytes were incubated with an aliquot of PAF-treated or untreated spermatozoa. Bars represent means $\pm$ SEM for six samples.

Fig. 5. The effect of PAF (10^{-14} mol l^{-1}), human follicular fluid (FF 1:4 final dilution) and progesterone (50 ng ml^{-1}) on the number of human spermatozoa penetrating hamster oocytes. Twenty-five oocytes were assessed with treated or untreated spermatozoa from the number of donors shown in parentheses above each bar. Bars represent means $\pm$ SEM; asterisks indicate significant differences between experimental and control groups ($P < 0.001$).

spermatozoa with PAF also increased the number of decondensed spermatozoa heads per egg 2.5-fold (Fig. 4b) compared with HSA controls ($P < 0.003$ Student's t test).

In a subsequent study, the effect of PAF (10^{-14} mol l^{-1} for 30 min) on oocyte penetration was compared with the effects of human follicular fluid (FF; 1:4 final dilution) and 50 ng progesterone ml^{-1} (Fig. 5). Treatment of human spermatozoa

Table 1. The effect of calcium on the percentage of oocytes containing decondensed sperm heads during the spermatozoa penetration assay (SPA) and on the percentage of acrosome-reacted (AR) spermatozoa after PAF treatment^a

Event	Control	+ Ca ²⁺ FF ^b	PAF ^b	Control	– Ca ²⁺ FF	PAF
AR (% reacted)	2.5 ± 2.0 ^c	21.0 ± 1.4 ^d	23.0 ± 0.9 ^d	3.0 ± 0.4	12.5 ± 1.3 ^{de}	2.5 ± 0.4 ^e
SPA (% penetrated)	33.3 ± 2.1	73.8 ± 2.6 ^d	69.8 ± 1.8 ^d	14.5 ± 1.8 ^e	23.2 ± 1.1 ^{de}	21.7 ± 1.7 ^{de}

^aSpermatozoa from the same donor were analysed in triplicate on three separate occasions.

^bFF: follicular fluid; PAF: platelet-activating factor.

^cResults are presented as means ± SEM.

^dExperimental value was significantly different from control value ($P < 0.01$; Student's *t* test).

^eExperimental value in the absence of calcium is significantly different from the experimental value in the presence of calcium ($P < 0.01$; Student's *t* test).

with PAF, follicular fluid and progesterone all appeared to significantly enhance the number of hamster eggs penetrated (76%, 74% and 80%, respectively) compared with diluent-treated controls (24% of oocytes penetrated) when analysed using one-way analysis of variance and unpaired *t* tests with Bonferroni adjustments ($P < 0.001$ for PAF-, follicular fluid-, or progesterone-enhanced penetration compared with penetration of hamster oocytes by diluent-treated control spermatozoa). The effect of follicular fluid on enhancement of oocyte penetration did not appear to be significantly different from the effects of progesterone or PAF ($P > 0.05$).

Role of calcium in PAF effects

The effect of calcium on follicular fluid- and PAF-induced changes in the acrosome reaction and in the sperm penetration assay was assessed using spermatozoa that had undergone an overnight capacitation in BWB that contained calcium or was nominally calcium free (Table 1). Follicular fluid and PAF significantly increased ($P < 0.01$; Student's *t* test) the acrosome reaction, by 8.2- and 9.2-fold, respectively, in the presence of calcium. However, in the relative absence of calcium, follicular fluid increased the percentage of spermatozoa that had lost their acrosomes by four times, whereas PAF failed to induce any increase in the acrosome reaction compared with that seen in control spermatozoa incubated in BWB with or without calcium. The increase in the acrosome reaction induced by follicular fluid in the nominal absence of calcium was significantly less ($P < 0.01$; Student's *t* test) than the acrosome reaction that was induced by follicular fluid in the presence of calcium.

When the effect of calcium on follicular fluid- and PAF-induced changes in the sperm penetration assay was examined (Table 1), the percentage of eggs containing decondensed spermatozoa heads increased 2.2-fold and 2.1-fold, respectively, in the presence of calcium. There was no significant difference between changes induced by follicular fluid in the sperm penetration assay and PAF-induced increases. In the absence of calcium, addition of follicular fluid and PAF also significantly increased ($P < 0.01$; Student's *t* test) the percentage of eggs containing decondensed spermatozoa heads compared with control samples incubated in BWB that did not contain calcium (1.6-fold and 1.5-fold increases after FF and PAF treatment). However, the percentage of eggs containing decondensed

spermatozoa heads was significantly lower ($P < 0.01$) in the nominal absence of calcium compared with the percentage in the presence of calcium after addition of both follicular fluid and PAF. Control values in the absence of calcium were also significantly lower ($P < 0.01$) than control values in the presence of calcium.

Evaluation of the PAF-induced acrosome reaction by electron microscopy

Treatment of spermatozoa with physiological concentrations of PAF induced changes in the acrosomal region of the spermatozoa head that appeared to be morphologically normal (Fig. 6) and were similar to those seen using follicular fluid (data not shown). Twenty-three per cent of the PAF-treated spermatozoa, 19% of the HFF, and 4% of the nontreated spermatozoa were acrosome reacted as confirmed with TEM and lectin binding.

Discussion

Platelet-activating factor is a unique alkyl-ether phosphocholine that appears to be involved in a wide spectrum of physiological events. Not only does it influence various pathophysiological responses associated with the inflammatory cascade as well as a multiplicity of physiological processes, but PAF also appears to play a significant role in most aspects of human reproduction. In the female, PAF modulates reproductive events from ovulation to parturition. Although less is known regarding the role of PAF in male reproduction, PAF is thought to be present in spermatozoa (Kumar *et al.*, 1988; Minhas *et al.*, 1988; Angle *et al.*, 1991) and is involved in certain aspects of sperm function including induction of the acrosome reaction (this study), the ability of spermatozoa to penetrate zona free hamster eggs (this study) and possibly spermatozoa motility (Ricker *et al.*, 1989; Helstrom *et al.*, 1991), although there are contradictory data regarding this final point (Angle *et al.*, 1991).

A number of variables may account for the reported differences in the literature with regard to the role of PAF in mediating sperm function. The method of isolating and quantifying PAF before use may modify its perceived dose-response effects. In addition, there appears to be a great deal of inter-laboratory variation regarding the source and concentration of

Fig. 6. Morphology of the human spermatozoon acrosome (a) before stimulation and (b) after stimulation by PAF (10^{-14} mol l⁻¹).

albumin used to resuspend PAF after purification. Finally, simple factors, such as the use of glass tubes or pipettes during the assay, may decrease the actual amount of PAF exposed to cells or tissues during an assay, as a consequence of the avidity of PAF binding to glass, thus affecting the apparent dose of biologically active PAF. Procedural variations between laboratories and the narrow range of PAF effectiveness may therefore account for reported differences in effects of PAF on sperm function.

PAF also appears to play a vital role in fertilization. Admixture of spermatozoa with PAF antagonists prior to artificial insemination does inhibit fertilization when structural analogues of PAF are used (Harper *et al.*, 1989). Furthermore, addition of PAF enhances the fertilization rate of mouse oocytes *in vitro* (Kuzan *et al.*, 1990; Minhas *et al.*, 1989) and administration of CV3988, one of the structural analogues of PAF (Minhas *et al.*, 1989) or WEB 2086 (Kuzan *et al.*, 1990), significantly decreases fertilization rates *in vitro*. As shown in this study, preincubation of human spermatozoa with PAF during the spermatozoa penetration assay increased both the number of decondensed sperm heads found per egg and the number of eggs containing decondensed spermatozoa. As spermatozoa from subfertile men contain significantly different amounts of PAF from those of normal donors (Angle *et al.*, 1991), these data support a role for PAF in normal sperm function and implicate a direct role for PAF in the fertilization process.

PAF that participates in the fertilization process may arise from one of several sources. PAF may come from the spermatozoa, since at least three groups have demonstrated that spermatozoa contain measurable amounts of PAF (Kumar *et al.*, 1988; Minhas *et al.*, 1988; Angle *et al.*, 1991). Thus, PAF present in spermatozoa may play a primary role in intracellular biochemistry of spermatozoa or may be released to act as a paracrine or autocrine mediator. Alternatively, PAF may arise from some component of the maternal system such as the uterus or ovary. Thus, although several studies have suggested that PAF is rarely found in either mouse or human unfertilized oocytes (Angle *et al.*, 1988b; Collier *et al.*, 1990; Punjabi *et al.*, 1990), PAF may be produced by the cumulus cells surrounding the newly ovulated oocyte in concentrations high enough to induce a physiological response in the spermatozoa as they transverse towards the zona pellucida. PAF has also been detected in uterine tissue of rats (Yasuda *et al.*, 1986), rabbits (Yasuda *et al.*, 1986; Angle *et al.*, 1988a) and humans (Alecozay *et al.*, 1989) and specifically in stromal but not glandular cells of the human uterus (Alecozay *et al.*, 1989). Consequently several different reproductive tissues and cell types apparently synthesize PAF.

However, even though PAF has been detected in several reproductive tissues, the kinetics of intracellular PAF synthesis, release and degradation remains relatively unknown. In some cell types, much of the intracellular PAF appears to remain within the cell rather than being released into the extracellular environment (Braquet *et al.*, 1987). Thus, although PAF has been found in spermatozoa, follicular fluid, the ovary and the uterus, whether the PAF from any of these sources may affect other cells of the reproductive tract *in vivo* remains relatively unexplored. Consequently, the role of PAF in intercellular communication, and thus its regulatory role in the fertilization process, has yet to be defined.

The precise mechanism whereby PAF induces an acrosomal response in spermatozoa is also unknown, but may be mediated via a membrane-associated PAF receptor. PAF receptors have been demonstrated in other cell types: high-affinity receptors have been found in human and rabbit platelets; human, bovine and guinea-pig neutrophils; rabbit and guinea-pig ileum; and human and guinea-pig lung membrane preparations (for references, see reviews by O'Flaherty, 1987 and Braquet *et al.*, 1987). It has been proposed that there are two PAF receptors in the neutrophil, one specific for acyl-PAF derivatives and one for alkyl-PAF derivatives (Levi *et al.*, 1989). Furthermore, monovalent as well as divalent cations and CTP have been shown to enhance the specific binding of PAF to its receptor in the platelet membrane system (Hwang *et al.*, 1986).

The data presented here reveal that the effects of PAF on the acrosome reaction and spermatozoa penetration of hamster oocytes are, at least in part, mediated by calcium, as the PAF-induced acrosome reaction was completely inhibited and spermatozoa penetration significantly depressed in the nominal absence of calcium in this study. Furthermore, while the acrosomal response to follicular fluid was not completely eliminated by removing calcium from the culture media, it was significantly decreased. This result suggests that the response to follicular fluid may also be regulated by calcium. The continued effect of follicular fluid on the acrosome response in the nominal absence of calcium may reflect additional exogenous calcium present in the follicular fluid itself. Thus PAF and human follicular fluid

may mediate the human acrosome reaction by similar calcium-dependent mechanisms.

PAF stimulates other known target cells to take up extracellular calcium, alter their concentration of cAMP in such cells, and turn over phosphatidylinositols (for review see O'Flaherty, 1987). PAF also stimulates GTPase activity in human platelets or their isolated membranes (Avdonin *et al.*, 1985). Many actions of PAF in other cell types require extracellular calcium and are inhibited by calcium antagonists (O'Flaherty, 1987). In addition to its role in the mobilization of extracellular calcium, PAF also induces the release of calcium from intracellular stores (Ingraham *et al.*, 1982). Consequently, it is possible that PAF may interact with specific receptors on the plasma membrane of the spermatozoa to induce a calcium influx, or mobilization of intracellular calcium, although definitive studies demonstrating an increase in intracellular calcium of spermatozoa after exposure to PAF have yet to be done.

In conclusion, these studies suggest that a better understanding of the role of PAF in the physiology of spermatozoa and spermatozoa-oocyte interactions may provide keys to new approaches to male contraception, and to the therapeutic use of PAF during the treatment of clinically subfertile spermatozoa during procedures such as *in vitro* fertilization.

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