Effect of platelet-activating factor on the electrophysiology of the human Fallopian tube: early mediation of embryo–maternal dialogue?

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Platelet-activating factor (PAF) is produced by preimplantation embryos and may be involved in the earliest stages of embryo–maternal dialogue. This study explored the potential effects of PAF acting as a signalling agent on human Fallopian tubal epithelial cells grown as a polarized layer in primary culture. The response of the tubal epithelium was assessed in terms of the transepithelial potential difference and short-circuit current (I_{sc}), which were recorded using a modified Ussing chamber. Resistance was calculated from the measurements of potential difference and I_{sc} . PAF (1.9 nmol to 1.9 μ mol l⁻¹) administered to the apical surface of the cells produced a marked, transient increase in both potential difference and I_{sc} in a dose-

dependent manner. The mode of action of PAF on the electrophysiological responses of human tubal epithelial cells was investigated. Blockers of Na⁺, K⁺ and voltage-operated Ca²⁺ channels had little effect on PAF action. However, incubation of the epithelial cells in Cl⁻-free medium or with a blocker of the Na⁺–K⁺–2Cl⁻ cotransporter (Furosemide) reduced the effect of PAF. Blockade of chloride–bicarbonate channels with 4-acetamido-4'-isothiocyano-2,2'-disulphonic acid (SITS) reduced the effect of low doses of PAF only. These results indicate that PAF influences the movement of chloride ions across the tubal epithelial cell and is a candidate molecule for initial embryo–maternal dialogue.

Introduction

The Fallopian tube is the site of ovum and sperm transport, fertilization and early embryo development. Tubal fluid, secreted by the epithelial cells lining the inner surface of the oviduct, provides the optimum environment for these processes, but the mechanism and regulation of its secretion are poorly understood (Leese *et al.*, 2001). The rate of secretion and composition of tubal fluid may vary to satisfy the different temporal requirements of the gametes and embryo. It is also possible that the gametes and embryos release signals that influence the function of tubal epithelial cells. For example, Ortiz *et al.* (1986) observed that hamster embryos were transported to the uterus faster than unfertilized oocytes indicating a role for local signals. One such candidate as a gamete/embryo–maternal signal is platelet-activating factor (PAF), which is produced by spermatozoa (Roudebush and Purnell, 2000) and by preimplantation embryos of mice (O' Neill, 1985), hamsters (Velasquez *et al.*, 1995) and cattle (Hansel *et al.*, 1989). Receptors for PAF are located in the endosalpinx of hamsters and mice (Stoddart *et al.*, 1996; Velasquez *et al.*, 1997, 2001; Lash and Legge, 2001). PAF has also been shown to increase

intracellular Ca²⁺ concentrations in cultured bovine oviduct endosalpingeal cells (Tiemann *et al.*, 1996) via influx of extracellular Ca²⁺. In other tissues, PAF action is mediated by prostaglandins (for a review, see Chao and Olson, 1993).

Fluid movements across epithelia are secondary to the movement of solutes, particularly ions. Such movement of ions results in an asymmetrical distribution of ionic charge and thus generates a transepithelial electrical potential difference that can be measured experimentally. Furthermore, the passage of ions across epithelia can be modulated using selective blockers of ion channels. Gott *et al.* (1988) and Dickens and Leese (1994) used a vascular perfusion technique to demonstrate that basal to apical movements of chloride ions were associated with tubal fluid secretion in the rabbit oviduct. These Cl⁻ fluxes were sensitive to blockade of Cl⁻–HCO₃⁻ exchange and Na⁺–K⁺–Cl⁻ cotransport (Gott *et al.*, 1988). This finding is consistent with the oviduct having a Cl⁻ secretory epithelium, as is also found in other tissues, such as the kidney proximal tubule and the airways (O'Grady *et al.*, 1987; Case *et al.*, 1989; Quinton, 1990). Rabbit and human oviduct epithelial cells, grown as a polarized layer in primary culture, also demonstrated greater chloride ion flux in the secretory (basal to apical) direction than in the absorptive (apical to basal) direction (Dickens *et al.*, 1993, 1996). In other studies it has been

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shown that the movement of chloride ions is a major factor in the generation of the transepithelial potential difference across cultured human Fallopian tubal epithelial cells and that agonists, such as extracellular ATP or PAF, are potent modulators of electrophysiological activity (Downing *et al.*, 1997, 1999; Reischl *et al.*, 1999, 2000). Downing *et al.* (1999) demonstrated that PAF has a greater effect on transepithelial potential difference and I_{SCC} when it is applied to the apical surface of the tubal epithelial cells rather than to the basal surface; this finding is consistent with a putative role as a gamete/embryo–maternal signalling molecule.

The present study examined the effect of blockers of Na^+ , K^+ , voltage operated Ca^{2+} and Cl^- channels on PAF-induced electrophysiological responses of primary cultures of human Fallopian tubal epithelial cells.

Materials and Methods

Human Fallopian tubes, at various stages of the menstrual cycle, were removed from pre-menopausal patients attending for hysterectomy at the Princess Royal Hospital (Hull). Permission was granted by the Hull and East Yorkshire Ethics and Clinical Trials Committee, and informed consent was always obtained from patients.

Fallopian tubes were donated by 27 women whose mean age was 40.3 ± 1.2 years (range 25–52 years). The patients were undergoing hysterectomy for menorrhagia, dysmenorrhoea, fibroids or sterilization. None of the patients had taken oral contraceptives or received other hormonal medication. Tubal disease had not been diagnosed. From the date of the last menstrual period, as given by the patient, Fallopian tubes were obtained from five patients who were in the follicular phase (days 1–14), ten patients who were in the luteal phase (days 15–28), two patients who were menstruating at the time of surgery and five patients who had not experienced menstruation for > 60 days (menopausal); the date of the last menstrual period was not available for four patients.

Epithelial cell culture

Epithelial cells were isolated according to the method of Dickens *et al.* (1993), which is a modification of methods devised by Glasser *et al.* (1988) and Kimber *et al.* (1993). Immediately after removal from the patient, the Fallopian tubes were washed in Hank's balanced salt solution without Ca^{2+} or Mg^{2+} (Ca^{2+} – Mg^{2+} –free HBSS, Gibco, Life Technologies Ltd, Paisley) and connective tissue was removed. The tubes were opened longitudinally, cut into 1 cm pieces and incubated in Ca^{2+} – Mg^{2+} –free HBSS containing 0.5% (w/v) type I trypsin (Sigma Chemical Co, Poole) and 2.7% (w/v) pancreatin (Gibco) for 1 h at 4°C. This procedure was followed by further incubation for 1 h at room temperature. The enzyme medium was removed, Ca^{2+} – Mg^{2+} –free HBSS added and the epithelial cells vortexed into suspension. After centrifugation at 250 *g* for 5 min, the cells were washed in Ca^{2+} – Mg^{2+} –free HBSS and centrifuged again

three times. After washing, cells were resuspended in pre-warmed, pre-gassed culture medium at a density of 1×10^6 cells ml^{-1} . Cell viability, as tested by the ability to exclude trypan blue (0.4%, w/v), was > 95%. Culture medium consisted of nutrient mixture F12 (Sigma) plus Dulbecco's modified Eagle's medium (DMEM) (1:1, v/v) (Sigma) containing 0.1% (w/v) BSA (ICN-Flow, Oxfordshire), 270.0 U penicillin ml^{-1} , 270.0 μg streptomycin ml^{-1} (Sigma), 20.0 μg fungizone ml^{-1} (Gibco), 2.5 mmol glutamine l^{-1} (Sigma), 2.5% (v/v) Nu-serum (ICN-Flow) and 2.5% (v/v) heat-inactivated fetal calf serum (Gibco). The cell suspension (250 μl) was placed in the top of Snapwell filters, 1.0 cm^2 , 0.4 μm pore size (Corning Costar Corporation, Cambridge, MA) coated with Pronectin F (Protein Polymer Technologies, Inc, San Diego, CA) in multiwell plates, and fresh medium was added under the filters. The cells were incubated in a humidified incubator at 37°C in 5% CO_2 in air. The medium above and below the filters was replaced every 48 h until the cells became confluent. Fallopian tubes from one patient usually yielded sufficient cells for 6–10 filters.

When the cells became confluent, the filters were placed in modified Ussing chambers (World Precision Instruments Inc, Sarasota, FL), and both surfaces of the cells were bathed with normal Krebs'–Ringer bicarbonate solution in 95% O_2 and 5% CO_2 at 37.5°C. The cells were alternately clamped at 0 mV for 5 s and +5 μA for 1 s using pairs of glass KCl electrodes. This procedure permitted almost simultaneous recording of short-circuit current (I_{SCC}) and potential difference. Resistance (R) was calculated from the measurements of potential difference and I_{SCC} . The normal Krebs'–Ringer bicarbonate solution contained: 118.0 mmol NaCl l^{-1} , 25.0 mmol NaHCO_3 l^{-1} , 4.74 mmol KCl l^{-1} , 1.19 mmol MgSO_4 l^{-1} , 1.17 mmol KH_2PO_4 l^{-1} , 1.17 mmol CaCl_2 l^{-1} , 5.5 mmol glucose l^{-1} gassed with 95% O_2 and 5% CO_2 . In the Cl^- -free medium, NaCl, KCl and CaCl_2 were replaced with sodium gluconate, potassium gluconate and calcium gluconate, respectively. Ion channel blocking agents were made up in normal Krebs'–Ringer (tetraethylammonium chloride (TEA)), methanol (verapamil) or DMSO (furosemide, amiloride) and 10 μl of stock solution was added to the bathing medium to achieve the appropriate final concentrations. SITS was made up in normal Krebs'–Ringer bicarbonate and 400 μl of the stock solution was added to produce the required final concentration in the bathing medium. PAF was made up in normal Krebs'–Ringer bicarbonate or Cl^- -free medium and added in 40 μl volumes to the apical bathing medium. The cells were washed in fresh Krebs'–Ringer bicarbonate and application of the same agonist was repeated after 20 mins, to prevent desensitization of the cells to agonists. When ion channel blocking agents were used, the cells were incubated in the presence of the blocker for 20 min before addition of PAF.

Where possible, one filter from the tubes of each patient was used as a control and a separate filter was used for each ion channel blocker/ Cl^- -free medium treatment group. Only one filter from each patient was allocated to each

Table 1. Resting values for potential difference (*PD*), short-circuit current (I_{sc}) and resistance (*R*) in cultured human Fallopian tubal epithelial cells

Day of menstrual cycle	Number of patients	<i>PD</i> (mV)	I_{sc} (μ A)	<i>R</i> (Ω cm ⁻²)
Days 1–14	5	5.4 $\pm$ 0.6	–28.7 $\pm$ 4.2	196.6 $\pm$ 18.9
Days 15–28	10	3.5 $\pm$ 0.3	–20.7 $\pm$ 2.3	178.8 $\pm$ 13.6
Menstruating	2	5.2	–18.3	287.4
Menopausal	5	3.6 $\pm$ 0.8	–18.4 $\pm$ 1.7	195.0 $\pm$ 25.4

Values are mean $\pm$ SEM.

Table 2. Change in potential difference (*PD*) and short-circuit current (I_{sc}) in response to platelet-activating factor (PAF) in cultured human Fallopian tubal epithelial cells

PAF (nmol l ⁻¹)	Parameter	Day of menstrual cycle		
		Days 1–14 (n = 4)	Days 15–28 (n = 4)	Other* (n = 6)
1.9	<i>PD</i> (mV)	1.0 $\pm$ 0.8	0.5 $\pm$ 0.3	0.4 $\pm$ 0.2
	I_{sc} (μ A)	5.4 $\pm$ 2.0	3.3 $\pm$ 2.0	1.2 $\pm$ 1.3
19.0	<i>PD</i> (mV)	0.6 $\pm$ 0.5	0.7 $\pm$ 0.3	1.0 $\pm$ 0.3
	I_{sc} (μ A)	3.7 $\pm$ 3.2	5.3 $\pm$ 1.6	5.5 $\pm$ 1.6
190.0	<i>PD</i> (mV)	1.0 $\pm$ 0.4	1.4 $\pm$ 0.2	1.3 $\pm$ 0.2
	I_{sc} (μ A)	7.8 $\pm$ 6.1	10.9 $\pm$ 3.2	7.8 $\pm$ 1.5
1900.0	<i>PD</i> (mV)	1.4 $\pm$ 0.3	1.9 $\pm$ 0.1	2.2 $\pm$ 0.5
	I_{sc} (μ A)	9.7 $\pm$ 2.3	18.1 $\pm$ 2.1	15.8 $\pm$ 4.7

Values are mean $\pm$ SEM.

*Other filters comprise menstruating (n = 2), menopausal (n = 2) and unknown day of menstrual cycle (n = 2).

treatment group. Each filter was used for one treatment only.

Amiloride, TEA, SITS, furosemide, verapamil and PAF were obtained from Sigma. Doses of ion channel blockers used in the present study were similar to those used in studies on airway, endometrial, kidney and other cultured epithelia (Gott *et al.*, 1988; Hanglow *et al.*, 1989; Zeitlin *et al.*, 1989; MacNaughton and Gall, 1991; Matthews *et al.*, 1993; Dickens and Leese, 1994; Jin and Hopfer, 1997; Deachapunya and O'Grady 1998) and were appropriate to produce selective blockade of the ion channel under investigation.

Results were analysed by Kruskal–Wallis test followed by Mann–Whitney U test (Dytham, 1999).

Results

Epithelial cells from human Fallopian tubes formed a polarized layer, which became confluent usually at days 5–6 of culture. The cells formed a rather leaky electrical system; mean values for resting potential difference, short-circuit current and resistance were 3.3 $\pm$ 0.2 mV, –22.3 $\pm$ 1.6 μ A and 165.8 $\pm$ 6.3 Ω cm⁻², respectively (n = 95 filters). The filters were used only if values for resistance of at least 100 Ω cm⁻² could be achieved. No difference in resting values was observed for tubes taken at various stages of the menstrual cycle (Table 1).

Effects of PAF on transepithelial potential difference and I_{sc}

Our previous study showed that PAF had a greater effect on potential difference and I_{sc} when applied to the apical surface of the epithelial cells rather than to the basal surface (Fig. 1). As in the previous study, PAF (1.9 nmol l⁻¹ to 1.9 μ mol l⁻¹, n = 14 filters) applied to the apical surface of the cells produced an increase in potential difference and I_{sc} in a dose-dependent manner (Fig. 2). There was no difference in response to PAF in epithelial cells taken from Fallopian tubes removed at different stages of the menstrual cycle (Table 2).

Effect of ion channel blockers on PAF action

Preincubation of the cultured epithelial cells for 20 min in Cl⁻-free medium significantly reduced the PAF-induced increase in potential difference and I_{sc} at all doses of PAF (Fig. 2) (potential difference: $P < 0.003$ at 1.9 nmol PAF l⁻¹, $P < 0.0004$ at 1.9 μ mol PAF l⁻¹; I_{sc} : $P < 0.01$ at 1.9 nmol PAF l⁻¹, $P < 0.0001$ at 1.9 μ mol PAF l⁻¹, Mann–Whitney U test) compared with untreated controls. Similarly, furosemide (100 μ mol l⁻¹), which blocks Na⁺–K⁺–2Cl⁻ cotransport, reduced the effect of PAF on potential difference ($P < 0.02$ at 1.9 nmol PAF l⁻¹, $P < 0.01$ at 19.0 nmol PAF l⁻¹ and $P < 0.02$ at 1.9 μ mol PAF l⁻¹) compared with the controls. I_{sc} response to PAF was reduced significantly at 19 nmol PAF l⁻¹ only ($P < 0.05$). SITS (1 mmol l⁻¹), which

Fig. 1. Change in transepithelial potential difference in cultured human oviductal cells with increasing concentration of platelet-activating factor (PAF) applied to either the apical (●, $n = 8$) or the basal surface (▲, $n = 7$) of the cells. Values are mean $\pm$ SEM. (Reproduced from *Biology of Reproduction* (1999) **61** 657–664 with kind permission from the Society for the Study of Reproduction).

blocks chloride–bicarbonate channels, reduced the PAF-stimulated increase in potential difference and I_{SCC} at low doses of PAF (potential difference: $P < 0.05$ at 1.9 nmol PAF l⁻¹; $P < 0.02$ at 19 nmol PAF l⁻¹) only, compared with controls. Preincubation with amiloride (10 $\mu\text{mol l}^{-1}$, Na⁺ channel blocker), TEA (25 mmol l⁻¹, K⁺ channel blocker) or verapamil (100 $\mu\text{mol l}^{-1}$, voltage-operated Ca²⁺ channel blocker) had little effect on PAF-induced increases in potential difference and I_{SCC} . The vehicles used to prepare solutions of the ion-blocking agents had no significant effect on the response of the epithelial cells to PAF (Fig. 3).

Discussion

Modulation of transepithelial permeability of ions occurs via alteration in the permeability of the tight junctions between cells or in the permeability of the apical and/or the basolateral membranes of the epithelial cells, resulting in a change in the rate or direction of movement of ions (Lewis *et al.*, 1995). Thus, agonists that alter ion permeability or movement across a secretory epithelium will influence the rate of fluid formation and secretion. Therefore, it is likely that PAF, which exerts its effects on tubal epithelial cell electrophysiology, modulates fluid formation and secretion in the human Fallopian tube. The present study has shown that the response of cultured human Fallopian tubal epithelial cells to apically applied PAF occurs primarily via changes in transepithelial flux of chloride ions. In secretory epithelia, chloride ion movements from the basal to apical poles of the cells play a significant role in providing the

driving force for the movement of fluid (O'Grady *et al.*, 1987; Case *et al.*, 1989; Quinton, 1990). Furosemide, which inhibits the Na⁺–K⁺–2Cl⁻ cotransporter was effective at inhibiting PAF-induced increases in potential difference and I_{SCC} in tubal epithelial cells in the present study. SITS, which blocks the Cl⁻–HCO₃⁻ antiporter, inhibited PAF action only at low concentrations of PAF (1.9–19.0 nmol PAF l⁻¹). At higher concentrations of PAF, activation of the Na⁺–K⁺–2Cl⁻ cotransporter may be sufficient to increase potential difference and, therefore, chloride ion movement, despite continued blockade of the Cl⁻–HCO₃⁻ antiporter. Verapamil was ineffective at inhibiting PAF-stimulated increases in potential difference and I_{SCC} , indicating that inward movement of Ca²⁺ is not required for initiation of Cl⁻ fluxes. It is possible that release of Ca²⁺ from intracellular stores is sufficient to ensure chloride ion movements.

The response of the cultured epithelial cells to PAF did not appear to be influenced by the stage of the menstrual cycle at the time of removal of the Fallopian tubes from the patient. However, it is likely that any influence of ovarian hormones may have diminished during culture of the epithelial cells. It is known that morphological features, such as cilia, are lost during culture (Bongso *et al.*, 1989; Henriksen *et al.*, 1990), although functional polarity is retained. Culture of the cells in the presence of oestrogen either prevents loss of cilia or induces their regrowth (Comer *et al.*, 1998).

Previous work from this laboratory has shown that, using this isolation method, primary cultures consist of cells that are epithelial in nature (Dickens *et al.*, 1996). Immunostaining showed the presence of secretory, basal and ciliated cells, with little or no contamination by stromal cells (Comer *et al.*, 1998). It would be expected that stromal cell contamination would prevent the formation of tight junctions between all cells, resulting in filters with low potential difference and resistance, which would not be used for experimentation. It is also likely that the different types of epithelial cell have different sensitivities to PAF and may be influenced to different extents by ovarian steroid hormones. It is not known whether the isolation procedure adversely affects the sensitivity of the cells, or the ion channels to PAF.

Tissue concentrations of PAF are governed by the equilibrium between biosynthesis and degradation by PAF–acetylhydrolase (Matsubara *et al.*, 1997). Uterine concentrations of PAF in the rabbit reach a peak of about 38 pmol g⁻¹ during early pregnancy (Angle *et al.*, 1988). In the human uterus, PAF concentrations are low, but are increased by progesterone and oestrogen (Alecozay *et al.*, 1989; 1991). Oviductal concentrations of PAF have not been determined; however, it is generally accepted that cells must be stimulated to produce PAF (Kasamo *et al.*, 1992). In other tissues, cells respond to PAF by producing more PAF in a positive feedback loop (Chao and Olson, 1993); thus local concentrations could be higher.

PAF is secreted by human spermatozoa. It is also secreted by the early developing embryos in hamsters (Velasquez

Fig. 2. (a) Change in potential difference with increasing concentration of platelet-activating factor (PAF) in cultured human Fallopian tubal epithelial cells in the absence (◆, $n = 14$) or presence of 100 $\mu\text{mol l}^{-1}$ furosemide (□, $n = 10$) or chloride-free medium (▲, $n = 11$). (b) Change in potential difference with increasing concentration of PAF in cultured human Fallopian tubal epithelial cells in the absence (◆, $n = 14$) or presence of 1 mmol 4-acetamido-4'-isothiocyano-stilbene-2,2'-disulphonic acid (SITS) l⁻¹ (■, $n = 10$) or 100 $\mu\text{mol l}^{-1}$ verapamil (△, $n = 10$). (c) Change in potential difference with increasing concentration of PAF in cultured human Fallopian tubal epithelial cells in the absence (◆, $n = 14$) or presence of 25 mmol tetraethylammonium chloride (TEA) l⁻¹ (○, $n = 10$) or 10 $\mu\text{mol l}^{-1}$ amiloride l⁻¹ (●, $n = 10$). (d) Change in short-circuit current with increasing concentration of PAF in cultured human Fallopian tubal epithelial cells in the absence (◆, $n = 14$) or presence of 100 $\mu\text{mol l}^{-1}$ furosemide l⁻¹ (□, $n = 10$) or chloride-free medium (▲, $n = 11$). (e) Change in short-circuit current with increasing concentration of PAF in cultured human Fallopian tubal epithelial cells in the absence (◆, $n = 14$) or presence of 1 mmol SITS l⁻¹ (■, $n = 10$) or 100 $\mu\text{mol l}^{-1}$ verapamil l⁻¹ (△, $n = 10$). (f) Change in short-circuit current with increasing concentration of PAF in cultured human Fallopian tubal epithelial cells in the absence (◆, $n = 14$) or presence of 25 mmol TEA l⁻¹ (○, $n = 10$) or 10 $\mu\text{mol l}^{-1}$ Amiloride l⁻¹ (●, $n = 10$). Values represent Fallopian mean $\pm$ SEM.

et al., 1995), cows (Hansel *et al.*, 1989) and mice (O'Neill, 1985). Rabbit embryos secrete PAF from the two-cell stage, producing nearly 40 ng PAF per embryo per 24 h on day 4 (Minhas *et al.*, 1993). Administration of PAF hastened the passage of oocytes to the uterus (Velasquez *et al.*, 1995),

whereas administration of PAF antagonists retarded the passage of embryos to the uterus. In the mouse, embryo-derived PAF promotes further embryonic development *in vitro* (Stoddart *et al.*, 1996) by a paracrine mechanism. Receptors for PAF have been found in oviduct epithelial

Fig. 3. (a) Change in potential difference and (b) change in short-circuit current with increasing concentration of platelet-activating factor (PAF) in cultured human Fallopian tubal epithelial cells in the absence (◆, $n = 14$) or presence of 2.5 μl methanol ml^{-1} (■, $n = 10$) or 2.5 μl DMSO ml^{-1} (△, $n = 10$). Values represent mean $\pm$ SEM.

cells in a number of species (Stoddart *et al.*, 1996; Velasquez *et al.*, 1997, 2001; Lash and Legge, 2001). Expression of mRNA encoding the PAF receptor is restricted to the endosalpinx and is most prominent in the sub-epithelial cells located in the mucosal folds that protrude into the lumen of the oviduct. This finding is in accord with the observation that PAF has greater effects on transepithelial potential difference when applied to the apical surface of tubal epithelial cells rather than to the basal surface. PAF released by gametes or embryos could influence the functions of tubal epithelial cells by stimulating chloride ion movement across the tubal epithelium and thereby increasing the rate of production, or modulating the composition, of oviductal fluid. PAF increases glycoprotein secretion in tracheal (Larivee *et al.*, 1994) and middle ear epithelial cells (Lin *et al.*, 1995), and may have a similar action on tubal epithelial cells. In this way gametes and embryos may control their immediate environment. Hunter (1994) suggested that, by increasing the viscosity of luminal fluid, oviduct glycoproteins could stabilize the micro-environments that surround the gametes and embryo, thus preventing dispersal of nutrients and ions. We propose that signal molecules, secreted in small amounts by the embryo, have a restricted, local action on the ciliated cells and smooth muscle to propel the embryo towards the uterus. Increased ciliary beat of hamster oviduct ciliated epithelial cells has been observed after addition of physiological concentrations of PAF *in vitro* (Hermoso and Villalon, 1995); however, whether PAF acts by increasing contractile activity of the myosalpinx is not known. If embryo-derived PAF acts on the myosalpinx, the endosalpinx may be acting as an intermediary in this embryo-maternal dialogue (Velasquez *et al.*, 2001). How such a signal received by tubal epithelial cells would be relayed to the

endometrium is unclear; however, PAF increases intracellular Ca^{2+} concentration in bovine oviduct epithelial cells by promoting influx of extracellular Ca^{2+} (Tiemann *et al.*, 1996) and also increases arachidonic acid and prostaglandin production by epithelial cells (for a review, see Chao and Olson, 1993). Both processes could be a mechanism for amplifying signals from the developing embryo.

The present study has shown that PAF, which appears to act predominately at the apical surface of the tubal epithelial cell, influences transepithelial potential differences by modulating Cl^{-} fluxes. Therefore, in humans, PAF may act as a signal from the gametes to the oviduct or the early embryo to the oviduct *in vivo*.

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References

- Alecozay AA, Casslen BG, Riehl RM, DeLeon FD, Harper MJK, Silva M, Nouchi TA and Hanahan DJ (1989) Platelet-activating factor (PAF) in human luteal phase endometrium *Biology of Reproduction* **41** 578–586
- Alecozay AA, Harper MJK, Schenken RS and Hanahan DJ (1991) Paracrine interactions between platelet-activating factor and prostaglandins in hormonally-treated human luteal phase endometrium *in vitro*. *Journal of Reproduction and Fertility* **91** 301–312
- Angle MJ, Jones MA, McManus LM, Pinckard RN and Harper MJK (1988) Platelet-activating factor in the rabbit uterus during early pregnancy *Journal of Reproduction and Fertility* **83** 711–722
- Bongso A, Ng SC, Fong CY, Anandakumar C, Marshall B, Edirisinghe R and Ratnam S (1992) Improved pregnancy rates after transfer of embryos grown in human Fallopian tubal co-culture *Fertility and Sterility* **58** 569–574
- Case RM, Lau KR, Steward M, Brown PD and Elliot AC (1989) Molecular mechanisms of transport: small and large molecules *Biochemical Society Transactions* **17** 803–805
- Chao W and Olson MS (1993) Platelet-activating factor: receptors and signal transduction *Biochemical Journal* **292** 617–629

- Comer MT, Leese HJ and Southgate J (1998) Induction of a differentiated ciliated cell phenotype in primary cultures of Fallopian tube epithelium *Human Reproduction* **13** 3114–3120
- Deachapunya C and O'Grady SM (1998) Regulation of chloride secretion across porcine endometrial epithelial cells by prostaglandin E_2 *Journal of Physiology (London)* **508** 31–47
- Dickens CJ and Leese HJ (1994) The regulation of rabbit oviduct fluid formation *Journal of Reproduction and Fertility* **100** 577–581
- Dickens CJ, Southgate J and Leese HJ (1993) Use of primary cultures of rabbit oviduct epithelial cells to study the ionic basis of tubal fluid formation *Journal of Reproduction and Fertility* **98** 603–610
- Dickens CJ, Comer MT, Southgate J and Leese HJ (1996) Human Fallopian tubal epithelial cells *in vitro*: establishment of polarity and potential role of intracellular calcium and extracellular ATP in fluid secretion *Human Reproduction* **11** 212–217
- Downing SJ, Maguiness SD, Watson A and Leese HJ (1997) Electrophysiological basis of human Fallopian tubal fluid formation *Journal of Reproduction and Fertility* **111** 29–34
- Downing SJ, Chambers EL, Maguiness SD, Watson A and Leese HJ (1999) Effect of inflammatory mediators on the electrophysiology of the human oviduct *Biology of Reproduction* **61** 657–664
- Dytham C (1999) The tests 1: test to look at differences. In *Choosing and Using Statistics A Biologist's Guide* pp 61–146 Blackwell Science Ltd, London, Edinburgh
- Glasser SR, Julian J, Decker GL, Tang J-P and Carson DD (1988) Development of morphological and functional polarity in primary cultures of immature rat uterine epithelial cells *Journal of Cell Biology* **107** 2409–2423
- Gott AL, Gray SM, James AF and Leese HJ (1988) The mechanism and control of rabbit oviduct fluid formation *Biology of Reproduction* **39** 758–763
- Hanglow AC, Bienenstock J and Perdue MH (1989) Effects of platelet-activating factor on ion transport in isolated rat jejunum *American Journal of Physiology* **257** G845–G850
- Hansel W, Stock A and Battista PJ (1989) Low molecular weight lipid-soluble luteotrophic factors produced by conceptuses in cows *Journal of Reproduction and Fertility Supplement* **37** 11–17
- Henriksen T, Tanbo T, Abyholm TH, Oppedal BR, Clausson OP and Hovig T (1990) Epithelial cells from human Fallopian tube in culture *Human Reproduction* **5** 25–31
- Hermoso MA and Villalon MJ (1995) Embryo-secreted factors increase the frequency of ciliary beat of hamster oviductal ciliated cells *in vitro*. *Biology of Reproduction* **52** S1–180
- Hunter RHF (1994) Modulation of gamete and embryonic microenvironments by oviduct glycoproteins *Molecular Reproduction and Development* **39** 176–181
- Jin W and Hopfer U (1997) Purinergic-mediated inhibition of Na^+K^+ -ATPase in proximal tubule cells: elevated cytosolic Ca^{2+} is not required *American Journal of Physiology* **272** C1169–C1177
- Kasamo M, Brandt M, Ishikawa M, Shimizu T and Harper MJK (1992) *In vitro* prostaglandin release from and platelet-activating factor accumulation in isolated endometrial cells from pregnant and pseudopregnant rabbits *Biology of Reproduction* **46** 829–845
- Kimber SJ, Waterhouse R and Lindenberg S (1993) *In vitro* models for implantation in the mammalian embryo. In *Preimplantation Embryo Development, Sero Symposium USA* pp 244–263 Ed. BD Bavister. Springer-Verlag, Amsterdam, New York
- Larivee P, Levine SJ, Martinez A, Wu T, Logun C and Shelhammer JH (1994) Platelet-activating factor induces airway mucin release via activation of protein kinase C: evidence for translocation of protein kinase C to membranes *American Journal of Respiratory Cell and Molecular Biology* **11** 199–205
- Lash GE and Legge M (2001) Localization and distribution of platelet activating factor receptors in the mouse ovary and oviduct during the estrous cycle and early pregnancy *American Journal of Reproduction and Immunology* **45** 123–127
- Leese HJ, Tay JI, Reischl J and Downing SJ (2001) Formation of Fallopian tubal fluid: physiology of a neglected epithelium *Reproduction* **121** 339–346
- Lewis SA, Berg JR and Kleine TJ (1995) Modulation of epithelial permeability by extracellular macromolecules *Physiological Reviews* **75** 561–589
- Lin J, Kim Y, Lees C and Juhn SK (1995) Effect of platelet-activating factor on secretion of mucous glycoprotein from chinchilla middle ear epithelial cells *in vitro*. *European Archives of Otorhinolaryngology* **252** 92–96
- MacNaughton WK and Gall DG (1991) Mechanisms of platelet-activating factor-induced electrolyte transport in the rat jejunum *European Journal of Pharmacology* **200** 17–23
- Matsubara T, Yasuda K, Johnston JM, Sanezumi M, Okada H, Matsuoka S and Kanzaki H (1997) Platelet-activating factor (PAF) and PAF acetylhydrolase activity in rat uterus and placenta during the late stages of pregnancy *Biology of Reproduction* **56** 885–890
- Matthews CJ, Thomas EJ, Redfern CPF and Hirst BH (1993) Ion transport by human endometrial epithelia *in vitro*. *Human Reproduction* **8** 1570–1575
- Minhas B, Zhu Y-P, Kim H-N, Burwinkel TH, Ripps BA and Buster JE (1993) Embryonic platelet activating factor production in the rabbit increases during the preimplantation phase *Journal of Assisted Reproduction and Genetics* **10** 366–370
- O'Grady SM, Palfrey HC and Field M (1987) Characteristics and functions of Na-K-Cl co-transport in epithelial tissues *American Journal of Physiology* **253** C177–C192
- O'Neill C (1985) Partial characterization of the embryo-derived platelet-activating factor in mice *Journal of Reproduction and Fertility* **75** 375–380
- Ortiz ME, Bedregal P, Carvajal MI and Croxatto HB (1986) Fertilized and unfertilized ova are transported at different rates by hamster oviduct *Biology of Reproduction* **34** 777–781
- Quinton PM (1990) Cystic fibrosis: a disease in electrolyte transport *FASEB Journal* **4** 2709–2717
- Reischl J, Downing SJ and Leese HJ (1999) Electrophysiological studies on bovine oviduct epithelial cells in primary culture *Journal of Reproduction and Fertility Abstract Series* **23** 24
- Reischl J, Downing SJ and Leese HJ (2000) Ion transport in bovine oviduct epithelial cells *Theriogenology* **53** 47
- Roudebush WE and Purnell ET (2000) Platelet-activating factor content in human spermatozoa and pregnancy outcome *Fertility and Sterility* **74** 257–260
- Stoddart NR, Wild AE and Fleming TP (1996) Stimulation of development *in vitro* by platelet activating factor receptor ligands released by mouse preimplantation embryos *Journal of Reproduction and Fertility* **108** 47–53
- Tiemann U, Neels P, Kuchenmeister U, Weels H and Spitschak M (1996) Effect of ATP and platelet-activating factor on intracellular calcium concentrations of cultured oviductal cells from cows *Journal of Reproduction and Fertility* **108** 1–9
- Velasquez L, Aguilera JG and Croxatto HB (1995) Possible role of platelet-activating factor (PAF) in embryonic signalling during oviductal transport in the hamster *Biology of Reproduction* **52** 1302–1306
- Velasquez LA, Ojeda SR and Croxatto CB (1997) Expression of platelet-activating factor receptor in the hamster oviduct: localization to the endosalpinx *Journal of Reproduction and Fertility* **109** 349–354
- Velasquez LA, Maisey K, Fernandez R, Valdes D, Cardenas H, Imrai M, Delgado J, Aguilera J and Croxatto HB (2001) PAF receptor and PAF acetylhydrolase expression in the endosalpinx of the human Fallopian tube: possible role of embryo-derived PAF in the control of embryo transport to the uterus *Human Reproduction* **16** 1583–1587
- Zeitlin PL, Wagner M, Markakis D, Loughlin GM and Guggino WB (1989) Steroid hormones: modulators of Na^+ absorption and Cl^- secretion in cultured tracheal epithelia *Proceedings National Academy of Sciences USA* **86** 2502–2505

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