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 ($I_{ss}$), which were recorded using a modified Ussing chamber. Resistance was calculated from the measurements of potential difference and $I_{ss}$. PAF (1.9 nmol to 1.9 μmol l$^{-1}$) administered to the apical surface of the cells produced a marked, transient increase in both potential difference and $I_{ss}$ 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 Ca$^{2+}$ 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'-isothiocyanostilbene-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.
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 \( I_{\text{sc}} \) 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 \( \text{Na}^+ \), \( \text{K}^+ \), voltage operated \( \text{Ca}^{2+} \) and \( \text{Cl}^- \) 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 menstruating at the luteal phase (days 15–28), two patients who were menstruating 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 \( \text{Ca}^{2+} \) or \( \text{Mg}^{2+} \) (\( \text{Ca}^{2+} \)-\( \text{Mg}^{2+} \)-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 \( \text{Ca}^{2+} \)-\( \text{Mg}^{2+} \)-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, \( \text{Ca}^{2+} \)-\( \text{Mg}^{2+} \)-free HBSS added and the epithelial cells vortexed into suspension. After centrifugation at 250 g for 5 min, the cells were washed in \( \text{Ca}^{2+} \)-\( \text{Mg}^{2+} \)-free HBSS and centrifuged again three times. After washing, cells were resuspended in pre-warmed, pre-gassed culture medium at a density of 1 \( \times \) 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 \( \mu \)l penicillin ml\(^{-1}\), 270.0 \( \mu \)g streptomycin ml\(^{-1}\) (Sigma), 20.0 \( \mu \)g 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 \mu l) was placed in the top of Snapwell filters, 1.0 cm\(^2\), 0.4 \( \mu \)m 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% \( \text{CO}_2 \) 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% \( \text{O}_2 \) and 5% \( \text{CO}_2 \) at 37.5°C. The cells were alternately clamped at 0 mV for 5 s and +5 \( \mu \)A for 1 s using pairs of glass KCl electrodes. This procedure permitted almost simultaneous recording of short-circuit current (\( I_{\text{sc}} \)) and potential difference. Resistance (\( R \)) was calculated from the measurements of potential difference and \( I_{\text{sc}} \). The normal Krebs’–Ringer bicarbonate solution contained: 118.0 mmol \( \text{NaCl} \) l\(^{-1}\), 25.0 mmol \( \text{NaHCO}_3 \) l\(^{-1}\), 4.74 mmol \( \text{KCl} \) l\(^{-1}\), 1.19 mmol \( \text{MgSO}_4 \) l\(^{-1}\), 1.17 mmol KH\(_2\)PO\(_4\) l\(^{-1}\), 1.17 mmol CaCl\(_2\) l\(^{-1}\), 5.5 mmol glucose l\(^{-1}\) gassed with 95% \( \text{O}_2 \) and 5% \( \text{CO}_2 \). In the \( \text{Cl}^- \)-free medium, \( \text{NaCl} \), 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 (tetraethyl-ammonium chloride (TEA)), methanol (verapamil) or DMSO (furosemide, amiloride) and 10 \( \mu \)l 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 \( \mu \)l 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 \( \text{Cl}^- \)-free medium and added in 40 \( \mu \)l 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/\( \text{Cl}^- \)-free medium treatment group. Only one filter from each patient was allocated to each
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, $-22.3 \pm 1.6$ μA 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).

<table>
<thead>
<tr>
<th>Day of menstrual cycle</th>
<th>Number of patients</th>
<th>PD (mV)</th>
<th>$I_{sc}$ (μA)</th>
<th>R (Ω cm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days 1–14</td>
<td>5</td>
<td>5.4 ± 0.6</td>
<td>-28.7 ± 4.2</td>
<td>196.6 ± 18.9</td>
</tr>
<tr>
<td>Days 15–28</td>
<td>10</td>
<td>3.5 ± 0.3</td>
<td>-20.7 ± 2.3</td>
<td>178.8 ± 13.6</td>
</tr>
<tr>
<td>Menstruating</td>
<td>2</td>
<td>5.2</td>
<td>-18.3</td>
<td>287.4</td>
</tr>
<tr>
<td>Menopausal</td>
<td>5</td>
<td>3.6 ± 0.8</td>
<td>-18.4 ± 1.7</td>
<td>195.0 ± 25.4</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

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

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

Values are mean ± SEM.

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

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 μmol 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 μmol PAF l$^{-1}$; $I_{sc}$: $P < 0.01$ at 1.9 nmol PAF l$^{-1}$, $P < 0.0001$ at 1.9 μmol PAF l$^{-1}$, Mann–Whitney U test) compared with untreated controls. Similarly, furosemide (100 μmol 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 19.0 μmol 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
blocks chloride–bicarbonate channels, reduced the PAF-stimulated increase in potential difference and $I_{\text{sc}}$ 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 mmol l$^{-1}$, Na$^+$ channel blocker), TEA (25 mmol l$^{-1}$, K$^+$ channel blocker) or verapamil (100 mmol l$^{-1}$, voltage-operated Ca$^{2+}$ channel blocker) had little effect on PAF-induced increases in potential difference and $I_{\text{sc}}$. 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 $I_{\text{sc}}$ in tubal epithelial cells in the present study. SITS, which blocks the Cl$^-$–HCO$_3^-$ 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$^-$–HCO$_3^-$ antiporter. Verapamil was ineffective at inhibiting PAF-stimulated increases in potential difference and $I_{\text{sc}}$, indicating that inward movement of Ca$^{2+}$ is not required for initiation of Cl$^-$ fluxes. It is possible that release of Ca$^{2+}$ 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; Quinton, 1990). 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...
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. 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 μmol furosemide l⁻¹ (▲, n = 11) or chloride-free medium (□, n = 10). (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 μmol verapamil l⁻¹ (△, 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 μmol 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 μmol furosemide l⁻¹ (▲, n = 11) or chloride-free medium (□, n = 10). (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 μmol 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 μmol Amiloride l⁻¹ (●, n = 10). Values represent Fallopian mean ± 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 subepithelial 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 microenvironments 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 Ca²⁺ concentration in bovine oviduct epithelial cells by promoting influx of extracellular Ca²⁺ (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
Chao W and Olson MS (1993) Platelet-activating factor: receptors and signal transduction Biochemical Journal 292 617–629

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 ± SEM.
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