Electrophysiological basis of human Fallopian tubal fluid formation

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A preparation for the maintenance of human Fallopian tubal epithelial cells as a polarized layer in primary culture was used to study the electrophysiological basis of tubal fluid formation in terms of the movement of Na^+ , K^+ and Cl^- ions. Transepithelial potential difference (PD) and short-circuit current (I_{sc}) were recorded by mounting the epithelial cells in a modified Ussing chamber. Resistance (R) was calculated from the measurements of PD and I_{sc} . The epithelia, although confluent, formed a 'leaky' electrical system and resistances greater than $300 \Omega \text{ cm}^{-2}$ were rarely achieved. The sodium channel blocker, amiloride ($100 \mu\text{mol l}^{-1}$), produced only small effects on PD and I_{sc} . The potassium channel blocker, tetraethylammonium chloride (TEA) (25 mmol l^{-1}), also produced small, but significant changes in PD , I_{sc} and R while the chloride channel blocker, 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS) (1 mmol l^{-1}), induced a marked increase in PD and I_{sc} , and a fall in R , when added to the basal surface of the cells. Bathing the apical surface of the cells with chloride-free medium also produced a marked increase in PD , I_{sc} and R ; bathing the basal surface of the cells with chloride-free medium produced a marked decrease in PD and I_{sc} . Extracellular ATP ($10 \mu\text{mol l}^{-1}$), added to either the apical or the basal surface of the cells, induced a transient increase in PD and I_{sc} and a decrease in R . Amiloride, TEA or furosemide had no effect on the response of the cells to ATP. SITS, applied to the apical surface, significantly reduced the response of the cells to ATP. We conclude that the major driving force for human tubal fluid formation is the transepithelial secretion of chloride ions into the oviduct lumen and that exogenous ATP is a potential modulator of secretion.

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, 1988). Fluid movements across epithelia are secondary to the movement of solutes, particularly ions. For understanding the basis of human tubal fluid formation, it is necessary to define which ion(s) moves preferentially in a secretory direction, that is from the basal to the apical side of the epithelial cells that border the lumen. Using a vascular perfusion technique, Gott *et al.* (1988) and Dickens and Leese (1994) found that basal to apical movements of chloride ions were associated with tubal fluid secretion in the rabbit oviduct. These chloride fluxes were sensitive to blockade of $\text{Cl}^-/\text{HCO}_3^-$ exchange and $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransport (Gott *et al.*, 1988) consistent with the oviduct being a Cl^- secretory epithelium as is found in other tissues such as the kidney proximal tubule and the airways. Dickens *et al.* (1993) then devised a method for maintaining rabbit oviduct epithelial cells as a polarized layer in primary culture. In this technique, oviducts were slit open and exposed to an enzyme digest medium and the epithelial cells released

were grown on collagen-impregnated permeable supports. The significance of this method is that the epithelial cells are maintained in their proper spatial orientation. Their basal surface, which in the intact tissue would be in contact with extracellular fluid, rests on the filter, and their apical surface, which borders the lumen *in vivo*, is exposed to the upper medium. Since the media on both sides of the cells are accessible, it is possible to mount the preparation in an Ussing chamber and to study transepithelial ionic and electrical events. Use of this preparation showed that chloride ion flux in the secretory (basal to apical) direction was greater than in the absorptive (apical to basal) direction and that transepithelial electrical potential difference (PD) increased in response to adrenergic agonists. Dickens *et al.* (1996a) then showed that human tubal epithelial cells could also be grown in a polarized manner.

Extracellular ATP is a potent Cl^- secretagogue in airway epithelial cells and has been shown to regulate chloride secretion in a variety of tissues (Chan *et al.*, 1996, 1997; Gorodeski *et al.*, 1996; Hwang *et al.*, 1996; Yamaya *et al.*, 1996). ATP also induces increases in intracellular calcium in epithelia, including airways (Mason *et al.*, 1991; Chan *et al.*, 1996, 1997), and oviduct (Cox and Leese, 1995; Dickens *et al.*, 1996a, 1996b). In cystic fibrosis, epithelial cells characteristically show reduced chloride secretion and thus reduced fluid secretion. The use of ATP and UTP as therapeutic agents in cystic fibrosis has been suggested (Hwang *et al.*, 1996).

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We have now used ion transport inhibitors in our polarized cell preparation to examine the electrophysiological basis of human tubal fluid formation in terms of Na^+ , K^+ and Cl^- ion movements and the response to extracellular ATP.

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 obtained.

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). The tubes were washed in Hank's balanced salt solution without calcium and magnesium (Ca- and Mg-free HBSS, Gibco, Life Technologies Ltd, Paisley) and connective tissue was removed. The tubes were opened longitudinally, chopped into 1 cm pieces and incubated in Ca- and Mg-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, followed by a further incubation for 1 h at room temperature. The enzyme medium was removed, Ca- and Mg-free HBSS added and the epithelial cells vortexed into suspension. After centrifugation at 250 g for 5 min, the cells were washed three times in Ca- and Mg-free HBSS. After centrifugation, cells were resuspended in prewarmed gassed culture medium at a density of 1×10^6 cells ml^{-1} . Cell viability, tested by the ability to exclude Trypan blue (0.4% w/v), was more than 95%.

For electrophysiological studies, 250 μl of the cell suspension was placed in the top of Snapwell filters, 1 cm^2 , 0.4 μm pore size (Corning Costar Corporation, Cambridge, MA) coated with Pronectin F (Protein Polymer Technologies, Inc., San Diego) in multiwell plates, and fresh medium was added under the filters. The cells were incubated in a humidified incubator at 37°C, gassed with 5% CO_2 in air. The medium was replaced above and below the filters every 48 h. When the cells became confluent, the filters were placed in modified Ussing chambers (World Precision Instruments, Inc., Sarasota), with both surfaces of the cells bathed with normal Krebs Ringer solution and gassed with 95% O_2 :5% CO_2 , at 37.5°C. Pairs of glass KCl electrodes were used to clamp the cells electrically alternately at 0 mV for 5 s and 5 μA for 1 s. This procedure permitted almost simultaneous recording of potential difference (PD), and short circuit current (I_{sc}). Resistance was calculated from the measurements of PD and I_{sc} . The normal Krebs Ringer bicarbonate solution contained: 118 mmol NaCl l^{-1} , 25 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} , 1 mmol glucose l^{-1} and was gassed with 95% O_2 :5% CO_2 . In chloride-free medium, Cl^- ions were replaced with gluconate. Drugs were made up in normal Krebs Ringer bicarbonate or chloride-free medium and added in volumes of 40 μl to either the basal or apical bathing medium and remained in the bathing medium for at least 30 min. The cells were washed and 20 min

Table 1. Response of cultured human Fallopian tube epithelial cells to ATP (10 μmol l^{-1})

Treatment	<i>n</i>	PD (mV)	I_{sc} (μA)	<i>R</i> (Ω cm^{-2})
Control	18	3.2 ± 0.3	-11.5 ± 1.11	96.0 ± 18.8
ATP, apical	18	$8.6 \pm 1.0^{***}$	$-47.4 \pm 5.8^{***}$	166.7 ± 13.0
Control	18	4.0 ± 0.4	-14.5 ± 1.3	207.7 ± 20.8
ATP, basal	18	$8.6 \pm 1.1^{***}$	$-40.6 \pm 4.7^{***}$	195.8 ± 18.8

*** $P < 0.001$ compared with control value, unpaired Student's *t* test.

allowed to elapse before application of the same agonist was repeated to prevent desensitization of the cells to agonists.

Amiloride, tetraethylammonium chloride (TEA), 4-acetamido-4'-isothiocyano-stilbene-2,2'-disulfonic acid (SITS), and furosemide were obtained from Sigma Chemical Co. Doses of ion channel blockers used in this study were similar to those used in studies on airway, endometrial, kidney and other cultured epithelia.

Results were analysed statistically by paired or unpaired Student's *t* tests.

Results

Epithelial cells from human Fallopian tubes formed a polarized layer, which became confluent usually after 5 to 6 days of culture. Although confluent, the cells formed a rather leaky electrical system; mean values for resting potential difference, short circuit current and resistance were 3.2 ± 0.2 mV, 18.9 ± 1.0 μA and 177.5 ± 7.0 Ω cm^{-2} , respectively ($n = 68$ filters). The filters were used only if values for resistance of at least approximately 100 Ω cm^{-2} could be achieved. The cells responded to ATP (10 μmol l^{-1}) applied either to the apical or the basal surface; there was a marked transient increase in PD and I_{sc} and a small decrease in resistance (Table 1). The peak response to ATP applied to the apical surface was PD: 168% increase ($P < 0.001$), I_{sc} : 312% increase, ($P < 0.001$) and *R*: 14.9% decrease. When ATP was applied to the basal surface the peak response was PD: 115% increase ($P < 0.001$), I_{sc} : 180% increase ($P < 0.001$) and *R*: 5.7% decrease. No effect of the stage of the menstrual cycle on resting PD and I_{sc} or on response to agonists was observed.

Effect of ion channel blockers on PD, I_{sc} and resistance

The sodium channel blocker, amiloride (10 μmol l^{-1}), applied to the basal surface of the epithelial cell layer, produced a significant increase in PD only ($P < 0.02$) (Table 2). When applied to the apical surface, a significant decrease in I_{sc} was observed ($P < 0.001$).

The potassium channel blocker, TEA (25 mmol l^{-1}) applied to the apical surface of the cells, induced a small but significant decline in PD ($P < 0.001$) and I_{sc} ($P < 0.001$) and a significant increase in *R* ($P < 0.05$) (Table 2). When applied to the basal cell surface, TEA induced an increase in PD ($P < 0.001$) and I_{sc} ($P < 0.001$) with a small reduction in *R* ($P < 0.05$).

The chloride channel blocker, SITS (1 mmol l^{-1}) added to the apical surface of the cell layer had little effect on PD, I_{sc} or

Table 2. Effect of amiloride ($10 \mu\text{mol l}^{-1}$) or tetraethylammonium chloride (TEA) (25mmol l^{-1}) on electrophysiological properties of cultured human Fallopian tube epithelial cells

Treatment	<i>n</i>	PD (mV)	I_{sc} (μA)	R (Ωcm^{-2})
Control	9	4.8 ± 0.7	-18.0 ± 2.1	211.1 ± 28.0
Amiloride, apical	9	4.4 ± 0.8	$-12.8 \pm 1.8^{***}$	250.1 ± 32.0
Control	5	4.5 ± 0.8	-14.8 ± 2.6	217.4 ± 18.7
Amiloride, basal	5	$6.2 \pm 1.1^{**}$	-17.8 ± 2.8	264.7 ± 14.2
Control	12	6.0 ± 0.4	-35.5 ± 3.3	152.4 ± 11.6
TEA, apical	12	$4.8 \pm 0.5^{***}$	$-25.5 \pm 2.9^{***}$	$169.0 \pm 14.3^*$
Control	12	4.9 ± 0.5	-25.2 ± 2.9	166.6 ± 11.5
TEA, basal	12	$6.1 \pm 0.5^{***}$	$-35.8 \pm 3.9^{***}$	$158.5 \pm 12.5^*$

* $P < 0.05$, ** $P < 0.02$, *** $P < 0.001$, compared with control value, paired Student's *t* test.

Fig. 1. Recordings of transepithelial potential difference (a) and short-circuit current (b) in cultured epithelial cells from human Fallopian tubes, during alternating voltage clamp at 0 mV for 5 s and current clamp at $5 \mu\text{A}$ for 1 s. Effect of 1 mmol 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS) l^{-1} applied to the basal surface of the cells.

R. When applied to the basal surface, SITS induced a multiphasic response consisting of an acute increase in PD ($P < 0.01$) and I_{sc} ($P < 0.01$), and a decrease in R ($P < 0.01$), followed by a slight decrease in PD and I_{sc} which was then followed by a more sustained increase in both PD and I_{sc} . After approximately 30 min, PD was still significantly raised ($P < 0.02$) but I_{sc} had declined to pre-SITS values (Fig. 1, Table 3).

Furosemide ($100 \mu\text{mol l}^{-1}$) had little effect when applied to the apical surface of the cells other than producing a small increase in R . Unlike SITS, furosemide did not elicit a biphasic response when applied to the basal surface of the cells, such that by 20–30 min, PD was unchanged; however, I_{sc} was significantly reduced ($P < 0.05$) and R was significantly increased ($P < 0.01$) (Table 3).

Furosemide ($100 \mu\text{mol l}^{-1}$) and SITS (1mmol l^{-1}) applied together to the apical surface of the cells had little effect other than a small, but significant increase in R ($P < 0.05$). Furosemide and SITS applied to the basal surface of the cells induced a transient increase in PD and I_{sc} , as with SITS alone. By 20–30 min, PD and I_{sc} values were similar to pretreatment values, although there appeared to be a small but significant increase in R ($P < 0.05$).

Effect of chloride-free medium

Replacing the medium bathing the apical surface of the cells with chloride-free medium resulted in an immediate fourfold increase in PD ($P < 0.01$) and a sixfold increase in I_{sc} ($P < 0.01$). Resistance was not significantly changed (Table 4, Fig. 2). By 20–30 min after application of chloride-free medium, PD had declined but was still three times higher than pretreatment values ($P < 0.01$). I_{sc} declined more rapidly although it remained 2.5 times higher than pretreatment values ($P < 0.05$). As a result, however, resistance increased significantly ($P < 0.002$) (Table 4).

Replacing the medium bathing the basal surface of the cells with chloride-free medium for 20 min produced a marked fall in PD to a negative value (Table 4, $P < 0.001$, Fig. 3) and the electrical polarity of I_{sc} also reversed ($P < 0.02$). When both surfaces of the cells were exposed to chloride-free medium, an increase in PD (Table 4, $P < 0.02$), and a marked increase in resistance ($P < 0.01$) were observed.

Effect of ion channel blockers and chloride-free medium on epithelial cell response to ATP

Amiloride, TEA or furosemide had no significant effect on the response of the epithelial cells to ATP ($10 \mu\text{mol l}^{-1}$), applied to either apical or basal surface. SITS, applied to the basal surface of the epithelial cells did not affect the response to ATP applied to the basal surface; however, when applied to the apical surface of the cells SITS significantly reduced the rise in PD and I_{sc} ($P < 0.05$) and the decrease in R ($P < 0.01$) normally observed in response to ATP applied apically (Table 5). Similarly, furosemide and SITS, applied together to the basal surface did not affect the response of the cells to ATP, but furosemide and SITS applied to the apical surface significantly

Table 3. Effect of 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS) (1 mmol l⁻¹), furosemide (100 µmol l⁻¹) or both drugs on electrophysiological properties of cultured human Fallopian tube epithelial cells

Treatment	n	PD (mV)	I _{sc} (µA)	R (Ω cm ⁻²)
Control	6	4.4 ± 1.2	- 24.0 ± 6.6	143.0 ± 12.8
SITS, basal, immediate	6	7.4 ± 1.9***	- 51.8 ± 12.9**	108.3 ± 22.3***
Control	6	4.2 ± 0.9	- 18.7 ± 2.9	176.6 ± 33.8
SITS, basal, 20 min	6	5.0 ± 1.0**	- 19.2 ± 2.5	219.9 ± 52.1
Control	10	4.5 ± 0.6	- 22.8 ± 3.1	165.1 ± 21.5
SITS, apical	10	4.2 ± 0.6	- 20.4 ± 2.5	173.8 ± 23.4
Control	10	4.9 ± 0.4	- 19.0 ± 1.8	211.6 ± 19.1
Furosemide, apical	10	5.2 ± 0.4	- 17.2 ± 2.4	243.9 ± 19.8**
Control	7	4.3 ± 0.4	- 18.3 ± 2.2	195.2 ± 20.8
Furosemide, basal	7	4.7 ± 0.5	- 14.4 ± 2.4*	246.3 ± 23.1***
Control	8	3.8 ± 0.5	- 17.0 ± 1.8	167.1 ± 18.1
Furosemide + SITS, apical	8	3.8 ± 0.6	- 14.8 ± 2.5	197.9 ± 27.7*
Control	7	3.2 ± 0.7	- 13.0 ± 2.2	170.6 ± 22.1
Furosemide + SITS, basal	7	3.4 ± 0.6	- 11.6 ± 1.7	204.9 ± 31.0*

*P < 0.05, **P < 0.02, ***P < 0.01 compared with control value, paired Student's *t* test.

Table 4. Effect of chloride-free medium on electrophysiological properties of cultured human Fallopian tube epithelial cells

Treatment	n	PD (mV)	I _{sc} S(µA)	R (Ω cm ⁻²)
Control	6	3.6 ± 0.7	- 13.8 ± 2.9	186.4 ± 22.3
Cl ⁻ free, apical, immediate	6	15.5 ± 2.0***	- 88.2 ± 13.9***	171.6 ± 13.8
Cl ⁻ free	6	11.1 ± 1.3***	- 36.7 ± 6.3*	279.5 ± 30.2 [†]
Control	6	4.9 ± 0.4	- 25.0 ± 3.2	170.0 ± 20.3
Cl ⁻ free, basal	6	- 1.4 ± 1.0 ^{††}	+ 15.2 ± 5.7**	159.6 ± 29.8
Control	4	3.5 ± 0.5	- 16.5 ± 2.6	162.3 ± 8.4
Cl ⁻ free, both sides	4	6.5 ± 0.5**	- 12.3 ± 0.5	374.8 ± 28.7***

*P < 0.05, **P < 0.02, ***P < 0.01, [†]P < 0.002, ^{††}P < 0.001, compared with control value, paired Student's *t* test.

reduced the response of the epithelial cells to ATP applied to the apical surface (Table 5). Chloride-free medium applied to either the apical or basal surface of the cells did not affect the response to ATP; however, bathing both surfaces of the cells with chloride-free medium significantly reduced the increase in PD in response to ATP applied to the apical surface of the cells.

Discussion

Previous studies using this culture method for human oviducts have demonstrated that a pure population of epithelial cells is obtained and that the cells form a polarized layer (Dickens *et al.*, 1995; Dickens *et al.*, 1996a). The oviduct epithelium is considered to be electrically 'leaky' (Leese, 1988), with high bidirectional fluxes of ions occurring, probably via paracellular junctions, and resistances above 300 Ω cm⁻² were rarely achieved. The role of ion fluxes in establishing the transepithelial PD and short circuit current was investigated using ion channel blockers. Amiloride, which inhibits Na⁺-H⁺ exchange, had a relatively small effect on PD and I_{sc} of the tubal epithelial cells, with maximum effects of the order of

a 10–15% change in PD only. This finding suggests that Na⁺-H⁺ exchange does not greatly influence transepithelial PD. TEA, a relatively non-specific blocker of K⁺ channels, showed greater, more consistent effects on the electrical properties of the cells. When applied to the apical surface, PD declined by 20% and I_{sc} by 30%; when applied to the basal surface, PD increased steadily by 30% and I_{sc} by 50% over 30 min. Movement of K⁺ ions may, therefore, play a role in controlling transepithelial PD. SITS, a blocker of Cl⁻-HCO₃⁻ exchange, had a marked initial effect when applied to the basal surface with the cells returning to equilibrium over 20–30 min. The effect of SITS was small when applied to the apical surface. Similarly, the influence of furosemide, a blocker of the Na⁺-K⁺-Cl⁻ transporter, was small at the apical surface of the cells. Unlike SITS, furosemide applied basally did not produce the marked, transient increase in PD and I_{sc}. After 30 min, however, furosemide had produced a significant increase in resistance, suggesting that movement of ions via the Na⁺-K⁺-Cl⁻ cotransporter was inhibited, particularly at the basal membrane. The effects of SITS and furosemide applied together were similar to those of SITS alone, at both the apical and basal surfaces, suggesting that there were no additive or

Fig. 2. Recording of transepithelial potential difference in cultured epithelial cells from human Fallopian tubes, during alternating voltage clamp at 0 mV for 5 s and current clamp at 5 μ A for 1 s. Effect of bathing the apical surface of the cells in chloride-free medium.

Fig. 3. Recording of transepithelial potential difference in cultured epithelial cells from human Fallopian tubes, during alternating voltage clamp at 0 mV for 5 s and current clamp at 5 μ A for 1 s. Effect of bathing the basal surface of the cells in chloride-free medium.

synergistic effects of the two Cl^- channel blockers. This finding suggests that Cl^- - HCO_3^- exchange plays an important role in determining transepithelial PD and that this activity occurs predominantly at the basal membrane.

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 fluid movement (O'Grady *et al.*, 1987; Case *et al.*, 1989; Quinton, 1990). A low intracellular Na^+ concentration is maintained by the Na^+ - K^+ -ATPase, with uptake of Cl^- via Na^+ - K^+ - Cl^- cotransporter occurring at the basolateral membrane. The apical membrane also becomes permeable to Cl^- , which allows the anion to move down an electrochemical gradient. The Cl^- ion movements generate an electrical force which drives Na^+ paracellularly through the tight junctions between cells into the lumen, creating a higher luminal osmotic pressure. Water

follows the ion movements towards the osmotic equilibrium and thus accumulates in the lumen (Quinton, 1990). Movements of chloride ions from basal to apical have been observed in the epithelium of the Fallopian tube consistent with it being a chloride secretory epithelium (Brunton and Brinster, 1971; Gott *et al.*, 1988; Dickens *et al.*, 1993, 1995) and our present data are in agreement with this. In vascularly perfused rabbit oviduct, inhibition of chloride ion transport decreased vascular to lumen Cl^- flux and reduced the rate of fluid secretion (Dickens and Leese, 1994).

The lack of effect of amiloride, in contrast to that observed in cultured human endometrial epithelium (Matthews *et al.*, 1993), suggests that Na^+ ion movements do not contribute substantially to the transepithelial potential difference in tubal epithelia. By contrast, the changes in transepithelial PD observed after TEA treatment suggest that K^+ movements play a significant role in generating the PD. TEA is a relatively non-specific blocker of K^+ channels; however, since furosemide had less effect on PD than did TEA, this would suggest that K^+ exerts its influence on transepithelial PD via channels other than the Na^+ - K^+ - Cl^- cotransporter. In this context, it is significant that James and Okada (1994) reported the presence of a Ca^{2+} -activated K^+ channel in the apical membrane of rabbit oviduct epithelial cells. It is likely that basal to apical movements of K^+ are important, since the concentration of this ion in oviduct fluid in a number of species is about three times that of plasma (Leese, 1988; Dickens and Leese, 1994). SITS appeared to have a marked, transient effect on transepithelial PD at the basal surface of the cells, suggesting, in human Fallopian tubal cells, that Cl^- - HCO_3^- exchange is an important short-term mediator of Cl^- movements. That the effect of SITS was transient suggests that alternative mechanisms of chloride uptake at the basal membrane can come into operation. Furosemide had no additive effects with SITS, nor did it prolong the effectiveness of SITS, suggesting that the control of ion movements via the Na^+ - K^+ - Cl^- co-transporter occurs later than via Cl^- - HCO_3^- exchange. The effects of chloride-free medium were more lasting; bathing the apical surface of the cells with chloride-free medium resulted in a fourfold increase in PD, presumably resulting from the increase in movement of Cl^- down the electrochemical gradient towards the apical surface; bathing the basal surface of the epithelial cells with chloride-free medium resulted in a marked reduction in transepithelial PD, such that a reversal of electrical polarity occurred. This finding suggests that the flux of Cl^- ions, in response to the reversed electrochemical gradient, becomes apical to basal, which would be characteristic of an absorptive epithelium or a volume-sensitive, osmoregulatory epithelium. These observations support the proposition that Cl^- movements, basal to apical, are a major factor determining transepithelial PD in normal Fallopian tube epithelial cells.

ATP induced a transient increase in PD and I_{sc} , as observed previously, in bovine (Cox and Leese, 1995) and human (Dickens *et al.*, 1996a) cultured oviduct cells. In the present study, we observed that the apical surface of the cells may be a little more sensitive to ATP than is the basal surface, as was found in endometrial epithelial cells (Chan *et al.*, 1997). ATP has been shown to influence the secretory activity of epithelial cells in a number of tissues (Mason *et al.*, 1991; Chan *et al.*,

Table 5. Effect of ion channel blockers or chloride-free medium on response of human Fallopian tube epithelial cells to ATP ($10 \mu\text{mol l}^{-1}$)

Treatment	n	Percentage change in parameter		
		PD (mV)	I_{sc} (μA)	R ($\Omega \text{ cm}^{-2}$)
ATP, apical	10	+ 151.1 $\pm$ 34.9	+ 301.9 $\pm$ 78.4	- 16.4 $\pm$ 2.6
SITS, apical, ATP, apical	10	+ 64.4 $\pm$ 19.3*	+ 103.4 $\pm$ 32.2*	- 6.7 $\pm$ 1.6***
ATP, apical	8	+ 139.7 $\pm$ 29.1	+ 299.1 $\pm$ 69.2	- 14.4 $\pm$ 3.1
SITS + furosemide, apical, ATP apical	8	+ 47.4 $\pm$ 14.8**	+ 87.7 $\pm$ 28.7**	- 8.4 $\pm$ 2.0
ATP, apical	4	+ 93.9 $\pm$ 16.7	+ 161.3 $\pm$ 27.1	- 12.2 $\pm$ 7.0
Cl ⁻ -free both sides, ATP apical	4	+ 43.1 $\pm$ 8.2*	+ 91.9 $\pm$ 19.7	- 9.4 $\pm$ 1.7

* $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$ compared with ATP response in the absence of ion channel blocker, unpaired Student's *t* test.
SITS: 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid.

1996; Gorodeski *et al.*, 1996; Hwang *et al.*, 1996; Yamaya *et al.*, 1996) acting via purinergic receptors. ATP was effective in the present system whether applied basally or apically. Neither amiloride nor TEA affected the response of the epithelial cells to ATP, suggesting that Na^+ and K^+ are not involved in this response, as was observed in endometrial epithelial cells (Chan *et al.*, 1997). SITS, with or without furosemide applied to the apical surface of the cells, reduced the response of the cells to apical ATP by a small, but significant amount. This finding suggests that Cl^- movement may be important in the effect of ATP on transepithelial PD. In other tissues, extracellular ATP has been shown to induce chloride secretion (Hwang *et al.*, 1996; Yamaya *et al.*, 1996). The enhanced chloride secretion has been shown to depend on an increase in intracellular Ca^{2+} concentration, either by release from intracellular stores or influx of extracellular Ca^{2+} (Mason *et al.*, 1991; Chan *et al.*, 1996, 1997; Yamaya *et al.*, 1996). A significant increase in intracellular Ca^{2+} has also been observed in response to ATP in cultured rabbit, bovine and human oviduct cells (Cox and Leese, 1995; Dickens *et al.*, 1996a,b). We have previously suggested that exogenous ATP, perhaps derived from the oviduct epithelium or from spermatozoa, promotes the secretion of glycoproteins and the activity of cilia (Dickens *et al.*, 1996a,b) and in this way regulates the environment for fertilization and early embryo development.

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