No effect of histamine on human bronchial epithelial cell

J.L. Devalia*, R.W.A. Godfrey**, R.J. Sapsford*,
N.J. Severs†, P.K. Jeffery**, R.J. Davies*

No effect of histamine on human bronchial epithelial cell permeability and tight junctional integrity in vitro. *J.L. Devalia, R.W.A. Godfrey, R.J. Sapsford, N.J. Severs, P.K. Jeffery, R.J. Davies. ©ERS Journals Ltd 1994.*
ABSTRACT: Both animal and human studies have suggested that histamine increases airway epithelial cell permeability *in vivo*. In order to study the effect of histamine on paracellular epithelial permeability and tight junctional integrity, we have cultured human bronchial epithelial cells to confluency and investigated the effect of topically applied 0.1–20.0 µM histamine.

Cultures were established on microporous membranes of tissue culture cell inserts and used for the assessment of: 1) transepithelial movement of radiolabelled mannitol (^{14}C-mannitol) and bovine serum albumin (^{14}C-BSA), in the luminal to serosal direction and 2) changes in electrical resistance of the cultures. Epithelial cell cultures were also established on plastic coverslips, in order to determine tight junction morphology by freeze-fracture electron microscopy, and to assess junctional integrity by lanthanum penetration, using thin sections.

Compared with untreated control cultures, 0.1–10 µM histamine did not significantly alter the movement of either ^{14}C-mannitol or ^{14}C-BSA across the epithelial cultures at any time during incubation, but caused an increase in the electrical resistance of the cultures, which was maximal by 6 h of incubation. The morphology of the tight junctions revealed by freeze-fracture and junctional integrity (the latter determined by the degree of lanthanum penetration) were similar in untreated control cultures and cultures incubated with histamine.

These studies indicate that histamine does not have a direct effect on paracellular bronchial epithelial permeability *in vitro*.
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*Dept of Respiratory Medicine, St. Bartholomew's Hospital, London, UK. **Dept of Lung Pathology, National Heart and Lung Institute, Royal Brompton Hospital, London, UK. †Dept of Cardiac Medicine, National Heart and Lung Institute, London, UK.

Correspondence: J.L. Devalia
Dept of Respiratory Medicine
St Bartholomew's Hospital
London EC1A 7BE
UK

Several studies have suggested that damage to the airway epithelium, causing either detachment of the cells or release of proinflammatory mediators by the cells, may play a role in the development of airway hyperreactivity [1–6]. Although there appears to be a close association between epithelial cell damage and hyperreactivity [7, 8], the specific mechanisms underlying the development of hyperreactivity are not well understood.

We and others have demonstrated that sputum obtained from patients undergoing acute exacerbations of asthma contain large amounts of histamine [9–11]. WHITE *et al.* [12] have reviewed the role of histamine in asthma and suggested that this biogenic amine plays a major contributory role in the various pathophysiological events which lead to airflow obstruction in asthma. Studies by BOUCHER *et al.* [13], have employed the movement of horseradish peroxidase across the airway epithelium as an index of increased permeability, and have demonstrated that histamine increases the permeability of airway epithelium in guinea-pigs *in vivo*. Similarly, studies in humans have demonstrated that histamine inhalation increases clearance of inhaled technetium-99m-labelled diethylenetriamine-pentaacetate (^{99m}Tc-DTPA) from the lungs, and suggested that this is a consequence of increased airway epithelial permeability [14, 15].

Studies in humans, however, have produced conflicting data with regard to the histamine receptor subtype responsible for this effect. Moreover, studies by CHEEMA *et al.* [16] have suggested that pulmonary clearance of ^{99m}Tc-DTPA may not be a suitable index for the study of bronchial epithelial permeability. These authors have investigated the binding and diffusion characteristics of ^{99m}Tc-DTPA in respiratory tract mucus-glycoprotein from patients with chronic bronchitis, and have demonstrated that this agent binds strongly to mucus and is, therefore, unlikely to move as freely and rapidly across the bronchial epithelium, as it does across the alveolar epithelium [16]. More recently, GODFREY and co-workers [17, 18], have characterized the freeze-fracture morphology of human bronchial epithelial tight junctions, which selectively control the paracellular movement of ions, macromolecules and water [17], and have demonstrated their lability in cystic fibrosis [18].

The aim of the present study was to investigate the effect of histamine on paracellular permeability of confluent human bronchial epithelial cell cultures *in vitro*, by examination of the luminal to serosal movement of radiolabelled markers and, in parallel, to assess the morphology by freeze-fracture and integrity of these junctions, in respect of the extent of their exclusion of luminal lanthanum.

Materials and methods

All chemicals and reagents were of tissue culture grade and, unless stated otherwise, were obtained from the Sigma Chemical Company (Dorset, UK).

Bronchial tissue

Bronchial tissue was obtained from 5 male and 4 female patients, mean age 61 yrs (range 41–74 yrs), who underwent lobectomy or pneumonectomy for lung cancer, at St Bartholomew's Hospital, London, UK. All patients were heavy smokers and were not known to be allergic to any common allergen, as indicated by patient history. Following resection, tissue which appeared to be macroscopically free of tumour was brought back to the laboratory and processed for tissue culture, within 0.5–1.0 h of resection.

Isolation, culture and identification of bronchial epithelial cells

Bronchial epithelial cells were cultured by an explant cell culture technique developed in our laboratory and described fully elsewhere [19]. Briefly, the epithelium was dissected away from the underlying tissue and following further dissection into smaller sections, approximately 1–2 mm^3 in size, was gently washed three times in prewarmed and pre-aerated sterile Medium 199 containing 1% (v/v) antibiotics/antimycotic solution. One or two sections were explanted into either 9 mm diameter Falcon® cell culture inserts, fused to transparent semipermeable (0.45 µm pore size) Cyclopore® polyethylene tetraphthalate track-etched membranes as the growth matrix (Becton Dickinson Ltd, Oxford, UK) or onto sterile Thermanox plastic coverslips as the growth matrix (Emitech Ltd, Ashford, Kent, UK), in 35 mm diameter Falcon® "Primaria" culture dishes (Becton Dickinson Ltd, Oxford, UK). Freshly prepared complete culture medium, 0.2 and 0.4 ml, containing antibiotics/antimycotic and several growth factors in Medium 199 [19] was added to each culture insert and insert well, respectively, and the explants were incubated at 37°C in a 5% CO_2 in air atmosphere. The explants in culture dishes were incubated at 37°C in a 5% CO_2 in air atmosphere in 1.5 ml of culture medium. All explants were observed for cell outgrowth after 2–3 days, and the medium was changed every 48 h until the cells had grown to confluency, normally by 2–3 weeks.

Epithelial phenotype was confirmed following examination of the cultures by: 1) light and electron microscopy and 2) indirect immunoperoxidase staining, using monoclonal antibody preparations CAM 5.2 (Becton Dickinson Ltd, Oxford, UK) and MCA 144 (Serotec, Oxford, UK). These antibodies detect the intermediate filament cytokeratins, which are commonly used as one of the most sensitive markers of epithelial cell differentiation.

Estimation of epithelial cell permeability

Changes in permeability of bronchial epithelial cell cultures were investigated by: 1) assessment of the movement, from the luminal to serosal aspect, of radiolabelled markers across confluent cultures grown in cell culture inserts: 2) measurement of changes in electrical resistance of these cultures, reflective of tight junction integrity; and 3) assessment of structural/functional changes in the tight junctions in similar cultures, but grown on plastic coverslips, to allow freeze-fracture and electron microscopy.

Assessment of radiolabelled marker movement and changes in electrical resistance. Primary explants were removed from all suitable confluent cultures prior to any experimental treatment and the cultures incubated further until the area of the culture insert membrane, left unoccupied on removal of the explant, was fully covered by newly generated cells. Fully confluent cultures were washed gently with fresh culture medium and then incubated at 37°C in a 5% CO_2 in air atmosphere for 30 min, in the presence of either 0.025 µCi ^{14}C-mannitol or 0.025 µCi ^{14}C-bovine serum albumin (^{14}C-BSA) (Amersham International plc, Amersham, UK) added to the luminal aspect of each culture. At the end of incubation the medium in each insert well, on the serosal aspect of each culture, was collected and analysed for total radioactivity by liquid scintillation counting in a Beckman LS1800 scintillation counter (Beckman-RIIC Ltd, High Wycombe, UK). When the total radioactivity passing through the culture was found to be less than 5% of the total for ^{14}C-mannitol and less than 0.5% of the total for ^{14}C-BSA added to the inserts at the beginning of the experiment, the experiment proceeded. Equal volumes of fresh medium containing different concentrations of histamine (ranging 0–10 µM) and 0.025 µCi ^{14}C-mannitol or 0.025 µCi ^{14}C-BSA were added to the luminal aspects of at least six separate cultures, for each histamine concentration, and the electrical resistance of each culture was measured with an EVOM ® micro volt-ohm-meter (World Precision Instruments, Owslebury, UK). All cultures were then incubated further at 37°C in a 5% CO_2 in air atmosphere, and the electrical resistance of each culture was measured again after 30 min, 1, 2, 4, 6 h and 24 h of incubation. The medium in each insert well was also collected at these time-points and replaced with 0.4 ml fresh culture medium. Cultures were also investigated similarly, after incubation in the presence of 0.5% Triton-X 100 or 0.1

μM phorbol dibutyrate (PDBU), as the positive control [20], and 0.025 μCi ^{14}C-BSA. The collected medium was analysed for total radioactivity and the biological activity of histamine by bioassay.

In a separate study, the effect of adding histamine to insert wells (*i.e.* on the serosal aspect of the epithelial cell cultures) was investigated on changes in electrical resistance and movement of ^{14}C-BSA from: 1) the luminal to serosal and; 2) the serosal to luminal aspects, of the epithelial cell cultures.

Prior to use, all incubation media containing histamine and the radiolabelled markers were monitored for pH and osmolality, using a Roebling "Automatik" osmometer (Camlab Ltd, Cambridge, UK). Only solutions found to have pH in the range 7.2–7.4 and osmolality in the range 300–315 mOsm·kg^{-1} H$_2$O were used, after sterilization by filtration through 0.2 μm pore size Minisart® syringe filters (Sartorius, Epsom, UK).

Assessment of epithelial tight junction morphology and integrity. Epithelial cells cultured on Thermanox plastic coverslips were used for investigation of the effect of histamine on tight junctions, as assessed by freeze-fracture and thin section electron microscopy. Prior to use, the explants were removed and the cultures washed gently with fresh prewarmed and pre-aerated medium. Cultures were then incubated in the absence or presence of 20 μM histamine for 15 min, and at the end of incubation washed again with fresh medium, prior to fixation. For freeze-fracture, all cultures were fixed for 1 h at 4°C in 3% glutaraldehyde solution buffered with 0.1 M sodium cacodylate, pH 7.4. For cryoprotection, the tissue was then transferred to 30% glycerol in cacodylate buffer and stored at 4°C for approximately 12 h. The technique developed by PAULI *et al.* [21], utilizing polyvinyl alcohol (PVA), was used to fracture the cultures. Pieces of coverslip were mounted cell-side down on PVA, and frozen in Freon. Fracturing was carried out by raising the knife from below a projecting portion of the coverslip, thereby flipping it away and directing the fracture into the cells. Freeze-fracture replicas were prepared at -115°C, with a vacuum greater than 10^{-6} m bar, in a Balzers BAF 400T apparatus. The standard unidirectionally shadowed platinum-carbon replicas were cleaned using sodium hypochlorite, and then mounted and viewed in a Philips EM 301 electron microscope.

For the investigation of junctional integrity utilizing lanthanum, a modified method of SHAKLAI and TAVASSOLI [22] was employed. Briefly, all cultures were fixed initially for 1 h at 4°C in 3% glutaraldehyde solution, pH 7.4, buffered with 0.1 M sodium cacodylate and containing 1% lanthanum. The tissue was then stored overnight in phosphate buffer containing 1% lanthanum (pH 7.2) at 4°C, and rinsed in phosphate buffer containing 1% lanthanum three times prior to post-fixation. Post-fixation was carried out in 1% osmium tetroxide in 0.1 M cacodylate buffer containing 1% lanthanum (pH 7.4) at room temperature. The tissue was then dehydrated, embedded in epoxy resin and thin sections examined in the electron microscope.

Bioassay for histamine

The biological activity of histamine in culture medium collected from cultures treated with 10 μM histamine at the beginning and end of each experiment was assessed by its contractility of Hartley guinea-pig gall-bladder tissue. Briefly, 300–400 g male animals were killed by cervical dislocation and the gall-bladders rapidly excised and placed into prewarmed and preaerated buffered Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl$_2$, 1.18 mM KH$_2$PO$_4$, 25 mM NaHCO$_3$, 1.18 mM MgSO$_4$. 7 H$_2$O and 5.6 mM glucose, pH 7.4). Strips were cut along the longitudinal axis of each gall-bladder and mounted in 10 ml organ baths under tensions of 1.0 g. The strips were connected to Model K30 force-displacement isometric transducers (Hugo Sachs Electronik KG, Germany) coupled to a Model WR3310 chart recorder (Graphtec Corp., Japan). Following equilibration for 60 min, the strips were contracted three times with a standard solution of final concentration 0.5 μM histamine, with a wash-out period of 30 min between each contraction, and then with 500 μl aliquots of culture media added into the 10 ml organ bath (*i.e.* ×20 dilution of the culture medium). Contractions elicited by the culture media samples were expressed as percentage of the mean contraction evoked by the standard histamine solution. Culture medium with or without 10 μM histamine, incubated under similar culture conditions for 24 h was used as control.

Statistical analysis

Individual data were analysed for skewness and expressed as mean±SEM for each treatment group. The significance of any differences in means of the different treatment groups, at each time-point, was compared by one way analysis of variance (ANOVA), followed by further analysis by Student's two sample t-test. Electrical resistance data were log-transformed and then subjected to similar statistical evaluation. All values of p<0.05 were considered to be statistically significant.

Results

Figure 1a and b shows a typical culture of human bronchial epithelial cells, growing on a tissue culture insert microporous membrane and stained for cytokeratin, which was not stained in control cultures incubated in the presence of an irrelevant antibody. Examination by light microscopy demonstrated that the cells grew out as layers of tightly-packed polygonal cells, many of which were ciliated. This confirmed our previous findings [19].

Effect of histamine on transepithelial migration of radiolabelled markers

Analysis of the movement of ^{14}C-mannitol, from the luminal to the serosal side, in control cultures demonstrated
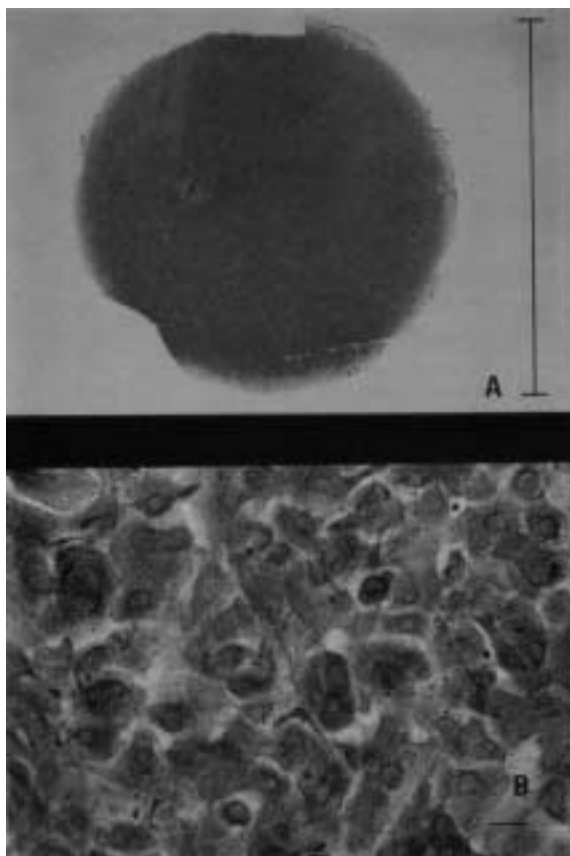
Fig. 1. – Human bronchial epithelial cells cultured in cell culture insert and stained with anti-cytokeratin antibody CAM 5.2. (A=magnification ×1; bar=8 mm; B=magnification ×600; bar=10 µm).

that, over the period 0.5–24 h of incubation, the passage of this marker across the culture increased from 3.5±0.2% to 68.8±2.2% of the total (fig. 2). Similar ¹⁴C-mannitol movement (4.1±0.5 to 77.7±1.9% of total) was observed in cultures incubated with the highest concentration of histamine (10 µm). The difference between the control and histamine-treated groups was not significantly different at any specific time during the incubation (fig. 2).

Analysis of the movement of ¹⁴C-BSA across the epithelial cultures demonstrated that from 0.5–24 h of incubation only 0.19±0.03% to 2.46±0.35% of the total moved across the untreated control cultures, and was not significantly affected by histamine at any concentration or at any time-point during incubation (fig. 3a). Incubation of the cultures in the presence of 0.5% Triton-X 100, however, demonstrated that the percentage of ¹⁴C-BSA moving across the cultures was significantly increased after 1 h of incubation ($p < 0.05$), and was increased nearly ninefold to 21.1± 2.48% ($p < 0.001$) after 24 h, when compared with control cultures (fig. 3a). Similarly, the movement of ¹⁴C-BSA in cultures incubated in the presence of 0.1 µM PDBU was significantly increased after 2 h of incubation ($p < 0.05$), and was increased by 2.5 fold to 6.12±0.59% ($p < 0.001$) after 24 h of incubation (fig. 3a).

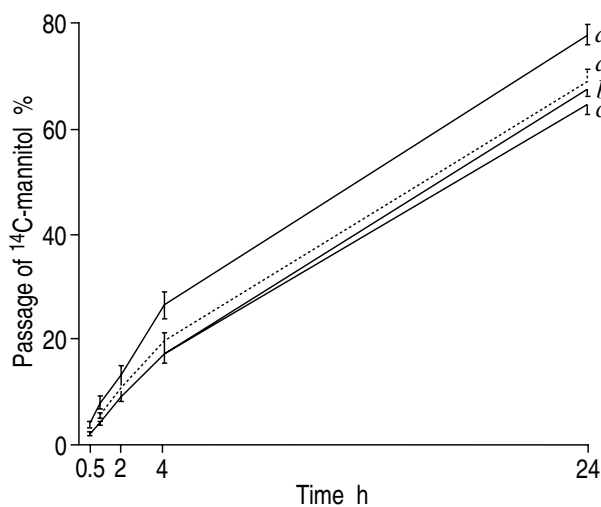Fig. 2. – The effect of histamine on the passage of ¹⁴C-mannitol, from luminal to serosal aspects of human bronchial epithelial cell cultures. Results are expressed as mean±SEM. *a*: untreated control cultures; *b*: +0.1 µM histamine; *c*: +1.0 µM histamine; *d*: +10 µM histamine).
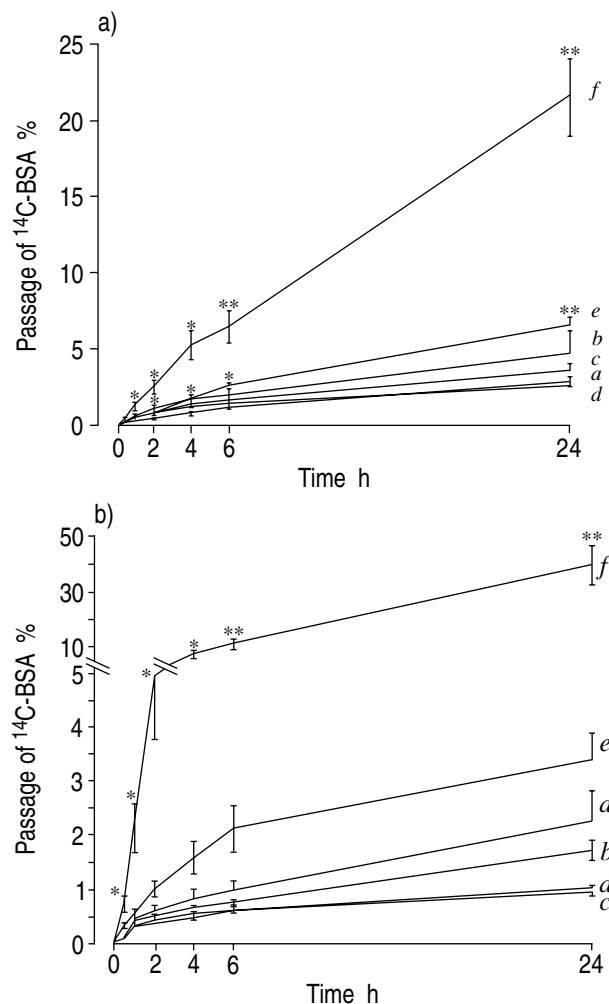
Fig. 3. – The effect of histamine added to: a) the luminal aspects of epithelial cell cultures; and b) the serosal aspects of epithelial cell cultures, on the movement of ¹⁴C-BSA from luminal to serosal aspects of human bronchial epithelial cell cultures. Results are expressed as mean±SEM. *a*: untreated control cultures; *b*: +0.1 µM histamine; *c*: +1.0 µM histamine; *d*: +10 µM histamine; *e*: +0.1 µM PDBU; *f*: +0.5% Triton-X 100. *: $p < 0.05$; **: $p < 0.001$. See text for significance of difference. BSA: bovine serum albumen; PDBU: phorbol dibutyrate.

Analysis of the movement of ^{14}C-BSA in epithelial cell cultures treated with histamine at the serosal aspects of the cultures (*i.e.* histamine added to the insert wells), demonstrated that this was not significantly altered, compared with control cultures (fig. 3b), and was similar to the effect observed in cultures treated with histamine at their luminal aspects. Similarly, treatment of the cultures with 0.1 µM PDBU at their serosal aspects did not significantly alter the movement of ^{14}C-BSA (3.47±0.55%, compared with 2.36±0.58% seen in control cultures) after 24 h of incubation. In contrast, treatment of the cultures with Triton-X 100 at their serosal aspects led to a significantly greater movement of 40.35±6.65% ^{14}C-BSA (p<0.001) after 24 h of incubation (fig. 3b).

The movement of ^{14}C-BSA from the serosal to the luminal aspects of the epithelial cultures, investigated under similar conditions, was also found not to be significantly affected by histamine treatment.
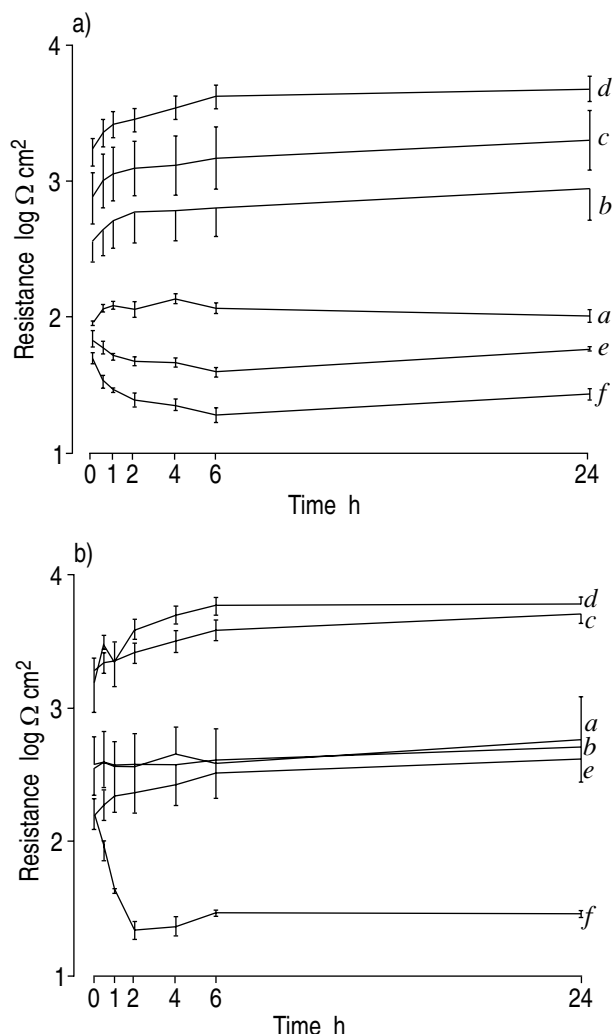

Fig. 4. – The effect of histamine added to a) the luminal aspects of epithelial cell cultures; and b) the serosal aspects of epithelial cell cultures, on the electrical resistance of human bronchial epithelial cell cultures. Results are expressed as mean±SEM. *a*: untreated control cultures; *b*: +0.1 µM histamine; *c*: +1.0 µM histamine; *d*: +10 µM histamine; *e*: +0.1 µM PDBU; *f*: +0.5% Triton-X 100. See text for significance of differences. PDBU: phorbol dibutyrate.

Effect of histamine on electrical resistance of epithelial cultures

Figure 4a demonstrates the effect of histamine, Triton-X 100 and PDBU on the electrical resistance of bronchial epithelial cell cultures, after correction for baseline resistance of the culture insert membrane and the appropriate incubation medium in the absence of the cells (mean=150.0 Ω). These studies demonstrated that incubation of the cells with 0.10–10 µM histamine significantly increased the electrical resistance of the cultures at all time-points investigated (p<0.01). In contrast, incubation of the cells with either 0.5% Triton-X 100 or 0.1 µM PDBU, led to a significant drop in the electrical resistance of the cultures at all time-points investigated (p<0.01).

Analysis of electrical resistance of epithelial cultures treated with histamine added to their serosal aspects demonstrated that 1.0 and 10 µM histamine significantly (p<0.05) increased the electrical resistance of the epithelial cell cultures, when compared with control cultures (fig. 4b), an effect similar to that noted in cultures treated with histamine at their luminal aspects (fig. 4a). In contrast, treatment with Triton-X 100 led to a significant drop in the electrical resistance from 0.5 h incubation onwards (p<0.01). Treatment of the cultures with PDBU at their serosal aspects did not, however, alter the electrical resistance of the cultures (fig. 4b).

Effect of histamine on contraction of guinea-pig gall-bladder

Bioassay for histamine demonstrated that complete culture medium containing 0.5 µM histamine (*i.e.* 500 µl aliquot of culture medium containing 10 µM histamine, mixed with medium in a 10 ml organ bath), evoked a contraction of guinea-pig gall-bladder tissue that was equivalent (100%) to that observed for a reference solution of 0.5 µM histamine (fig. 5). Analysis of medium collected from cultures after 24 h of incubation in the presence of 10 µM histamine demonstrated that this medium still retained the ability to contract the guinea-pig gall-bladder, and evoked a similar response as that observed for the complete culture medium prior to and 24 h after incubation, in the absence or presence of epithelial cells, and equivalent (100%) to that observed for the reference histamine solution (fig. 5).

Effect of histamine on epithelial tight junction integrity

Transmission electron microscopy of ultra-thin sections of epithelial cultures demonstrated that these comprised 2–3 overlapping layers of squamoid cells, which varied in overall thickness by 1–4 µm. Numerous microvilli were present on the luminal cell membranes, but cilia were not detected. The overall ultrastructure of untreated control and histamine-treated cultures was similar.

Studies with lanthanum tracer placed on the luminal aspect demonstrated that the tracer was clearly visible as
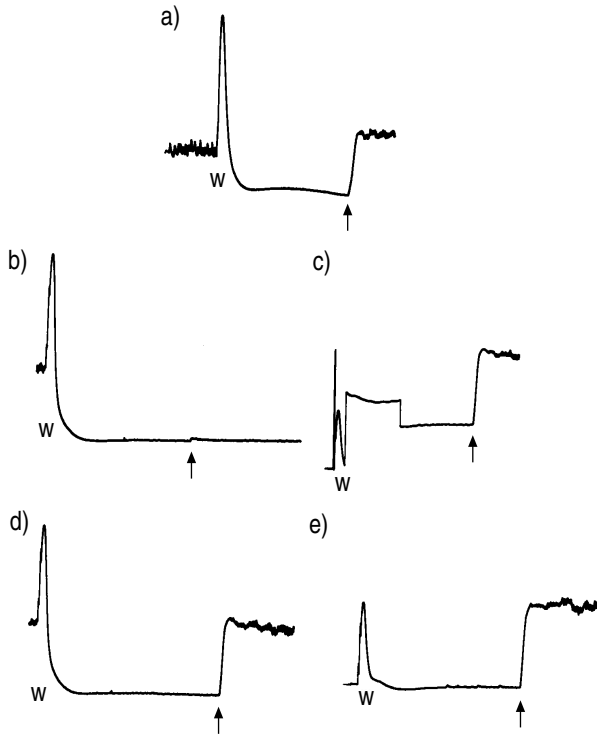
Fig. 5. – Representative tracings of contractions evoked in guinea-pig gall-bladder tissue by: a) reference 0.5 µM histamine solution; b) culture medium without histamine; c) culture medium with 10 µM histamine; culture medium after 24 h incubation in the: d) presence; or e) absence of epithelial cells, in vitro. w: organ bath wash-out, (↑): point of addition of test solution to the organ bath.

an electron-dense layer remaining on the luminal aspect of the epithelial cultures (fig. 6a). Lanthanum penetrated into the luminal clefts at intercellular boundries, but did not penetrate below the tight junctions, in the majority (approximately 95%) of the cultures studied (fig. 6b and c). Infrequently, however, the tracer did gain
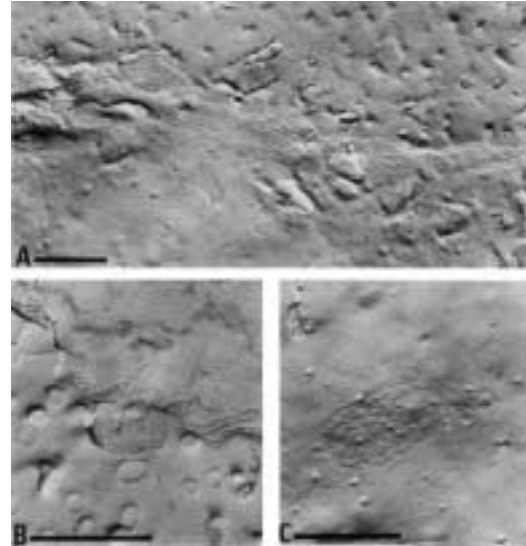
Fig. 7. – Electron micrographs of human bronchial epithelial cells illustrating the morphology of the tight junctions found in untreated control cultures, as revealed by freeze-fracture. A) Tight junctional expanses appearing as a well-defined belt composed of a number of junctional strands with numerous interconnections. (Scale bar=1 µm). B) Tight junctional expanses with less well-organized structure than in (A), showing fewer strands comprising the "belt" and fewer strand interconnections. (Scale bar=1 µm). C) Isolated foci of proliferation of junctional strands distant from and not associated with a junctional belt. (Scale bar=1 µm).
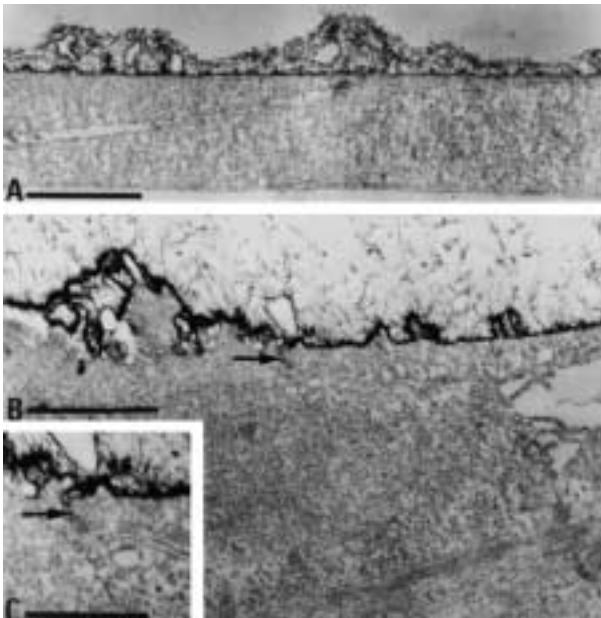

Fig. 6. – Transmission electron micrographs of human bronchial epithelial cells treated with lanthanum. A) Low power view of an untreated control culture illustrating several overlapping cells with large intracellular spaces. A band of lanthanum is clearly visible on the "luminal" edge of the cells. (Scale bar=5 µm). B) High power view of a culture treated with 20 µM histamine, demonstrating that the passage of lanthanum is limited at the site of the tight junction (arrow). (Scale bar=0.5 µm). C) High power micrograph of tight junction (arrow) shown in (b). (Scale bar=0.25 µm).
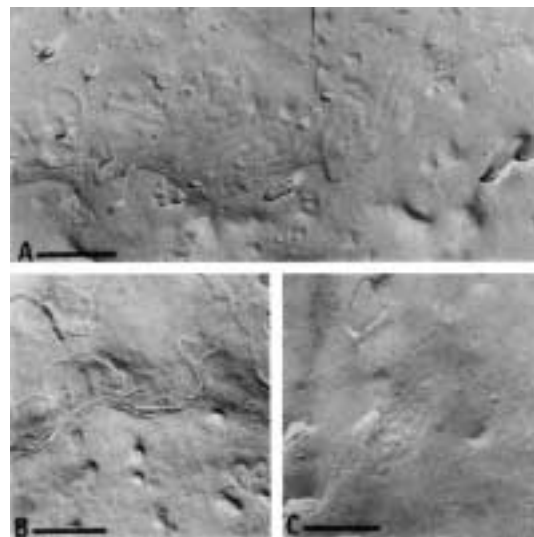

Fig. 8. – Electron micrographs of human bronchial epithelial cells illustrating the morphology of the tight junctions found in cultures treated with 20 µM histamine, as revealed by freeze-fracture. A) Tight junctional expanses appearing as a well-defined belt composed of a number of junctional strands with numerous interconnections. (Scale bar=0.5 µm). B) Tight junctional expanses with less well-organized structure than in (A), showing fewer strands comprising the "belt" and fewer strand interconnections. (Scale bar=0.5 µm). C) Isolated foci of proliferation of junctional strands distant from and not associated with a junctional belt. (Scale bar=0.5 µm).

access to spaces below the tight junctions, but this was always associated with cell necrosis. There were, however, no obvious differences in the degree of lanthanum penetration of the intercellular spaces of untreated control culture group and the culture group treated with 20 µM histamine.

Freeze-fracture replicas obtained both from the untreated control cultures and histamine-treated cultures revealed large expanses of fractured cell membrane. At low magnifications, the individual outlines of the cells were clearly visible, and numerous small microvilli (diameter 250 nm) were frequently present. The morphology, frequency and extent of the tight junctional elements were found to be similar both in the untreated control culture group and the histamine-treated culture group, and conformed to several distinct spatial arrangements: 1) junctional elements arranged in a belt-like structure comprised of well interconnected strands (figs 7a and 8a); 2) less well-organized junctional belts comprising fewer, less frequently connected strands, and with numerous free-ending strands (figs 7b and 8b); and 3) isolated foci of junctional strands distant from and not connected to an organized belt (figs 7c and 8c). The isolated foci of junctional strands occurred frequently and varied in appearance, ranging from relatively simple structures involving few strands, with few interconnections, to much more complicated arrangements involving numerous interconnected strands.

Discussion

Our studies have demonstrated that histamine did not significantly increase the movement of either ^{14}C-mannitol or ^{14}C-BSA across the epithelial cell cultures, but significantly increased the electrical resistance of the cultures. In contrast, 0.1 µM PDBU, a compound known to increase paracellular permeability in epithelial cell lines [20], had the opposite effect and led to a significant decrease in the electrical resistance of the epithelial cell cultures and a significant increase in the passage of ^{14}C-BSA across the epithelial cell cultures treated at their luminal aspects. The movement of ^{14}C-BSA from the luminal to the serosal aspects of the epithelial cultures was not, however, altered when the cultures were treated with PDBU at their serosal aspects; probably, as a consequence of the inaccessibility of this agent to the tricellular junctions lying at the luminal surfaces of the epithelial cultures. These results suggest that histamine does not directly lead to an increase in paracellular permeability of epithelial cells *in vitro*.

Although the passage of ^{14}C-mannitol across the epithelial cultures was found to be greater than that of ^{14}C-BSA, the overall passage of either marker was not significantly altered, in either control cultures or histamine-treated cultures, at any time during incubation. This suggests that the difference observed in the total flux of the two radiolabelled markers was likely to be a consequence of the difference in their molecular size (mannitol 182.2 Da; albumin 69,000 Da). The integrity of the epithelial tight junctions, as assessed by the degree of lanthanum penetration into the intercellular spaces, was also not affected by treatment of the cultures with up to 20 µM histamine, neither was the freeze-fracture morphology of the epithelial tight junctions altered sufficiently for morphological detection. These structural studies complement our functional findings of the effect of histamine on the movement of radiolabelled markers across the epithelial cultures.

The finding of a lack of effect of histamine on epithelial permeability in the present *in vitro* study is in contrast to that reported by others investigating the effect of inhaled histamine both on epithelial [14, 15] and endothelial permeability [12], *in vivo*. It is unlikely that the lack of effect in epithelial permeability reported in our studies was a consequence of an absence of histamine receptors on epithelial cells, since we have previously demonstrated that these cells express functionally active H-1 and H-2 histamine receptors *in vitro* [23]. Additionally, it is unlikely that the lack of effect was a consequence of either degradation or inactivation of histamine in the culture system, since conditioned medium collected from cultures after 24 h incubation was found to be as effective as either a reference solution of histamine or histamine-containing culture medium incubated for 24 h, in its ability to contract guinea-pig gall-bladder tissue *in vitro*. The observation, however, that addition of histamine to either the luminal or serosal aspects of the cultures had similar effects both on the movement of ^{14}C-BSA and the electrical resistance of the epithelial cultures, suggests that histamine receptors are probably not polarized to either the luminal or basal surface of the epithelial cells *in vitro*. This would, however, need to be confirmed by specific receptor-binding studies.

The finding of increased epithelial permeability following histamine inhalation *in vivo*, can be reconciled with our *in vitro* findings, however, if the submucosal capillary endothelium rather than the luminal epithelium is considered to be the major site of histamine activity. Studies investigating the effects of histamine on vascular permeability both in intact tissue and cultured endothelial cells have demonstrated that histamine leads to endothelial cell contraction and subsequent formation of gaps of up to 1 µm in diameter between adjacent cells, resulting in extravasation of macromolecules [24]. PERSSON *et al.* [25] have recently reviewed the mechanisms of endothelial permeability resulting from provocation of the airway mucosa by different agents, and suggested that this is a noninjurious and a fully reversible process, which of itself leads to reversible permeability of the epithelial barrier. These authors have suggested that the plasma exudate produced as a consequence of increased endothelial permeability leads to an increase in the hydrostatic pressure at the basolateral aspects of the epithelial cells and creates intercellular pathways for its own entry into the lumen.

More recently, KONDO *et al.* [26] have investigated changes in permeability of dog tracheal epithelial sheets, mounted in Ussing chambers, and confluent tracheal epithelial cell cultures *in vitro*, in response to hydrostatic pressure. These authors have demonstrated that

application of hydrostatic pressure only from the submucosal to the mucosal side, but not in the opposite direction, significantly increased both: 1) the transepithelial flux of radiolabelled markers (^{3}H-mannitol and fluorescein isothiocyanate (FITC)-labelled BSA); and 2) the electrical properties of the tissues (conductance and electrical resistance studied under short-circuit and open-circuit conditions, respectively). Furthermore, these authors have demonstrated that the changes in transepithelial flux and electrical properties of the epithelial cell cultures correlate well with morphological changes, such as dilatation of lateral intercellular spaces, disruption of tight junctions and submucosal oedema; and suggest that, in asthma, submucosal oedema-associated structural changes may be a prelude to epithelial shedding.

In conclusion, the present studies have demonstrated that explant-derived human bronchial epithelial cells can be grown to confluence on semipermeable membranes *in vitro* and can be used as a suitable model for the investigation of agents which could influence paracellular epithelial permeability. Using this model, our studies suggest that contrary to the currently held view, histamine is unlikely to affect human bronchial epithelial cell permeability directly *in vivo*, but may do so indirectly, *via* generation of increased submucosal hydrostatic pressure resulting from increased endothelial permeability and the ensuing plasma leakage.

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