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# Glucocorticoids mediate reduction of epithelial acetylcholine content in the airways of rats and humans

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**Abstract**

The cholinergic system in rat and human airways and the effects of glucocorticoids were investigated by assay of choline acetyltransferase activity, by high-pressure liquid chromatography measurement of acetylcholine, and by anti-choline acetyltransferase immunocyto-/histochemistry. Human bronchi were obtained at surgery from patients with lung cancer. Group 1 patients did not suffer from additional lung diseases and had not been treated with glucocorticoids. Group 2 patients, who suffered in addition to lung cancer from chronic obstructive bronchitis, had been treated for at least 6 weeks before surgery with four puffs of flusinolid daily. Isolated bronchial epithelial cells as well as intact surface epithelium of human bronchi expressed choline acetyltransferase immunoreactivity and choline acetyltransferase enzyme activity ($3 \pm 1$ nmol/mg protein per h). Ciliated epithelial cells showed strong choline acetyltransferase immunoreactivity at the basal body and the roolet of cilia. Surface epithelium in group 1 and 2 bronchi contained $23 \pm 6$ ($n = 14$) and $1.8 \pm 0.3$ pmol/g acetylcholine) ($n = 7$, $P < 0.001$), respectively, whereas the transmural acetylcholine content did not differ significantly between both groups. The amount of choline acetyltransferase immunoreactivity appeared similar in the surface epithelium of both groups. In an animal (rat) study the effects of oral dexamethasone (3 mg/day, 1 week) on choline acetyltransferase activity and acetylcholine levels were investigated. Dexamethasone treatment reduced epithelial acetylcholine in the airways and small intestine by about 80% and inhibited epithelial choline acetyltransferase activity. In conclusion, epithelial cells of human airways possess components of the cholinergic system, i.e., contain the synthesizing enzyme choline acetyltransferase and store acetylcholine. The data obtained from the animal study indicate that glucocorticoids can inhibit epithelial acetylcholine. 

*Keywords:* Cholinergic system; Non-neuronal; (Human); (Rat); Epithelial acetylcholine; Ciliated cell; Glucocorticoid

## 1. Introduction

Acetylcholine was the first signalling molecule characterized as a neurotransmitter (Loewi, 1921). In addition, acetylcholine is found in non-neuronal mammalian cells (Sastry and Sadavongvivad, 1979). Recent findings demonstrate that acetylcholine is expressed in the vast majority of surface epithelial cells in human (Klapproth et al., 1997b; Wessler et al., 1998). For example, choline acetyltransferase activity and acetylcholine were demonstrated in epithelial cells of human airways and of the alimentary tract (oral mucosa, small and large intestine, gall bladder). In addition, keratinocytes of human skin express choline acetyltransferase and contain acetylcholine, and evidence has been published that epithelial acetylcholine acts as a local hormone (Grando et al., 1993a,b, 1996; Klapproth et al., 1997a,b). In the present study we further characterized the cholinergic system in human airway surface epithelium and investigated whether glucocorticoid treatment affects this system. Glucocorticoids represent a mainstay in the treatment of inflammatory airway disease, and these drugs may affect the non-neuronal cholinergic system. The airway epithelial acetylcholine content was compared in two groups of lung cancer patients. Group 1 patients had not been treated and group 2 patients, who suffered additionally from chronic

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obstructive bronchitis, had been treated with an inhalative glucocorticoid before surgery. In an animal study we evaluated whether dexamethasone, given via drinking water, affects the cholinergic system expressed in the airways and intestinal surface cells of rats.

## 2. Materials and methods

### 2.1. Patients and preparation of bronchi and epithelial cells

The protocol for obtaining human tissue was approved by the state ethics review board for human studies (Landesärztekammer Rheinland-Pfalz, Germany). Lung tissue was obtained from patients with lung cancer after thoracotomy. Patients received atropine (0.5 mg) as premedication, i.e., roughly 4 h before bronchial tissue was isolated. Tumor-free bronchi (diameter: 12 to 3 mm) were prepared as described (Reinheimer et al., 1996; Klapproth et al., 1997b), placed in oxygenated salt solution and washed vigorously (composition in mM: 125 NaCl, 23.8 $NaHCO_3$, 5.05 glucose, 2.68 KCl, 1.80 $CaCl_2$, 1.04 $MgCl_2$, 0.54 $NaH_2PO_4$, 0.06 ascorbic acid, and 0.001 choline chloride; pH 7.3). Lung tissue was classified into two groups as indicated in the Introduction. Group 1 bronchi were obtained from 14 patients (9 male and 5 female, $58 \pm 3$ years). Group 2 bronchi were obtained from 7 patients (5 male and 2 female, $61 \pm 4$ years) who, in addition to lung cancer, suffered from chronic obstructive bronchitis and had been treated for at least 6 weeks before surgery with flusinolid (0.25 mg/puff, 4 puffs/day). Acetylcholine and choline acetyltransferase activity were measured in mechanically or enzymatically isolated surface cells. For mechanical isolation the luminal surface of bronchi was gently rubbed (5 s) with a cotton-tipped applicator (Q-tip) without penetrating the basal membrane and thereafter the Q-tips were placed immediately in 15% formic acid in acetone (v/v) (Reinheimer et al., 1996; Klapproth et al., 1997b). Enzymatic isolation of epithelial cells was performed by a 2-h (36°C) or 24-h (4°C) incubation of human bronchi with 0.1% pronase in Dulbecco's modified Eagle's medium–Ham's F-12 (DMEM/F12) medium (Reinheimer et al., 1996; Klapproth et al., 1997b). Thereafter, cells were collected by centrifugation (5 min, 200 rpm) and washed with 3 ml phosphate-buffered saline. The resuspended cell pellet was exposed (10 min) to 0.5% trypsin, washed again (3) then seeded for subsequent culturing or snap frozen for immunocytochemistry. Isolated cells expressed cytokeratin immunoreactivity identifying them as epithelial cells, whereas they were negative for neuronal markers (see Fig. 1).

For culturing, cells were seeded on 6-well plates ($10^6$ per well) in DMEM/F12 medium. Culture medium contained 60 $\mu$M choline, 100 IE/ml penicillin and 100 IE/ml streptomycin and was supplemented with some growth factors (5% fetal calf serum, epidermal growth factor 20 ng/ml, bovine insulin 10 $\mu$g/ml, transferrin 5 $\mu$g/ml, cholera toxin 10 ng/ml, trans-retinoic acid 100 nM; atmosphere of 95 vol.% $O_2$ and 5 vol.% $CO_2$ at 37°C). After a culture period of 24 h epithelial cells were washed and lysed for measuring acetylcholine and choline acetyltransferase enzyme activity. Culturing was limited to 24 h, because periods longer than 24 h resulted in a marked decrease in acetylcholine synthesis.

### 2.2. Measurement of acetylcholine and choline acetyltransferase enzyme activity

Acetylcholine was extracted from applied Q-tips, cultured cells or pieces of homogenized bronchi (Ultra Turrax®, 2 min at full speed) in 0.5–1.5 ml 15% formic acid in acetone (v/v). Acetylcholine was measured by cationic exchange high-pressure liquid chromatography (HPLC) with electrochemical detection as described previously (Wessler et al., 1995, Reinheimer et al., 1996; Klapproth et al., 1997b). Epithelial acetylcholine extracted from Q-tips was related to the wet weight of the bronchus from which the surface epithelium was removed and normalized to 1 g; epithelial acetylcholine extracted from cultured cells was related to the cell number. The choline acetyltransferase assay was performed as described recently (Ričný et al., 1995; Reinheimer et al., 1996). Freshly isolated or cultured epithelial cells were placed (15 min) in ice-cold 0.5–1 ml extraction buffer (10 mM $Na_2HPO_4$, 100 mM NaCl, 2 mM EDTA, 0.5% (v/v) Triton X-100), centrifuged (3 min; 12 000 rpm, 0°C) and an aliquot of the supernatant (20 $\mu$l) was then added to the assay buffer containing 8 mM choline chloride, 0.1 mM physostigmine and 0.2 mM [$^3$H]acetyl CoA (Ričný et al., 1995; Reinheimer et al., 1996). The effect of the choline acetyltransferase-selective inhibitor bromoacetylcholine was tested (Tuček, 1982). Protein content of the samples was measured according to Smith et al. (1985).

### 2.3. Immunocyto-/histochemistry

Enzymatically isolated epithelial cells or intact bronchi were snap frozen in liquid nitrogen and 4-$\mu$m cryosections were prepared for immunocyto-/histochemistry or cytospin preparations (epithelial cells) were established. The expression of the choline acetyltransferase protein was investigated by applying monoclonal or polyclonal anti-choline acetyltransferase antibodies (Figs. 2 and 3). The specificity of the polyclonal choline acetyltransferase antiserum has been tested previously (Schemann et al., 1993a,b; Klapproth et al., 1997b). Primary antibodies were incubated at room temperature for 1 h and subsequently at 4°C for 12 h. Secondary antibodies were added for 10–30 min at room temperature. Staining was achieved by incubation

(10–20 min) in chromogen solution containing either 0.8% 3,3-diaminobenzidine (peroxidase system) or 0.5% naphthol-AS-biphosphate and fast TR-salt (alkaline phosphatase system). Sections were counterstained with hemalaun. For all samples negative controls were performed, consisting of the substitution of the primary antibody with an irrelevant murine antibody (for example, see Fig. 2C).

The following primary antibodies were used: monoclonal mouse immunoglobulin (Ig) G1 anti-choline acetyltransferase antibody (dilution 1:500/2000); polyclonal rabbit anti-choline acetyltransferase antibody (dilution 1:800/2000; Schemann et al., 1993a,b; Klapproth et al., 1997b); monoclonal mouse anti-cytokeratin 8 antibody (AM5.2; dilution 1:15); polyclonal mouse anti-S100 antibody (dilution 1:2000); monoclonal mouse anti-neurofilament antibody (dilution 1:20). The following secondary antibodies were applied: rabbit anti-mouse or goat anti-rabbit IgG biotinylated antibody in combination with avidin biotinylated-peroxidase complex (dilution 1:50); goat anti-rabbit IgG antibody–alkaline phosphatase conjugate (dilution 1:100).

### 2.4. Animal study

Sprague–Dawley rats (200–250 g) of either sex were maintained under standard laboratory conditions on 12-h day/night cycles with ad libitum access to food and water. Rats were treated (1 week) with dexamethasone applied via the drinking water (2 mg/100 ml water, corresponding to a daily dose of about 3 mg/kg); control rats were kept under identical conditions but without dexamethasone. Rats were killed by exsanguination and the trachea and intestine were removed. Surface acetylcholine and choline acetyltransferase activity were investigated by taking Q-tip samples from the luminal surface of the trachea and the intestine; transmural acetylcholine and choline acetyltransferase activity were measured after mechanical homogenization of the tissue. For acetylcholine measurement tissue was homogenized by means of an Ultra-Turrax in 2 ml 15% formic acid in acetone (v/v). For choline acetyltransferase measurement tissue was snap frozen in liquid nitrogen, pulverized and then extracted into 5 × volume of the extraction buffer (see Section 2.3). Measurements were performed as described above. Morphology was checked by histological inspection of respective specimens of the trachea and intestine stained with hematoxylin and eosin.

### 2.5. Statistical analysis

Results are given as means ± S.E.M. with n indicating the number of experiments. The significance of differences was evaluated by the computer program Instat® (Student's *t*-test; Dunnett test); $P$ values of $< 0.05$ were regarded as significant.

### 2.6. Drugs and special chemicals

The following drugs and chemicals were used: Acetyl [$^3$H] CoA (choline acetyltransferase-assay, specific radioactivity: 7.4 GBq/mmol; Du Pont, Bad-Homburg, Germany); biotin–peroxidase complex (Boehringer Mannheim, Mannheim, Germany); bovine insulin (Sigma Chemie, München, Germany); bromoacetylcholine bromide (RBI Biotrend, Köln, Germany); cholera toxin (Sigma; dexamethasone (Sigma); DMEM/F12 medium (Sigma); epidermal growth factor (Eurobio, Raunheim, Germany); fetal calf serum (Eurobio); goat anti-rabbit IgG-biotinylated antibody (Boehringer Mannheim); goat anti-rabbit IgG antibody–alkaline phosphatase conjugate (Boehringer Mannheim); monoclonal mouse IgG1 anti-choline acetyltransferase antibody (Boehringer Ingelheim Bioproducts, Heidelberg, Germany); monoclonal mouse anti-cytokeratin 8 antibody (AM5.2, Becton-Dickinson, Neckargemünd, Germany); monoclonal mouse anti-neurofilament antibody (Monosan, Saubio, The Netherlands); polyclonal mouse anti-S100 antibody (Dako, Hamburg, Germany); physostigmine sulfate (Sigma); penicillin (Sigma); rabbit anti-mouse IgG-biotinylated antibody (Boehringer Mannheim); streptomycin (Sigma); transferrin (Sigma).

## 3. Results

### 3.1. Choline acetyltransferase and acetylcholine in human airway epithelium (group 1 bronchi)

Cells, isolated from human bronchi, were cytokeratin-positive but negative for neuronal markers like neurofilament or S100 (Fig. 1). The epithelial cells expressed specific and intense choline acetyltransferase immunoreactivity, as demonstrated with both the mono- and polyclonal anti-choline acetyltransferase antibodies (Fig. 2). Ciliated epithelial cells expressed strong choline acetyltransferase immunoreactivity at the basal body and rootlet of the cilia, i.e., at the apical part of the cells where the cilia are embedded (Fig. 2B). Choline acetyltransferase immunoreactivity was also demonstrated in the intact surface epithelium by using mono- or polyclonal anti-choline acetyltransferase antibodies (Fig. 3). In line with the positive choline acetyltransferase immunoreactivity, epithelial cells showed choline acetyltransferase enzyme activity, detected either immediately after cell isolation or after 24 h culture ($3.0 \pm 1.2$ ($n = 4$) and $1.9 \pm 0.4$ ($n = 3$) nmol/mg protein per h, respectively; group 1 bronchi). Bromoacetylcholine (30 $\mu$M) inhibited choline acetyltransferase activity in freshly isolated epithelial cells by more than 85% ($0.4 \pm 0.2$ nmol/mg protein per h; $n = 4$).

Q-tip extracts taken from bronchial surface epithelium contained acetylcholine ($23 \pm 6$ pmol/g bronchus, $n = 14$, group 1). Acetylcholine was also detected in cultured (24

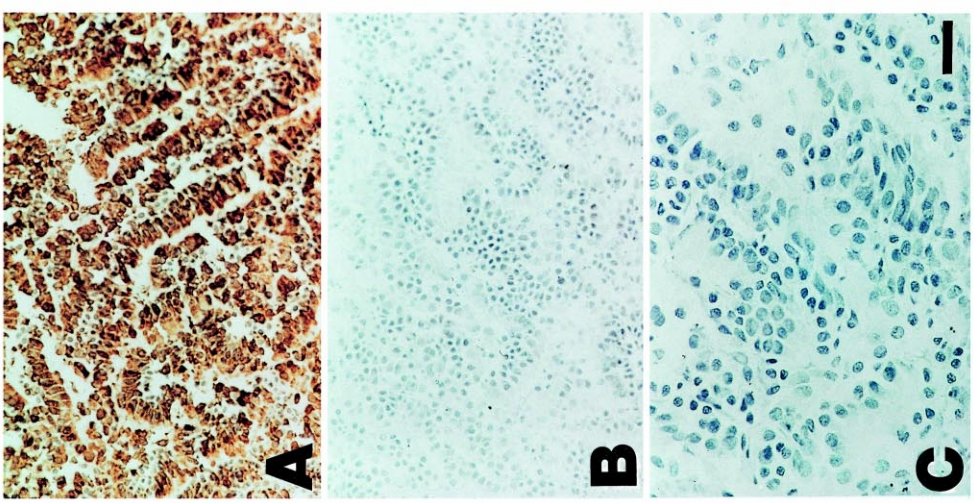


Fig. 1

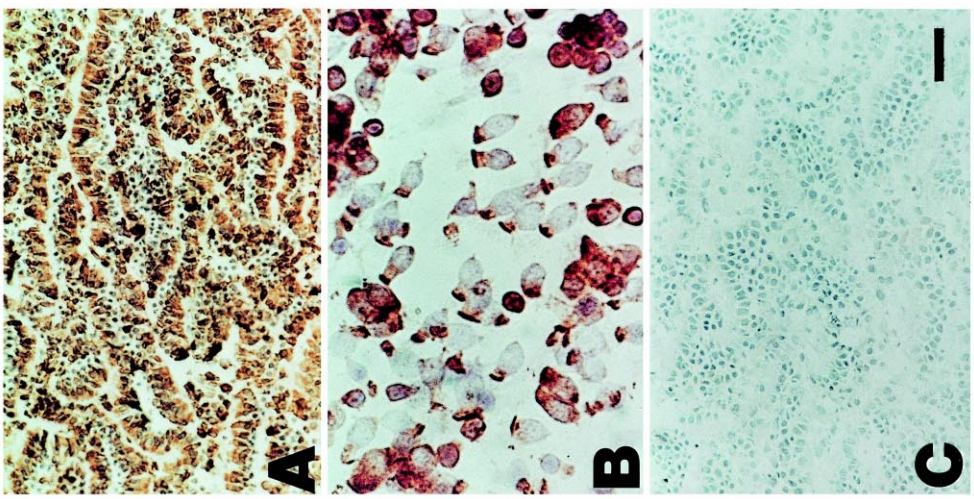


Fig. 2

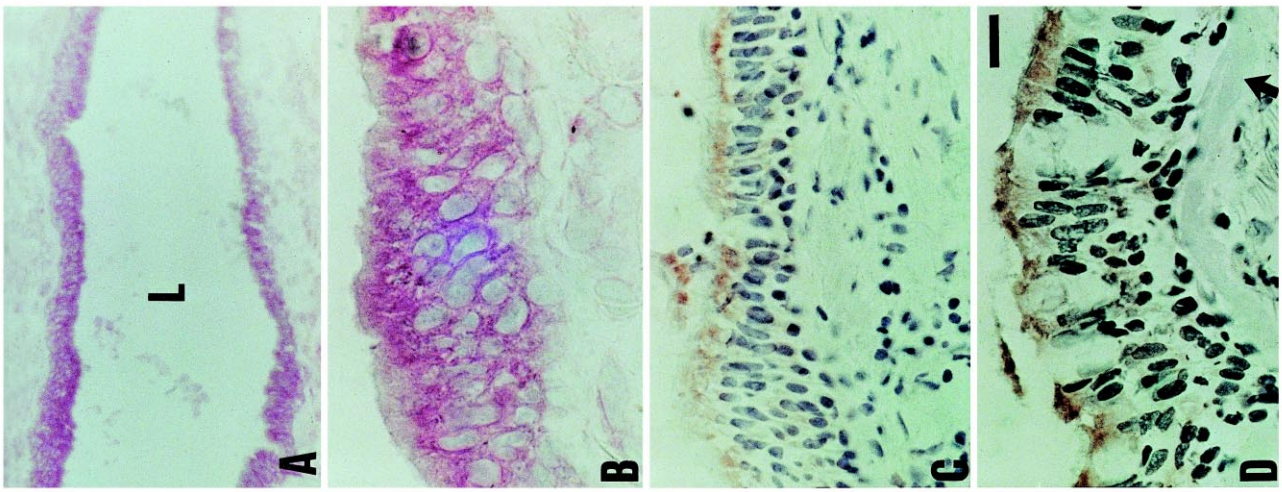


Fig. 3

Table 1
The effect of dexamethasone on choline acetyltransferase activity and acetylcholine content in rat trachea and intestine

| Tissue | Acetylcholine (nmol/g tissue) | | ChAT (nmol/mg protein per h) | |
|---|---|---|---|---|
| | Control | Dexa | Control | Dexa |
| Trachea | | | | |
| Surface epithelium | 2.80 ± 0.50 (8) | 0.65 ± 0.15 (6)[c] | 47.0 ± 13.0 (5) | 19.0 ± 8.0 (5) |
| Transmural | 12.50 ± 2.30 (3) | 3.40 ± 1.20 (4)[a] | 190.0 ± 60.0 (5) | 50.0 ± 12.0 (4) |
| Small intestine | | | | |
| Surface epithelium | 1.10 ± 0.10 (5) | 0.10 ± 0.04 (6)[c] | 30.0 ± 3.9 (5) | 14.0 ± 3.0 (5)[b] |
| Transmural | 15.00 ± 2.40 (6) | 10.70 ± 1.57 (5) | 148.05 ± 5.0 (6) | 99.0 ± 20.0 (5) |
| Large intestine | | | | |
| Surface epithelium | 1.50 ± 0.20 (8) | 0.90 ± 0.30 (5) | 60.0 ± 6.0 (4) | 29.0 ± 4.0 (5)[b] |
| Transmural | 18.20 ± 3.30 (4) | 17.00 ± 2.80 (4) | 250.0 ± 90.0 (5) | 130.0 ± 34.0 |

Rats were treated with dexamethasone (Dexa) added to the drinking water (2 mg/100 ml water corresponding to a daily dose of about 3 mg/kg); control rats were kept under identical conditions without dexamethasone. After 1 week, rats were killed by exsanguination, the trachea and intestine were removed and biochemical analysis was performed as described. Epithelial acetylcholine content is related to the wet weight of the tissue (trachea, intestine) from which the surface epithelium was removed by means of a Q-tip and normalized to 1 g. Transmural acetylcholine content is related to the wet weight of the homogenized tissue and normalized to 1 g.
ChAT: choline acetyltransferase.
Shown are the means ± S.E.M., number of rats in parentheses.
Significance of difference from the respective control: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.005$.

h) epithelial cells ($7 \pm 2$ pmol/$10^6$ cells, $n = 3$, group 1). For comparison between epithelial and transmural acetylcholine content, the content of the whole bronchial wall was also measured. The bronchial wall contained $2600 \pm 500$ pmol/g acetylcholine ($n = 14$, group 1).

### 3.2. Epithelial acetylcholine after glucocorticoid treatment (group 2 bronchi)

The surface epithelium obtained from group 2 bronchi showed significant choline acetyltransferase immunoreactivity (Fig. 3D). However, Q-tip extracts taken from group 2 bronchi contained substantially less acetylcholine than those of group 1 bronchi ($1.8 \pm 0.3$ pmol/g, $n = 7$; significantly different from group 1 bronchi $P < 0.001$), whereas the acetylcholine content of the bronchial wall did not differ significantly between the two groups ($2100 \pm 300$ pmol/g; $n = 7$). The reduced epithelial acetylcholine content may be caused by glucocorticoid treatment or by the chronic obstructive airway disease itself. Since the surface epithelium of airways and intestine contains acetylcholine in both rats and humans (Reinheimer et al., 1996; Klapproth et al., 1997b), the animal study was carried out to shed more light on this issue.

Fig. 1. Immunocytochemistry of epithelial cells isolated from human bronchi. Enzymatically isolated human bronchial epithelial cells were prepared for immunocytochemistry (4-$\mu$m cryosections; see Section 2). (A) Cytokeratin-8 immunoreactivity, positive brown staining (magnification ×200). A typical result of two experiments is shown. (B) Neurofilament immunoreactivity, negative (×200); (C) S100 immunoreactivity, negative (×400). Biotinylated secondary antibodies were applied throughout (peroxidase system). Bar (given in C) represents 100 $\mu$m in A, B and 50 $\mu$m in C.

Fig. 2. Choline acetyltransferase immunoreactivity in epithelial cells isolated from human bronchi. Enzymatically isolated human bronchial epithelial cells were prepared for immunocytochemistry (4-$\mu$m cryosections; see Section 2). (A) primary antibody was the monoclonal mouse $IgG_1$ anti-choline acetyltransferase antibody (magnification ×200; positive brown staining). A typical result of two experiments is shown. (B) primary antibody was the polyclonal rabbit anti-choline acetyltransferase antibody (×1000; positive brown staining); (C) control experiment, primary antibody was an irrelevant murine antibody, negative (×200). Biotinylated secondary antibodies were applied throughout (peroxidase system). Bar (given in C) represents 100 $\mu$m in A, C and 20 $\mu$m in B.

Fig. 3. Choline acetyltransferase immunoreactivity in the surface epithelium of human bronchi. Human bronchi were snap frozen immediately after surgical dissection and prepared as described in Section 2. (A,B) primary antibody was the polyclonal rabbit anti-choline acetyltransferase antibody; secondary antibody was the goat anti-rabbit IgG antibody–alkaline phosphatase conjugate (alkaline phosphatase system). Substantial choline acetyltransferase immunoreactivity (pink staining) was expressed by surface epithelial cells (A: magnification ×200; B: ×1200). (C,D) Primary antibody was the monoclonal mouse IgG1 anti-choline acetyltransferase antibody; secondary antibody was rabbit anti-mouse IgG biotinylated antibody (peroxidase system). Significant choline acetyltransferase immunoreactivity (brown staining) was found in group 1 (C, ×1000) and group 2 bronchi (D, ×1000). L: bronchial lumen; arrow in D indicates the thickened basal membrane. Typical results of 2–4 experiments are shown. Bar (given in D) represents 100 $\mu$m in A, 17 $\mu$m in B, and 20 $\mu$m in C, D.

### 3.3. Animal study

In dexamethasone-treated animals the surface epithelium of the trachea or intestine remained unchanged, as demonstrated by histological inspection of respective specimens (not shown). However, dexamethasone treatment was followed by a substantial reduction in the surface content of acetylcholine, particularly in the trachea and small intestine (Table 1). The transmural acetylcholine content of the trachea was also reduced in dexamethasone-treated rats (Table 1). Sufficient tissue was available in the animal study to investigate choline acetyltransferase activity. Dexamethasone tended to inhibit choline acetyltransferase activity in all tissues but a statistically significant decline was observed only for the surface epithelium of the intestine (Table 1).

## 4. Discussion

### 4.1. Epithelial acetylcholine

Recently we have reported that acetylcholine is widely expressed in epithelial surface cells of mammals including humans (Klapproth et al., 1997b; Wessler et al., 1998). The present experiments further characterize the cholinergic system in the surface epithelium of human airways. Choline acetyltransferase immunoreactivity was visualized in the bronchial surface epithelium by applying mono- or polyclonal anti-choline acetyltransferase antibodies. In particular, choline acetyltransferase immunoreactivity was found in ciliated cells at the apical part, i.e., within the transition zone between free cilium and basal body and in the intact surface epithelium. In line with the positive anti-choline acetyltransferase immune response, choline acetyltransferase enzyme activity and acetylcholine were demonstrated in freshly isolated and in cultured epithelial cells. Taken together, these observations indicate that the human airway surface epithelium have a cholinergic system that is independent of neuronal structures. It is known that cholinergic neurons do not terminate within the surface epithelium of human airways (Jeffery and Reid, 1973; Adriaensen and Scheuermann, 1993).

Surface epithelium contributes about 10% to the mass of the bronchial wall (Reinheimer et al., 1996), but the epithelial acetylcholine content represents a minute fraction (1%) of the transmural acetylcholine content. This poor relation, however, does not imply a lack of biological significance. The following cellular effects of acetylcholine at epithelial cells should be considered or have already been demonstrated. The pattern of choline acetyltransferase immunoreactivity in ciliated cells provides strong morphological evidence for a functional role of epithelial acetylcholine in regulating ciliary activity. A functional role of applied acetylcholine in ciliary activity has already been demonstrated (Sastry and Sadavongvivad, 1979; Wanner et al., 1996). Biopsies of human airways have been used to demonstrate stimulatory and depressive effects on ciliary activity of applied muscarinic receptor agonists and antagonists, respectively (Sastry and Sadavongvivad, 1979; Wong et al., 1988a,b; Wanner et al., 1996). Most likely the inhibitory effects of the antagonists alone (Wong et al., 1988a,b) indicate that epithelial acetylcholine is directly involved in ciliary activity. In dog trachea, capsaicin increased the ciliary beat frequency, and this effect was blocked by the muscarinic receptor antagonist ipratropium (Eljamal et al., 1994).

Acetylcholine, by stimulation of muscarinic and nicotinic receptors, can promote the growth of human bronchial epithelial cells, corneal epithelial cells and human skin keratinocytes (Cavanagh and Colley, 1989; Grando et al., 1993a,b, 1996; Klapproth et al., 1997b). In sheep tracheal epithelium, acetylcholine transiently increases the short-circuit current indicative of apical chloride secretion (Acevedo, 1994), an effect also observed in intestinal epithelial cells (Stewart and Turnberg, 1989; Chandan et al., 1991). Goblet cell secretion may also be affected by non-neuronal acetylcholine as it is known that goblet cells are endowed with muscarinic receptors and respond to muscarinic agonists (Marin, 1994). Thus, it appears likely that epithelial acetylcholine is directly involved in the regulation of chloride secretion. Finally, experimental evidence has been presented that acetylcholine can affect the barrier and immune function of mucosal surfaces (Grando et al., 1993a,b, 1996; Klapproth et al., 1997b; Reinheimer et al., 1997; Wessler et al., 1997, 1998).

### 4.2. Effects of glucocorticoids

The group 2 patients, who suffered from chronic obstructive bronchitis in addition to lung cancer, had been treated with inhaled flusinolid. This was not the case for group 1 patients. Q-tip extracts taken from bronchi of group 2 patients contained less acetylcholine than those of group 1 patients. Thus, flusinolid treatment may have induced the decrease in epithelial acetylcholine content, although the chronic airway disease itself could have been the cause for this reduction. The animal study was performed to discriminate between the two possibilities. Histological inspection of sections of the trachea and the small/large intestine of dexamethasone-treated rats did not indicate relevant morphological changes in the surface epithelia but showed a reduction in the intestinal Peyer's plaques. Dexamethasone, however, caused a substantial reduction in the surface acetylcholine content in the trachea and small intestine. Therefore, we conclude from the animal study that glucocorticoid treatment reduces epithelial acetylcholine. This conclusion implies that the inhalation of flunisolid may be, at least partly, responsible for

the decrease in epithelial acetylcholine content, in addition to the known morphological alterations associated with the chronic airway disease.

The underlying cellular mechanisms leading to a reduced acetylcholine content have to be studied in more detail. Nevertheless, the present results allow some suggestions to be made. Choline acetyltransferase immunoreactivity appeared similar in group 1 and 2 bronchi. Obviously, choline acetyltransferase should also be studied in epithelial cells taken from glucocorticoid-treated patients but the amount of bronchial tissue from these patients was limited and enzymatic separation of epithelial cells could not be performed. In the animal study, dexamethasone appeared to decrease choline acetyltransferase activity, but this effect failed to reach statistical significance in the trachea. A significant inhibition was observed in the intestinal surface epithelium. Most likely, systemically applied glucocorticoids can decrease choline acetyltransferase activity by inhibiting protein expression or enzyme activity. Glucocorticoids may also interfere with the storage and inactivation of acetylcholine, i.e., cholinesterase activity may be upregulated by glucocorticoids.

In contrast to the substantial decrease in epithelial acetylcholine content the amount of acetylcholine stored in the whole bronchial wall did not differ between group 1 and 2 bronchi. This finding shows that inhalative glucocorticoid treatment does not significantly affect acetylcholine beyond the basal membrane, a finding already reported in a previous study (Řičný et al., 1995). However, in the animal study in which dexamethasone was added to the drinking water, both the epithelial and transmural acetylcholine content in the trachea was reduced by about 80%. Thus, the systemic application of glucocorticoids may affect the cholinergic system in the whole airway wall, in contrast to the situation when these drugs are applied topically.

Currently it is unknown whether the inhibition of epithelial acetylcholine contributes to the therapeutic effects of glucocorticoids, i.e., the reduction of hyperresponsiveness and mucosal inflammation. Additionally, it remains to be investigated whether the decrease in epithelial acetylcholine content modifies ciliary activity. The anti-inflammatory effects of glucocorticoids are dominant, and oral glucocorticoid therapy has been shown to improve mucociliary clearance in patients with asthma (Agnew et al., 1984). In patients with diffuse fibrosing alveolitis, oral prednisolone has been found to reduce enhanced mucociliary clearance (Labrune et al., 1994). Patients with pulmonary sarcoidosis treated with inhaled glucocorticoids show a greater impairment of mucociliary transport than patients either not treated with steroids or treated with oral glucocorticoids (Hasani et al., 1992).

In conclusion, the present experiments demonstrate the existence of a non-neuronal cholinergic system in human airway surface epithelium and an inhibitory effect of glucocorticoids on epithelial acetylcholine. These data identify a new cellular target of glucocorticoids, i.e., the interaction with the non-neuronal cholinergic system.

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