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Adrenergic stimulation lowers the proportion of tracheal goblet cells containing fucosylated glycoconjugates in rabbits

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Intravenous administration of epinephrine is widely used in emergency medicine. Since such an administration of epinephrine conspicuously lowered the proportion of sialylated glycoconjugate-containing tracheal goblet cells, we evaluated the proportion of fucosylated glycoconjugate-containing tracheal goblet cells after the same treatment as well. The proportion of fucosylated glycoconjugates mostly influences rheologic properties of the airways' mucus. Six New Zealand White rabbit males were intravenously administered with epinephrine in the dose of 10 μg per 1 kg of body weight. Material for lectin histochemistry was collected 5 min and 20 min post exposure, respectively. Lectins of Ulex europaeus (ULE-I) detecting α(1-2)-linked fucose and of Aleuria aurantia (AAL) detecting α(1-3)-, α(1-4)-, and α(1-6)-linked fucose were used both individually and simultaneously. The proportion of total goblet cells containing fucosylated glycoconjugates decreased from 44.2±22.1% in controls to 3.2±4.0% and 9.2±7.8%, respectively, in treated animals. The proportion of goblet cells with ULE-I-positive content decreased from 38.9±19.9% to 0.3±0.7% and 4.3±4.5%, respectively. The proportion of goblet cells with AAL-positive content decreased from 14.6±7.5% to 2.9±4.3% and 9.2±7.8%, respectively. In the rabbit tracheal epithelium, adrenergic stimulation dramatically lowered the proportion of goblet cells containing fucosylated glycoconjugates. This decrease was mostly at the expense of α(1-2) fucosylated glycoconjugates and begun to recover 20 min post exposure.

Key words: epinephrine, lectin histochemistry, airways' epithelium, goblet cells, fucosylation.

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Introduction

Castells et al. (1991) have revealed fucosylated glycoconjugates in both secretory granules and cell membranes. Such glycoconjugates, secreted as components of airways' mucus, contribute substantially to its viscoelastic properties as evidenced by Finkbeiner (1999) and Majima et al. (1999). Both secreted and bound to cellular surfaces, they also serve as adhesion sites for various antigens (Adam et al., 1997; Gebert & Posselt, 1997; López-Ferrer et al., 2000). Increased proportion of fucosylated glycoconjugates in the airways' secretion has been described in sinusitis (Otori et al., 1998), chronic bronchitis (Finkbeiner, 1999; Freney et al., 2001), asthma (Finkbeiner, 1999), cystic fibrosis (Finkbeiner, 1999; Glick et al., 2001) and acute bronchiolitis in rats (Ishihara, 2002). In adult beings, the α(1-2) linkage of fucose to galactose is the most common (Sato et al., 1986; Biol-N'Garagba et al., 2002). Fucosylation in other linkages, α(1-3), α(1-4), and α(1-6), respectively, to N-acetyl glucosamine, increases under pathological conditions like inflammation (Otori et al., 1998; Blander et al., 1999; Ishihara, 2002) or cystic fibrosis (Glick et al., 2001) at the expense of α(1-2) position and sialylation as well.

Intravenous administration of epinephrine in the dose 10 μg per 1 kg of body weight is used in status asthmaticus in children as well as in controlling anaphylactic shock (0.3–0.5 mg pro toto) or in cardiac arrest (20 μg per 1 kg of body weight). Such an administration can immediately influence the environment within the airways. Guyton & Hall (1996) have described a slight to moderate increase in the volume of secretion as the primary direct result of adrenergic stimulation of secretory elements. In general, the mucus-secreting cells release most often concentrated secretion with changed composition. As a dual effect, vasoconstriction follows reducing rates of secretion. As demonstrated by Konrádová et al. (1999), tracheal goblet cells in rabbits have been overstimulated and damaged after administration of the same dose of epinephrine as in the present study. Since adrenergic stimulation lowers the proportion of sialylated glycoconjugate-containing tracheal goblet cells significantly (Vajner et al., 2001), we have decided to evaluate the proportion of fucosylated glycoconjugate-containing goblet cells as well.

Material and methods

Ten SPF New Zealand White male rabbits (Charles River, Sulzfeld, Germany) of the average body weight 2,420±507 g were used. Six of them were intravenously (i.v.) administered with 10 μg/kg of epinephrine (Adrenalin, Léčiva, Praha, Czech Republic) and divided into two groups of three animals. The material was collected 5 minutes and 20 minutes post exposure, respectively. Four rabbits served as untreated healthy controls; the material was collected immediately after the induction of anaesthesia.

The middle portions of tracheae between the 15^{th} and 20^{th} tracheal rings were formalin-fixed, paraffin-embedded, and cut at 5–7 μm. The combined staining method of Alcian Blue (AB) at pH 2.5 followed by PAS-reaction according to Mowry & Winkler (1956) was used to reveal both total acidic and neutral glycoconjugates, i.e. to reveal total number of goblet cells. Thus, in each given method, we evaluated 398 goblet cells in controls; 313 and 346 goblet cells were evaluated in experimental animals, respectively. To detect fucosylated glycoconjugates, the methods of *in situ* lectin histochemistry were used. The legume lectin of *Ulex europaeus* (ULE-I), detecting terminal or branched fucose (Fuc) linked α(1-2) to an oligosaccharide, and the ascomycete orange-peel mushroom *Aleuria aurantia* lectin (AAL), detecting preferentially α(1-6), but also α(1-3) and α(1-4), linked fucose residues (Spicer & Schulte, 1992), were employed (Vector Laboratories, Inc., Burlingame, USA). Sections were dewaxed by xylene and rehydrated in graded alcohol series; no further delipidation was performed. The endogenous peroxidase (Px) was blocked and sections were incubated with biotinylated ULE-I or biotinylated AAL or both lectins simultaneously in concentrations 30 μg/mL for 60 min. Then, the sections were incubated with the solution of streptavidin-horseradish Px conjugate (Vector Laboratories, Inc., Burlingame, USA) in the concentration 2 μg/mL for 45 minutes, followed by Sigma *FAST*$^{\text{TM}}$ DAB Peroxidase Substrate Tablets visualisation (Sigma-Aldrich Chemie, Deisenhofen, Germany) enhanced by $CuSO_4$. Blocking of endogenous Px was verified by omitting the first step of the method. Specific lectin binding was verified by 15-minute incubation of lectins with control substrate – 0.2 M L-fucose, preceding the incubation with sections (Castells et al., 1991). We evaluated only goblet cells containing well-developed granules with the positive reaction in each method used. The granules had to fill at least 2/3 of a goblet cell. Simultaneous use of both lectins made possible identification of goblet cells containing glycoconjugates positive for both lectins; the overlap of AAL- and ULE-I-positive goblet cells could be thus calculated. Venn's diagram enabled the calculation of goblet cells containing ULE-I-positive granules only and goblet cells containing AAL-positive granules only, respectively, as well.

For statistical evaluation, relative values of the five categories of goblet cells, revealed by individual methods (total goblet cells, ULE-I-positive gob-

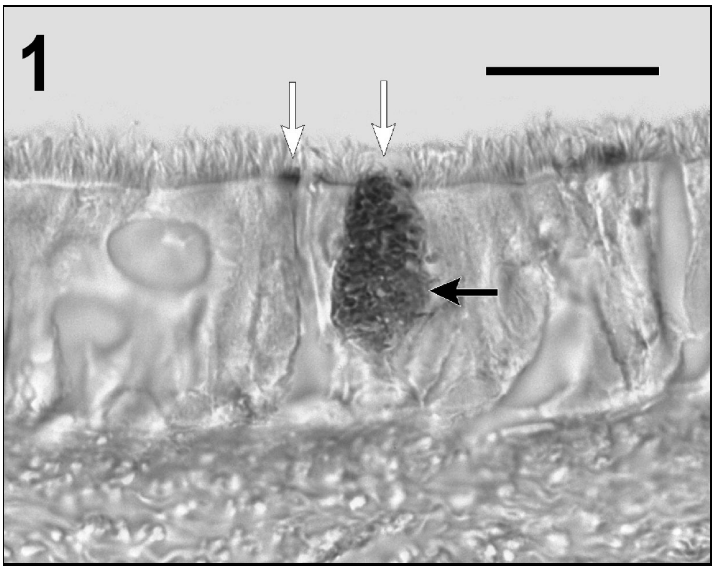

Fig. 1. Goblet cell (black arrow) containing ULE-I-positive mucous granules in a control rabbit. White arrows indicate ULE-I-positive surface of goblet cells. Bar = 20 μm.

let cells, AAL-positive goblet cells, total lectin-positive goblet cells, and goblet cells with calculated overlap of the ULE-I-AAL-positive reaction) were evaluated by the chi-square test of homogeneity in frequency tables, using the Yates' correction in low frequencies when appropriate (Statistica, v.6.0 software). The significance of differences between fucosylated glycoconjugate-detecting methods was tested by the matched t-test, Spearman rank correlation, matched sign test, and Wilcoxon's paired test (BMDP new system software).

The experimental procedures were performed under general anaesthesia (ketamine 35 mg/kg and xylazine 5 mg/kg intramuscularly) and after local subcutaneous infiltration of the ventral cervical field with procaine. the procedure was approved by the Animals Protection Expert Commission of the Faculty.

Results

The tracheae of both control and treated rabbits were lined with the pseudostratified columnar ciliated epithelium composed mostly of ciliated, goblet, and basal cells. The height of the epithelium was approximately 30 μm. The distribution of secretory elements was irregular.

Using conventional histochemical methods, the secretory elements revealed typical staining patterns according to the type of glycoconjugates they contained. PAS-positive mucous granules were stained magenta; Alcian Blue stained mucous granules blue. some goblet cells exhibited various shades of violet colour; these cells were counted as containing acidic glycoconjugates in the mixture with neutral ones.

The appearance of goblet cells reacting with lectins used was the same in both control and treated rabbits. The positive reaction of ULE-I (Figs 1,2) was featured with the conspicuous staining of mucous granules in an individual goblet cell, either in the whole volume of a granule, or as a densely contrasted ring; staining of the ciliary border was restricted to the close vicinity of apical surfaces of goblet cells. AAL-reaction depicted individual mucous granules as dark rings. The ciliary border was always densely stained, too (Fig. 3).

In healthy control rabbits, we revealed 38.9$\pm$ 19.9% of ULE-I-positive goblet cells and 14.6$\pm$ 7.5% of AAL-positive goblet cells. The simultaneous use of both lectins revealed 44.2$\pm$22.1% of total goblet cells (Fig. 4). PAS-positive goblet cells represented 1.5$\pm$2.4% of total goblet cells. Having compared the sum of percentages of goblet cells positive for both lectins used individually with the percentages of goblet cells positive for both lectin simultaneously using the Venn's diagram (Fig. 5), the calculation gave 29.6% of goblet cells containing ULE-I-positive granules only and 5.3% of goblet cells containing AAL-positive granules only, respectively. Goblet cells containing granules positive for both lectins represented 9.3%.

Five minutes after the administration of epinephrine, 0.3$\pm$0.7% of goblet cells with ULE-I-positive granules and 2.9$\pm$4.3% of goblet cells with AAL-positive granules were found. The simultaneous use of both lectins revealed 3.2$\pm$4.0% of total

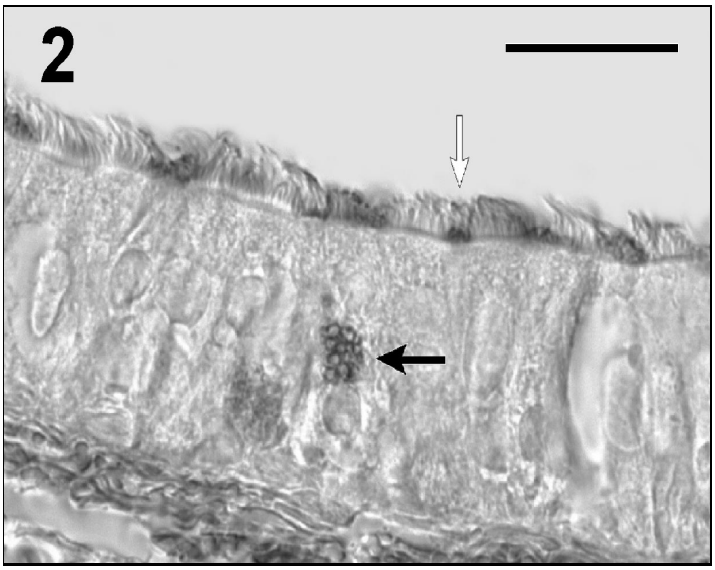

Fig. 2. Goblet cell (black arrow) containing ULE-I-positive mucous granules 20 min after administration of epinephrine – recovery of the granule formation. White arrow indicates ULE-I-positive surface of another goblet cell. Bar = 20 μm.

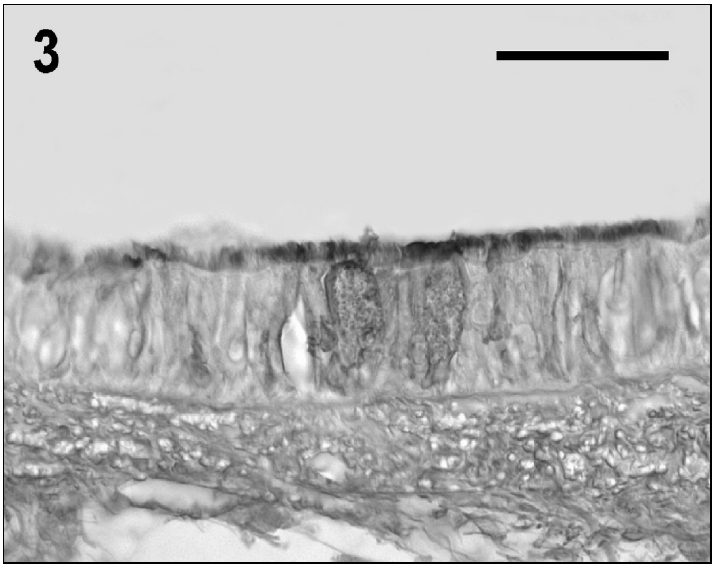

Fig. 3. Goblet cells containing AAL-positive mucous granules 20 min after administration of epinephrine. Note the intensive staining of the ciliary border. Bar = 50 μm.

goblet cells (Fig. 4). PAS-positive goblet cells disappeared completely from the tracheal epithelium. Having compared the sum of percentages of goblet cells positive for both lectins used individually with the percentages of goblet cells positive for both lectin simultaneously using the Venn's diagram (Fig. 5), the calculation gave 0.3% of goblet cells containing ULE-I-positive granules only and 2.9% of goblet cells containing AAL-positive granules only, respectively. Goblet cells containing granules positive for both lectins were not identified at all.

Twenty minutes after the administration of epinephrine, 4.3±4.5% of goblet cells with ULE-I-positive granules and 9.2±7.8% of goblet cells with AAL-positive granules were found. The simultaneous use of both lectins revealed 9.2±7.8% of total goblet cells (Fig. 4). PAS-positive goblet cells did not still reappear in the tracheal epithelium. Having compared the sum of percentages of goblet cells positive for both lectins used individually with the percentages of goblet cells positive for both lectin simultaneously using the Venn's diagram (Fig. 5), the calculation gave no goblet cells

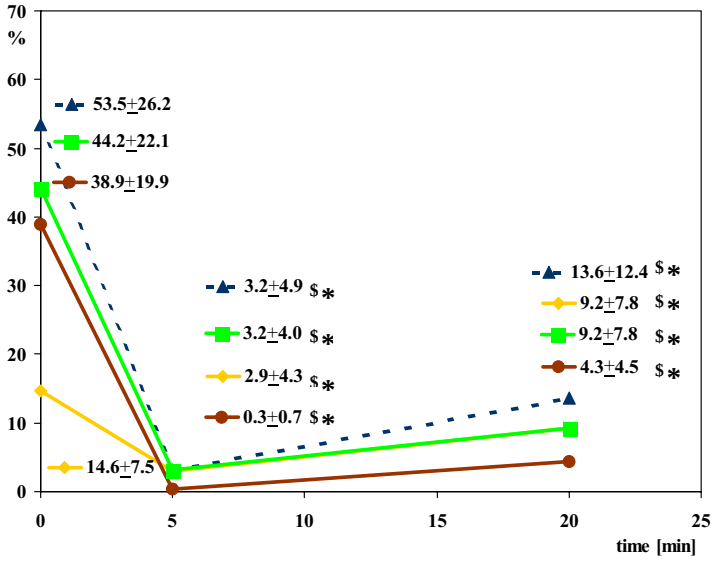

Fig. 4. Changes in percentage of goblet cells in the tracheal epithelium containing fucosylated glycoconjugates 5 min and 20 min after administration of epinephrine. Lectin histochemistry. Sum = the sum of percentages of goblet cells detected individually, sim = the percentage of goblet cells detected simultaneously. Means ± SD.
* Corresponding values (5 and 20 min) significantly differ ($a = 0.05$) from each other
$ Corresponding values (5 and 20 min) significantly differ ($a = 0.05$) from controls (0 min)

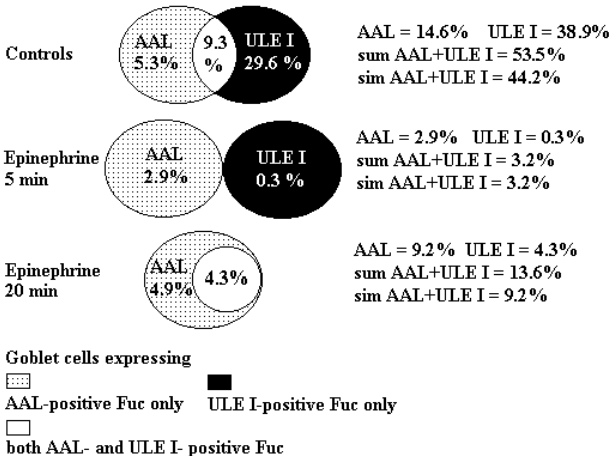

Fig. 5. Proportions of goblet cells containing AAL-positive granules only, ULE-I-positive granules only, and both lectin-positive granules. Venn's diagram. Sum = the sum of percentages of goblet cells detected individually, sim = the percentage of goblet cells detected simultaneously.

containing ULE-I-positive granules only and 4.9% of goblet cells containing AAL-positive granules only, respectively. Goblet cells containing granules positive for both lectins represented 4.3%.

The statistical significance of differences between individual groups of goblet cells is given in Figure 4.

Discussion

The methods used allowed us to give the proportion of goblet cells containing granules with α(1-2)-fucosylated glycoconjugates and goblet cells containing granules with α(1-6)-, α(1-3)-, and α(1-4)-fucosylated glycoconjugates directly. Spicer et al. (1983) and Robinson et al. (1986) held PAS-reaction as specific for fucose moieties. Since PAS-positive goblet cells represented only minor population in controls or disappeared completely in administered animals, it should be concluded that fucosylated glycoconjugates were components of mucous granules containing also acidic glycoconjugates. This opinion was supported by both conventional and lectin histochemistry. Staining patterns of mucous granules and ciliary border could implicate for airways' epithelium that α(1-2)-fucosylation in healthy adult beings prevails in secretions (Mariassy et al., 1988); on the other hand next types of fucosylation were more expressed in membrane bound glycoconjugates. The significant decrease of proportion of goblet cells containing fucosylated glycoconjugates

could be a result of releasing those glycoconjugates into tracheal secretion after adrenergic stimulation. This was in accordance with findings on the increased viscoelasticity of tracheal mucus after β-adrenergic stimulation (KING & ANGUS, 1981).

Quantification of goblet cells containing individual fucosylated glycoconjugates in controls corresponded with the fact that α(1-2)-fucosylated glycoconjugates were mostly found in adults under physiological conditions (SATO et al., 1986; BIOL-N'GARAGBA et al., 2002). The shifts in percentages of ULE-I-positive goblet cells and AAL-positive goblet cells might reflect the shift in glycosylation because GLICK et al. (2001) supposed erroneous compartmentalisation in the Golgi complex under pathological conditions; substrates thus might meet other fucosyltransferases earlier than α(1-2) fucosyltransferase or sialyltransferases. Alternative explanation could be based on the selective release of ULE-I-positive mucous granules after stimulation of secretory elements. Both above-mentioned mechanisms could be the background of findings by VAJNER et al. (2001) concerning changes in proportion of goblet cells containing sulphated and sialylated glycoconjugates, respectively. Ultrastructural findings in the tracheal epithelium after the i.v. administration of epinephrine (KONRÁDOVÁ et al. 1999), that indicate the overstimulation of majority of secretory elements resulting even in their damage and degeneration, could be a picture of selective release of secretion from goblet cells containing fucosylated glycoconjugates, too. This finding also supported the premise that fucosylated glycoconjugate-containing mucus played the protective role against some microbes preventing them to adhere on cell surfaces (ADAM et al., 1997; GEBERT & POSSELT, 1997; LÓPEZ-FERRER et al., 2000).

We could conclude that the i.v. administration of epinephrine dramatically lowered the proportion of goblet cells containing fucosylated glycoconjugates. This decrease was mostly at the expense of α(1-2)-fucosylated glycoconjugates and lasted even 20 min post exposure with only slight shift to recovery.

Such changes can substantially influence the inner environment of airways, e.g. viscoelastic properties of the airways' mucus and mucosal barrier functions. Thus, it is necessary to count on these effects after the emergency use of epinephrine as well as in patients suffering from chronic respiratory diseases.

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