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EFFECT OF BRONCHIAL MUCUS OF BRONCHIAL ASTHMA PATIENTS AND CHRONIC BRONCHITIS ON CILIARY ACTIVITY OF CILIATED EPITHELIAL CELLS

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Defects of ciliary motility of ciliated epithelial cells in the bronchi may be primary in character and may arise as a result of organic changes in the contractile apparatus of the cilia (the tubulin-dynein complex), as is found in Kartagener's syndrome [4]. Meanwhile, disturbance of ciliary motility may be secondary and due to functional (reversible) changes arising as a result of inflammatory or allergic processes, increased viscosity of the mucus, and other pathological conditions. The possibility of the appearance of various factors influencing ciliary motility in the ciliated epithelium of patients with bronchopulmonary pathology has been widely discussed in the literature. The presence of glycoprotein factors, inhibiting ciliary motility in the ciliated epithelium in vitro in biopsy material taken from animal tissues has been observed to be present in the serum [2, 13, 14], blood plasma [5], and lymphocytes and mononuclear leukocytes [15] of patients with mucoviscidosis, bronchial asthma, and various respiratory and autoimmune diseases. In mucoviscidosis, the patient's saliva also possesses inhibitory activity [2]. Some species of microorganisms, which are agents of bronchopulmonary diseases, can produce compounds inhibiting ciliary motility [7]. Among the list of substances capable of disturbing normal ciliary functions may be mentioned the major basic protein (MBP) of eosinophils, which accumulates in the blood and sputum of patients with lung diseases [8]. Nevertheless, the information given on factors disturbing ciliary motility in the ciliated epithelium is contradictory in character and has not always been confirmed by analysis of biopsy material from the human mucosa [11]. This may indicate that the inhibitory effect observed is partly the result of species-specific incompatibility [10]. The most encouraging results have been obtained by the investigation of the inhibiting activity of sputum. The sputum of patients with bronchial asthma [5, 6] and bronchiectasis [12] has been found to contain factors other than MBP and the blood glycoproteins which reduce ciliary motility of the ciliated epithelium in vitro in experiments with biopsy material both from animal tissues and from the human mucosa. The cilioinhibitory action of sputum in bronchial asthma is reversible, it does not cause ultrastructural changes in the cilia, and the intensity of its manifestation depends on the patient's clinical state [5, 6]. As an original clinical material, sputum corresponds more closely than others to the medium in which the

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cilia of the bronchial ciliated epithelium actually beat. However, this material likewise is not homogeneous, for it may be contaminated with saliva and gastric juice.

This paper describes the study of the ciliostatic activity of bronchial secretion and of the possible mechanism of this activity in various forms of bronchial pathology.

EXPERIMENTAL METHOD

Specimens of bronchial secretion obtained during diagnostic fiberoptic bronchoscopy under local anesthesia were studied. The bronchial secretion was withdrawn through the carefully treated and intact first channel of a BF-2TR two-channel fibroscope (Olympus, Japan) directly into a centrifuge tube, and then centrifuged as described in [6] at 30,000*g* for 40 min at 5°C to separate the liquid part of the secretion from floccules of mucus and other solid impurities. The supernatant was carefully drawn off and its pH measured. Only specimens with pH $\geq$ 6.5 were used for analysis of their ciliostatic activity in order to exclude any inherent inhibitory effect of this parameter on motility of the cilia [5]. The volume of the specimens thus obtained varied from 0.1 to 1.5 ml. If necessary the material obtained was frozen and kept at −18°C for 2-3 weeks, without any effect on its activity. The ciliostatic activity of the bronchial secretion was tested in a system with biopsy material obtained from the frog's palatal mucosa by the method in [6] with certain modifications. Pieces of mucosa measuring 3 × 3 mm were placed in wells of Terasaki's planchets, covered with Ringer's solution for cold-blooded animals in the control, and with the test specimen of bronchial secretion in the experiment; the wells were tightly covered with a coverslip and motility of the cilia was noted along the edge of the preparation by phase-contrast microscopy under a magnification of 50 times. Observations were made at room temperature. The specimen of bronchial secretion was considered to possess ciliostatic activity if it caused total arrest of the cilia in the preparation in the course of 120 min or less. For each specimen the time of complete arrest of the cilia was recorded. In the control preparation no visible changes in motility of the cilia took place during 120 min. The investigation of the effect of the bronchial secretion on reactivation of the cell models of ciliated epithelium was carried out on glycerol models of frog palatal epithelium, obtained by the method in [1]. Pieces of epithelium measuring 5 × 5 mm, rendered permeable by glycerol, were thoroughly washed to remove the glycerol in a solution containing 20 mM sodium phosphate, pH 7.0, 120 mM KCl, and 5 mM $MgCl_2$ (rinsing solution − RS). Rinsed specimens of epithelial models were incubated for 15 min in samples of bronchial secretion, diluted 1:1 with a solution twice as concentrated as RS. Control specimens of modeless were incubated for the same length of time in RS. The models in the control and experiment were reactivated by the addition of ATP solution to the incubation medium up to a final concentration of 5 mM, after which scrapings of mucus were quickly obtained from the surface of the epithelium on a slide, 1 or 2 drops of the corresponding incubation medium with ATP were added, and the slide was examined under the phase-contrast microscope. The inhibitory effect was assessed at four levels: +) reactivation as in the control, ±) inhibition of reactivation by 50% (about 50% of the models did not become reactivated), ∓) considerable inhibition of reactivation (70-80% of the models were not reactivated), −) total inhibition of reactivation.

EXPERIMENTAL RESULTS

Two groups of patients were investigated. Group 1 consisted of 30 patients with bronchial asthma (infectious-allergic and noninfectious allergic forms). In 26 patients of this group (15 in a stage of exacerbation, 12 in a stage of relative remission) specimens of bronchial secretion showed ciliostatic activity to a varied degree: the time for total arrest of the cilia varied from 10 to 120 min. In four patients (one in the stage of relative remission, three in the remission stage) specimens of bronchial secretion did not possess ciliostatic activity. The results are in full agreement with data obtained by analysis of the sputum of patients with bronchial asthma [5, 6].

Group 2 consisted of 15 patients with chronic catarrhal bronchitis. In 12 specimens of bronchial secretion ciliostatic activity was found (seven from patients in a stage of exacerbation, five from patients in a stage of relative remission). The time for total arrest of the cilia under the influence of bronchial secretion from patients with bronchitis varied within the same limits as in patients with asthma. Specimens of bronchial secretion from three patients did not exhibit activity (two in a stage of relative remission, one in a remission stage). Incidentally, no ciliostatic activity was found in the sputum of patients with chronic bronchopulmonary diseases without an asthmatic syndrome [6], although unlike in our investigation, the patients tested were in a stable clinical state and not in a state of exacerbation.

The cytostatic activity of the bronchial secretion discovered both in asthma and in bronchitis still continued if the specimens of secretion were diluted 1:1 or 1:2 with Ringer's solution or RS.

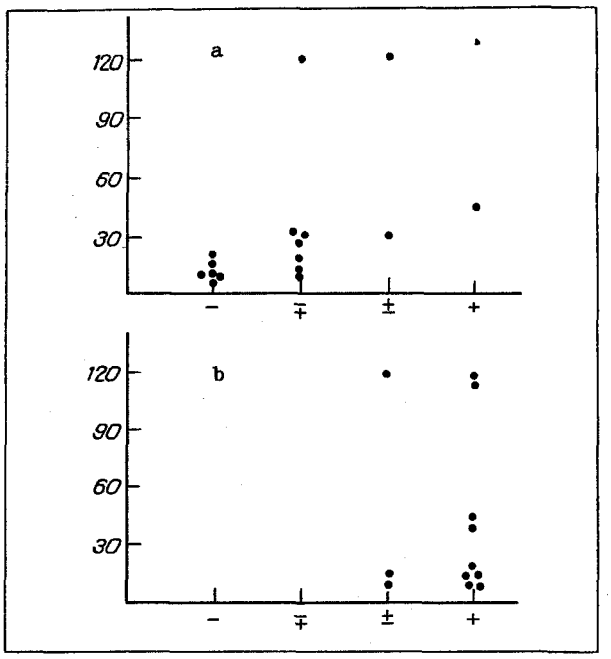

Fig. 1. The effect of bronchial secretion on motility of cilia belonging to ciliated epithelial cells and on reactivation of glycerol models of these cells: a) bronchial asthma, b) chronic bronchitis. Abscissa, degree of inhibitory effect of same specimens of secretion on reactivation of models (in conventional units); ordinate, time of total arrest of cilia in preparation under the influence of test specimens of bronchial secretion (in min; ciliostatic activity).

To study the mechanism of the ciliostatic activity, the effect of specimens of bronchial secretion was studied on motility of cilia belonging to cell models under the influence of exogenous ATP. According to [1], if any factor induces a disturbance of ciliary motility in a living cell, and also in a model of that cell, it can be concluded that this factor acts directly on the contractile apparatus of the cilia (the tubulin-dynein complex). If, however, in a reactivated model preparation, unlike in the living cell, no disturbance of ciliary motility takes place, the action of the factor is aimed not at the contractile apparatus, but at other cell systems connected with it. Altogether 16 specimens of bronchial secretion possessing ciliostatic activity from patients with bronchial asthma and 12 active specimens of secretion from patients with bronchitis were studied. The results of this investigation are given in Fig. 1. They show that specimens of bronchial secretion possessing ciliostatic activity, obtained from patients with bronchial asthma, significantly inhibited reactivation of the models, possible evidence that the activity thus discovered acts directly on the tubulin-dynein complex of the cilia. A similar mechanism was clearly established for activity determining the inhibitory action of seminal fluid on motility of sperm in asthenospermia [9]. By contrast, in bronchitis specimens of bronchial secretion possessing ciliostatic activity had virtually no effect on reactivation of the models of ciliated epithelium, possible evidence that they act by a different mechanism.

In three patients with bronchial asthma and two patients with bronchitis (all in a stage of exacerbation of the disease) the ciliostatic activity of the bronchial secretion and the effect of the secretion on reactivation of the models in the course of traditional treatment were investigated. A positive clinical trend both in asthma and in bronchitis was accompanied by a fall of ciliostatic activity, expressed as an increase in the time required to produce total arrest of the cilia in the test preparation. None of the preparations of bronchial secretion from patients with bronchitis had any effect on reactivation of the models. In asthma, weakening of ciliostatic activity of the secretion correlated with weakening of the inhibitory action on reactivation of the models. Specimens of bronchial secretion of patients with asthma, in which no ciliostatic activity was found, did not inhibit reactivation of the models. These results may be evidence of the reversible character of the disturbance of ciliary motility on ciliated epithelium under the influence of the ciliostatic activity of the bronchial secretion in the forms of bronchopulmonary pathology studied.

The investigation thus showed that the bronchial secretion of patients with bronchial asthma and chronic bronchitis may possess ciliostatic activity. Activity was found with the greatest frequency during clinical exacerbations and is evidently reversible in character. The mechanisms of action of the ciliostatic activity differ in asthma and in bronchitis.

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