logically active substances from target cells (mast cells) of the allergic reaction, and by a decrease in the sensitivity of the effector tissues to the liberated mediators of anaphylaxis and, in particular, to histamine.

These findings will serve as the basis for future research aimed at conducting clinical trials of MIBX.

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ADRENERGIC AND CHOLINERGIC STRUCTURES OF THE LUNGS IN NORMAL GUINEA PIGS AND IN GUINEA PIGS WITH EXPERIMENTAL BRONCHIAL ASTHMA

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The morphological and functional state of the adrenergic and cholinergic innervation of the bronchopulmonary apparatus of guinea pigs was studied by luminescence and histochemical methods under normal conditions and during the course of sensitization (subcutaneous and inhalation methods) by the allergen of the mite *Dermatophagoides pteronyssinus*. Considerable excitation of the adrenergic innervation and a fall in acetylcholinesterase activity were observed in the bronchopulmonary tissue during sensitization of the animals.

KEY WORDS: *allergy; bronchial asthma; adrenergic system; cholinergic system.*

There is clinical evidence in the literature [4-6, 12, 14, 15] of the pathogenetic role of the allergenic component of the mite *Dermatophagoides pteronyssinus* in the development of bronchial asthma and other allergic diseases in man. It is claimed that the pathogenesis of allergic bronchial asthma is based on the hereditary or acquired blockage of the β-adrenergic receptors of the bronchopulmonary apparatus [1, 2]. The β-adrenergic theory of development of bronchial asthma is a significant addition to the allergic theory of its development.

The aim of the present investigation was to study the state of the adrenergic and cholinergic structures of the bronchopulmonary apparatus in normal guinea pigs and during the development of sensitization of the animals (produced by different methods) by the allergen of the mite *Dermatophagoides pteronyssinus*.

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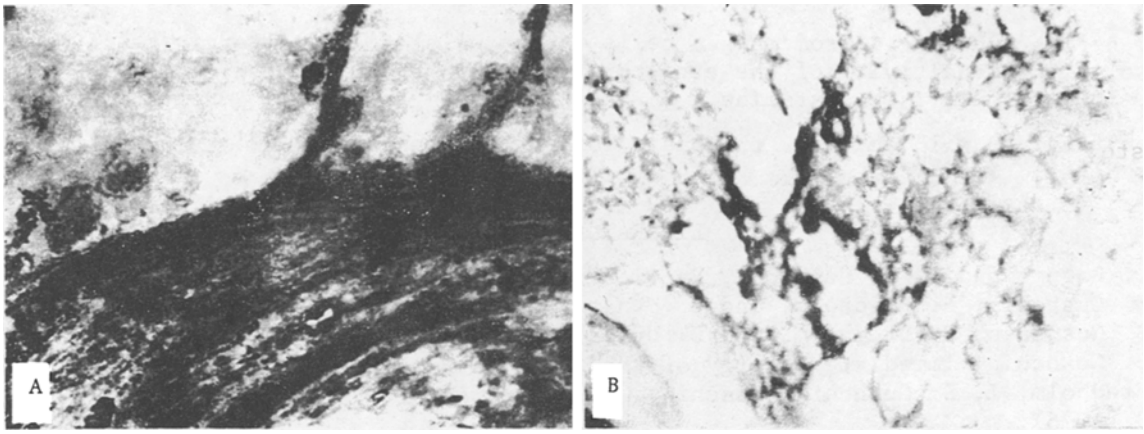

Fig. 1. Distribution of ACE in wall of bronchus of intact guinea pig (A) and in lung tissue of guinea pig after reacting inhalation (B). A) Objective 90×, homal 3×; B) objective 40×, homal 3×.

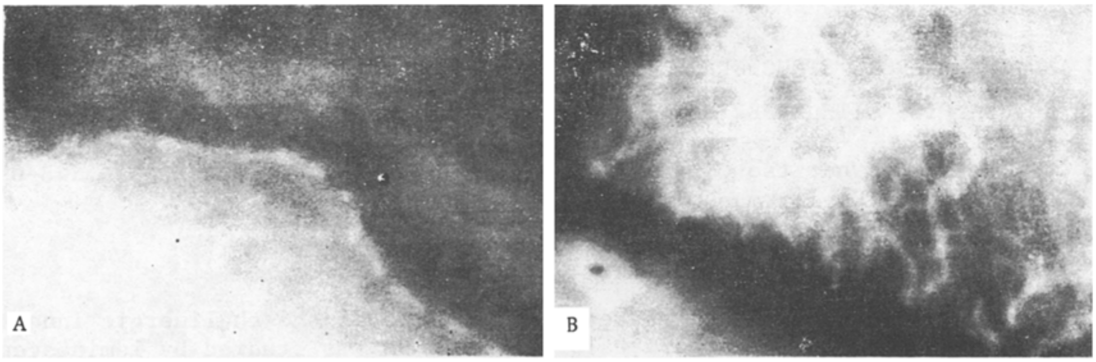

Fig. 2. Single terminal adrenergic fiber and diffuse distribution of catecholamines in lung tissue (A), and plexus of elastic fibers against background of diffuse fluorescence of catecholamines in submucosa of bronchus (B) of guinea pig after reacting inhalation. Objective 90×, homal 3×.

EXPERIMENTAL METHOD

Experiments were carried out on 56 guinea pigs of both sexes. The control group consisted of 10 intact animals; 16 guinea pigs were sensitized with *D. pteronyssinus* allergen by subcutaneous injection and 30 guinea pigs received the same allergen by inhalation as an aerosol. The allergen was prepared from mites grown in an incubator [5, 9]. The mass of mites, after treatment with ether, was covered with Kok's fluid with phenol, pH 7.2, in the ratio of 5:100 and extracted for 2-3 days in a refrigerator. After centrifugation at 6000 rpm for 30 min the allergen was filtered through a finely porous filter under sterile conditions. Each batch of allergen was kept in field ampuls in the refrigerator for not more than 15 days. For inhalation sensitization the allergen was diluted from 6:100 to 10:100. For subcutaneous sensitization five injections of increasing doses of a 5% solution of the allergen were given. Inhalation sensitization was carried out in a chamber 0.8 m^3 in volume (with the tube of the atomizer fixed into the side and with the air supply through a hole in the floor). The aerosol was dispersed by means of the UI-2 universal inhaler with a working pressure of 1.2 kg/cm^2. The particle size of the aerosol varied from 0.5 to 5.0 μ. Sensitization by the inhalation method consisted of eight cycles. Each cycle consisted of two daily inhalations for 60 min each. The reacting inhalation was given 14 days after the end of the last cycle.

The external respiration of the animals was recorded by no-contact chamber pneumography on the ÉLKAR-2 instrument [8].

The state of the cholinergic structure and the acetylcholinesterase (ACE) activity of the bronchopulmonary tissue of the guinea pigs were investigated by a histochemical method [13]. Adrenergic structures were studied by a modified [3] luminescence method [10, 11].

EXPERIMENTAL RESULTS

Some immunogenic changes in guinea pigs after sensitization by the allergen of the mite *D. pteronyssinus* were described previously [7].

Histochemical investigation of the bronchopulmonary tissue of the intact guinea pigs revealed quantitative predominance of cholinergic over adrenergic innervation structures. Both in the alveolar system and in the walls of the bronchi and bronchioles ACE was distributed fairly uniformly (Fig. 1A). Comparatively large cholinergic nerve trunks approached the adventitia of the bronchi and bronchioles, where they formed dense plexuses in a circular direction.

The adrenergic innervation of the bronchi and bronchioles was apparently inconstant in character, whereas in the alveolar tissue of the lung adrenergic nervous structures were seen extremely rarely. The lung tissue itself gave a bright luminescence, probably due to the presence of elastic fibers (autoluminescence). A few solitary luminescent mast cells were found. As a rule the cytoplasm of the mast cells was filled with finely granular material with whitish-yellow luminescence, and in the middle of the cell the nucleus could be seen as a dark area. Considerable ACE activity also was observed in the blood cells in the lumen of the vessels.

After the third sensitization, corresponding to the 14th day after the first subcutaneous injection of the allergen, adrenergic structures were more frequently found in the bronchopulmonary tissue of the animals. At the height of sensitization, i.e., on the 14th day after the fifth sensitizing injection of the allergen, the number of cholinergic structures observed was considerably reduced, possibly on account of a decrease in the activity of the enzyme not only in the alveolar system, but also in the walls of the bronchi and bronchioles.

In the course of sensitization, the background luminescence of the lung tissue became green or even dark green, evidence of the appearance of catecholamines. Nerve fibers with varicose outlines were frequently seen approaching the adventitia of the bronchi and bronchioles. At the height of sensitization adrenergic nerve plexuses in the walls of the bronchi and bronchioles were observed much more frequently than normally or in the course of sensitization. Mast cells were appreciably more numerous than normally in the lung tissue, chiefly around the bronchi and bronchioles.

In the case of inhalation sensitization the changes were similar but were more marked. In the course of sensitization, irregular intensive green luminescence of the lung tissue was observed, indicating a diffuse distribution of catecholamines. Against the background of this luminescence, many mast cells of different sizes, with signs of degranulation, could be seen. Degranulating mast cells were frequently found also in the lumen of the bronchioles.

Disturbances of external respiration of the guinea pigs, associated with bronchospasm, developed after the second and third cycles of sensitization and increased appreciably with each inhalation. Two of the experimental guinea pigs (8%) died in the course of sensitization from anaphylaxis with features of asphyxia. After the reacting inhalation six guinea pigs (20%) died as a result of severe asphyxia. In the remaining animals disturbances of external respiration due to bronchospasm were observed.

On histochemical investigation of lung tissue taken at the height of bronchospasm, the parenchyma of the lung consisted of areas of focal emphysema alternating with areas of atelectasis. The muscle bundles of the bronchi and bronchioles were thickened and contracted and their mucous membrane was folded. Activity of ACE was sharply reduced both in the alveolar system and in the wall of the bronchi and bronchioles (Fig. 1B). The number of detectable adrenergic nerve fibers was only rarely increased. Solitary luminescent nerve fibers with a varicose outline were observed in both the peribronchial and the interalveolar tissue (Fig. 2A). In the course of sensitization and, in particular, after the reacting inhalation, terminal plexuses of adrenergic nerve fibers and the elastic tissue of the bronchi were detected with steadily increasing frequency (Fig. 2B).

The preliminary conclusion can thus be drawn that in healthy guinea pigs the cholinergic system is morphologically predominant over the adrenergic system. In the course of sensitization (especially by the inhalation method) the activity of the adrenergic system increases significantly, probably in connection with adaptive and compensatory reactions of the body. The death of some animals after the reacting inhalation, associated with severe bronchospasm and, probably, with a considerable fall of the blood pressure, may be connected with a disturbance of the sensitivity of the adrenergic receptors.

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