SENSITIVITY OF THE SMOOTH MUSCLES OF THE GUINEA PIG TRACHEA TO BACTERIAL ALLERGENS

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Bacterial allergy is a leading component in the pathogenesis of several diseases of the human respiratory tract: rhinosinusopathies, chronic bronchitis, infectious-allergic bronchial asthma [2, 5]. However, methods of estimating the specific activity of bacterial allergens that are widely used for the diagnosis and treatment of infectious-allergic diseases are extremely limited in number [10]. The main test used to verify the specific action of infectious allergens in patients with diseases of infectious-allergic nature or in sensitized animals is still the skin test.

However, a positive skin test is not always direct proof of an allergic state of smooth-muscle organs [4, 6, 7], and for that reason the reaction of anaphylactic contraction of smooth muscles of isolated organs (uterus, segment of small intestine, bronchus) has been used by many workers to study the specific action of allergens of noninfectious and infectious origin on cells of a sensitized organ [14, 15].

The Schultz–Dale reaction with bacterial allergens was used in a number of investigations [4, 7, 8]. The authors cited found a high degree of specificity of the resulting contraction of smooth muscles of the small intestine of sensitized guinea pigs.

In diseases of the respiratory tract and, in particular, in infectious asthma, an allergic reaction of the smooth-muscle elements of the bronchus is observed, and accordingly preparations of the tracheobronchial chain of sensitized animal provide the optimal model with which to study the specific action of an allergen on bronchial tissues.

The aim of the present investigation was to study the possibility of using the test of anaphylactic contraction of the tracheal chain (TC) of guinea pigs in order to evaluate the biological activity of bacterial allergens. Allergens of nonpathogenic neisserias (*Neisseria perflava*), allergy to which, according to data in the literature [3], is found in most patients with infectious asthma, were used as the test object.

EXPERIMENTAL METHOD

Cultures of *N. perflava*, strains Nos. 3 and 13, *Klebsiella pneumoniae* strain D-va, and *Staphylococcus aureus* strain No. 3, isolated from patients with infectious asthma, were used. The basis for preparation of the bacterial allergens was the method of growing cultures on solid nutrient media covered with cellophane disks [13]. By means of a sterile spatula an 18-h culture was distributed uniformly over the surface of a cellophane disk. The seeded plates were kept for 20 h at 37°C. The cultures were then washed off with physiological saline. Films for checking purity were then made from the bacterial suspension and the concentration of the suspension determined against a bacteriological turbidity standard, supplied by the L. A. Tarasevich State Research Institute for Standardization and Control of Medical Biological Preparations.

The reactions of anaphylactic contraction of TC of sensitized guinea pigs was carried out with material from a disintegrated neisserial culture. The neisserias were disintegrated by treatment with ultrasound, after which the destroyed cells were removed by centrifugation. The protein content in the supernatant was determined by Lowry's method.

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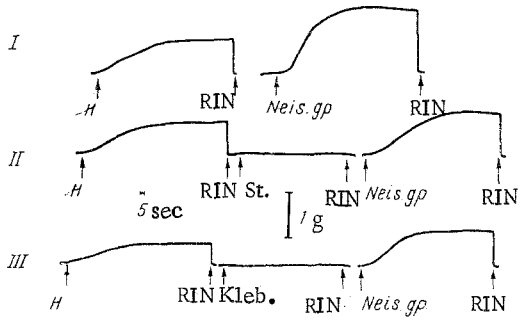

Fig. 1. Anaphylactic reactions of TC from guinea pigs sensitized with living culture of neisserias to various bacterial allergens. I) Reaction to histamine (H) and specific allergen (neisseria — Neis.); II) reaction to histamine (H), nonspecific (staphylococcus — St.), and specific allergen; III) response to histamine (H), nonspecific (klebsiella — Kleb.), and specific allergen. RIN) Rinsing with specific solution.

Thirty guinea pigs were sensitized by two injections of 1 and 2 billion bacterial cells of a 24-h culture of neisserias respectively into the hind footpads.

The degree of sensitization of the animals was assessed by the active cutaneous anaphylaxis test (ACA) according to [16], by reproduction of anaphylactic shock and estimation of the shock index as in [17].

Titrated neisserial antigen, injected intradermally into a sensitized animal in a volume of 0.1 ml, was used for the ACA test. An injection of 0.25 ml Evans' blue was given intravenously 30 min later. The reaction was read 30-40 min later, according to the diameter of the stained spot.

The state of anaphylaxis in the animals was confirmed by a morphological control. When a marked degree of sensitization of the guinea pigs to non-pathogenic neisserias had been obtained, the tracheas were removed from a group of animals, preparations of TC obtained, and the reaction of anaphylactic contraction of TC to bacterial allergens carried out. The control group consisted of 19 intact animals.

TC preparations were obtained by the method in [12]. Smooth-muscle contractions were recorded under isometric conditions on a two-channel plethysmographic apparatus with type P-42 SKhÉ ink recorder [1]. The dose of specific allergen in a volume of 0.2 ml was added to the bath with TC in an amount sufficient to give a final concentration of allergen of 100 µg protein/ml. The magnitude of the anaphylactic reaction of the smooth-muscle preparation was determined by the equation $a:b = x:y$, where a is the standard concentration of histamine (in ng); b the tension developed by TC during the action of a standard concentration of histamine (in g); x the magnitude of the anaphylactic reaction (in ng), and y the tension developed by TC during the action of the specific allergen (in g).

EXPERIMENTAL RESULTS

The sensitivity of smooth muscles of the guinea pig TC to neisserial allergens was estimated in experiments on animals sensitized by neisserias. The degree of sensitization was first recorded in guinea pigs of parallel groups by reproduction of anaphylaxis in the ACA test, because this test could be used to judge the presence of allergic reactions of immediate type.

The state of sensitization was studied in the animals of three groups sensitized with a living culture of neisserias. Animals of group 1 (n = 10) were given an intravenous injection of the reacting dose of neisserial allergen at optimal times of sensitization. Anaphylactic reactions were recorded on the 11th-15th days after the beginning of sensitization. However, these reactions reached their maximal levels on the 21st-25th day of sensitization. The shock index according to [17] in guinea pigs sensitized with nonpathogenic neisserias reached 2.8 ± 0.8. At autopsy on the animals dying from anaphylactic shock, emphysema of

the lungs and hemorrhages on their surface were observed. The morphological control on sections of the lung and bronchi confirmed the typical picture of anaphylactic shock (emphysematous changes, a response of bronchospasm).

In the animals of group 2 (n = 10) sensitivity of the skin to neisserial allergen was estimated in the ACA test. The mean diameter of the stained spot on the 21st day of sensitization was 14.2 ± 1.0 mm.

These tests thus confirmed the presence of allergic reactions of immediate type in animals sensitized with neisserias.

In the guinea pigs of group 3 in the same series of experiments the trachea was isolated and sensitivity of TC estimated. The mean value of the anaphylactic reactions of tracheal preparations from sensitized guinea pigs (21st-25th days) to contact with neisserial allergens was 165 ± 11% relative to contraction of the same chain to histamine (Fig. 1, I). TC of intact animals did not react to contact with neisserial allergens.

Addition of nonspecific allergens of *St. aureus* or *Kl. pneumoniae* to the bath containing the solution with TC of animals sensitized with neisserias likewise was not accompanied by any anaphylactic contraction (Fig. 1, II, III).

These investigations with production of anaphylactic contraction of TC of guinea pigs sensitized with neisserias in response to bacterial allergens thus demonstrate that the test can be used to assess the biological activity of bacterial allergens. The specific character of anaphylactic contraction of TC is confirmed by the absence of a response of TC of intact animals to the same preparations and of TC of guinea pigs sensitized with neisserias to allergens of *St. aureus* and *K. pneumoniae* which, according to data in the literature [3], are active sensitizers of the human bronchopulmonary system.

The absence of crossed reactions is evidence that the test of anaphylactic contraction of TC is a method of determining allergen-specific bacterial antigens.

Accordingly anaphylactic contraction of TC in this case can be regarded as a model with which to study the mechanism of bronchospasm in response to bacterial allergens.

LITERATURE CITED

1. T. A. Avdeeva, Byull. Éksp. Biol. Med., No. 11, 118 (1969).
2. A. D. Ado, in: Special Allergology [in Russian], Moscow (1976), p. 57.
3. A. D. Ado and V. N. Fedoseeva, Sov. Med., No. 11, 3 (1973).
4. N. D. Beklemishev and G. S. Sukhodoeva, Clinical and Experimental Aspects of Allergy to Bacteria [in Russian], Moscow (1979).
5. P. K. Bultatov and G. B. Fedoseev, Bronchial Asthma [in Russian], Leningrad (1975), p. 62.
6. A. T. Kravchenko and V. A. Fradkin, in: Bronchial Asthma [in Russian], Moscow (1969), p. 25.
7. A. T. Kravchenko and N. V. Galanova, The Third Factor in Acquired Immunity: Immunity and Allergy of Cells [in Russian], Moscow (1948).
8. G. S. Sukhodoeva, "Comparative analysis of different types of allergy to bacterial antigens," Doctoral Dissertation, Alma-Ata (1974).
9. V. N. Fedoseeva and V. G. Chitaeva, in: Bronchial Asthma [in Russian], Moscow (1974), p. 276.
10. V. A. Fradkin, Allergens [in Russian], Moscow (1978).
11. V. G. Chitaeva, "The connection between polyallergic reactions and common antigenic determinants of microorganisms," Candidate's Dissertation, Moscow (1978).
12. A. Akcasu, Arch. Int. Pharmacol. Ther., 122, 201 (1959).
13. L. Bechoenkova et al., Allergy Asthma, 5, 386 (1959).
14. P. D. Joner, M. Wall, et al., J. Allergy, 53, 261 (1974).
15. K. M. Lulich, I. M. Papadimitrou, and I. W. Paterson, Clin. Exp. Pharm. Phys., 6, 625 (1979).
16. Z. Ovary, Acta Allerg., 6, Suppl. 3, 170 (1958).
17. W. Weigle, C. Cochrane, and E. J. Dixon, Immunology, 85, 469 (1960).