ACETYLCHOLINE-INDUCED LYMPHOCYTE MOBILITY IN INTACT AND SENSITIZED MICE

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Neurotransmitters are known to play an important role in the development of allergic reactions. A theory ascribing the key role in the pathogenesis of bronchial asthma to changes in tissue β-adrenoreceptors has been put forward [11]. It has recently been shown that in allergic reactions changes take place not only in the adrenoreceptor apparatus, but also in receptors for prostaglandins and acetylcholine [8, 9]. The writers showed previously that parallel with the development of allergic hypersensitivity of the tissues, they also develop sensitivity to acetylcholine [1, 2]. Various workers have shown that acetylcholine may have a considerable influence on the state of lymphocyte membrane function and on activity of membrane-bound enzymes [3, 6, 10]. At the same time, we know that the physicochemical state of lymphocyte membranes may change both during the formation of a state of sensitization and under the influence of the reacting dose of antigen [7]. All these factors necessitate a study of interaction between acetylcholine receptors and other receptors of the lymphocyte membrane and changes in the sensitivity of lymphocytes to this agent in the course of sensitization.

The object of this investigation was to study the sensitivity of mouse spleen lymphocytes to acetylcholine in the course of sensitization to the protein antigen ovalbumin and under the influence of the reacting dose of antigen on lymphocytes.

EXPERIMENTAL METHOD

Altogether 50 BALB/c mice of both sexes weighing 20-25 g were used. The animals were immunized with a single intraperitoneal injection of 50 μg ovalbium (the lyophilized powder, from Reakhim, USSR) with 5 mg aluminum hydroxide gel as adjuvant. To obtain lymphocytes the animals were decapitated at different periods of sensitization and their spleen was homogenized gently in medium 199, and then filtered through Kapron to remove debris [4]. The spleen cells from each animal, in 5 ml medium 199, were then layered on 3 ml of Ficoll–Verografin solution with a density of 1.090 and centrifuged for 30 min at 1500 rpm [5]. The isolated lymphocytes were washed in medium 199 by centrifugation twice at 1200 rpm for 7 min and the cell concentration adjusted to 1×10^6 cells/ml. Aliquots of 0.2 ml of lymphocyte suspension were incubated in a humid chamber at 37°C for 10 min in plastic dishes in the presence of different concentrations of acetylcholine or antigen. The cells were then fixed with 5% formalin and the number of mobile forms counted among 200 lymphocytes, using phase-contrast microscopy [9].

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The isolated lymphocytes preserved a high level of viability, and the number of living cells did not fall below 96% in any experiment. Spontaneously mobile forms in intact animals accounted for 10-12%, in good agreement with data in the literature [9]. Sensitization was accompanied by an increase in mobility of the splenic lymphocytes by 1.5 times. Morphologically, large blast forms with ameboid mobility and also lymphocytes with other forms of move-

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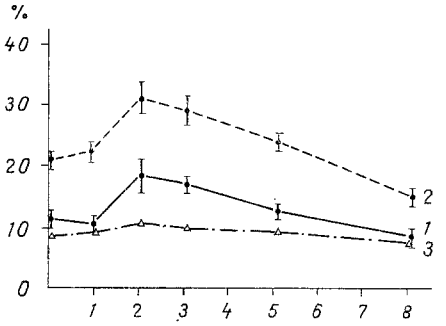

Fig. 1. Action of acetylcholine (10^{-8} M) on mobility of splenic lymphocytes of mice depending on time of sensitization. Abscissa, time of sensitization (in days); ordinate, percentage of mobile forms of lymphocytes. 1) Background mobility of lymphocytes; 2) acetylcholine-induced mobility of lymphocytes; 3) difference between acetylcholine-induced and background mobility.

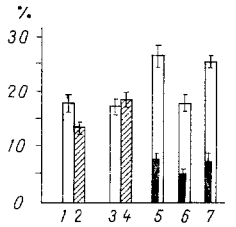

Fig. 2. Action of antigen and acetylcholine on mobility of splenic lymphocytes on 3rd day of sensitization of mice. Vertical axis, percentage of mobile forms of lymphocytes; horizontal axis, substances tested. 1) Control; 2) antigen (100 μg/ml, incubation for 15 min); 3) mobility of lymphocytes 1 h after action of antigen; 4) repeated action of antigen in same dose; 5) acetylcholine (10^{-8} M); 6) antigen (incubation for 15 min with acetylcholine in concentration of 10^{-8} M); 7) incubation for 1 h after action of antigen and acetylcholine (10^{-8} M). Black columns denote increase in lymphocyte mobility under the influence of acetylcholine.

ment could be distinguished. Under the influence of acetylcholine the number of mobile forms was twice that found in intact animals (Fig. 1). Maximal stimulation of lymphocyte mobility was achieved in the presence of acetylcholine in a concentration of 10^{-9} M, and this value was independent of the period of sensitization.

Although the difference between background and acetylcholine-induced mobility was not significantly changed by sensitization, it should be pointed out that during its action on the same cell substrate an increase in spontaneous mobility could lead to depression of mobility induced by exogenous substances, as has been shown in the case of activation of B lymphocytes by antiimmunoglobulin serum [9]. Accordingly, the absolute number of mobile forms under the influence of acetylcholine at different times of sensitization must be regarded as decisive. This parameter reaches its maximum on the 2nd-3rd day of sensitization, but by the 8th day all parameters studied return to normal.

An increase in the number of mobile forms of lymphocytes during sensitization, under conditions of acetylcholine induction, reflects the increasing sensitivity of lymphocytes to acetylcholine during sensitization, although redistribution of B and T lymphocytes in the lymphoid organs during the immune response may play a definite role in this situation, for it has been shown that it is the B lymphocytes which are mobile cells under the influence of acetylcholine [10].

The effect of the reacting dose of antigen on splenic lymphocytes on the 3rd day of sensitization was accompanied by some decrease in lymphocyte mobility, which was restored after incubation for 1 h; a second injection of antigen had no effect (Fig. 2).

The effect of acetylcholine following the action of the antigen was accompanied by a smaller increase in mobility than when acetylcholine acted on intact cells; this can be interpreted as the result of action of acetylcholine on the same substrate as that on which the antigen acted. The sensitivity of the cells to acetylcholine was restored 1 h after the action of the antigen, whereas the antigen itself no longer exerted any effect. Loss of sensitivity to the antigen was probably connected with its action in blocking the lymphocyte receptors. An important role here was evidently played by processes of "cap formation" on antigen-sensitive immunoglobulin receptors. Since sensitivity to acetylcholine was unchanged under these circumstances, receptors for antigen and for acetylcholine cannot be closely connected on the lymphocytes membrane.

Similar changes in antigen-induced and acetylcholine-induced lymphocyte mobility also were observed on the 8th day of sensitization, although they were less pronounced.

In the course of sensitization spontaneous mobility of mouse spleen lymphocytes thus increases, as also does the acetylcholine-induced mobility of the lymphocytes; this is probably the result of an increase in sensitivity of the lymphocytes to acetylcholine during the development of sensitization. Both antigen and acetylcholine act on the same cell substrate, although receptors for the antigen and for acetylcholine on the lymphocyte membrane are not interconnected.

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