PROTEIN SPECTRA AND PHOSPHOLIPID COMPOSITION OF ACETYLCHOLINE RECEPTOR-RICH MEMBRANES FROM SKELETAL MUSCLES OF SENSITIZED RATS

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Functional activity of acetylcholine receptors, like that of receptor proteins in general, is closely connected with the physicochemical state of membrane phospholipids [2, 7, 13]. In allergic reactions phospholipid metabolism in target cell membranes during fixation of reagins of the IgE type on their surface has received most study [1, 11, 14, 16, 19]. Relations between structural changes in proteins and phospholipids in cell membranes actively binding cholinotropic agents has virtually not been studied. As regards microbial sensitization, this problem has not hitherto been considered.

The aim of this investigation was to study the effect of sensitization with neisserial allergen in protein spectra and phospholipid composition of acetylcholine-rich membranes (ARM).

EXPERIMENTAL METHOD

Experiments were carried out on 56 August rats. The 36 animals of group 1 were sensitized with a living culture of *Neisseria perflava* by the scheme in [3]. The allergen was injected subcutaneously in a concentration of 5×10^6 bacterial cells with an equal volume of incomplete stimulator at intervals of 3-4 days. The course of sensitization consisted of three injections. The degree of sensitization of the animals was estimated by the usual methods at optimal times of sensitization from the 21st to the 32nd day after the last injection of allergen. Cutaneous sensitivity of the animals to specific allergen was estimated from the diameter of the area of erythema in the active cutaneous anaphylaxis test [15] and from titers of reagin-like antibodies determined in rat blood in the passive cutaneous anaphylaxis test [15]. The control group consisted of 20 intact rats.

ARM were isolated from skeletal muscle cells of intact and sensitized animals by the method in [17], using ultracentrifugation in a sucrose gradient. According to Sobel, ARM are membranes with high binding activity with the α-toxin of *Naja nigricollis* (4000 nmoles/g protein). The biological activity of the membranes was assayed by binding with choline derivatives: acetylcholine, phosphorylcholine in the presence of blockers — Diplacin† and benzohexonium, by spectrofluorometry [8] and radiometry [6], using [^{3}H]acetylcholine (2.7 Ci/mmole; from Amersham Corporation, England).

The protein spectra of the membranes were studied by polyacrylamide gel (PAG) electrophoresis [12] and their electrophoretic mobility was estimated from the R_f value — the coefficient of similarity of the fractions [5], and the molecular weights of the membrane proteins were determined [12].

Separation of phospholipids into individual fractions was done by thin-layer chromatography on Woelm G silica-gel (East Germany) [18]. Lipid phosphorus in individual fractions was determined quantitatively by the method in [9]. When calculating mean values, results obtained in each group of animals were used. Statistical analysis of the results followed the Kolmogorov—Smirnov procedure [4].

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†1,3-Di(β-platyneciniumethoxy)-benzene hydrochloride.

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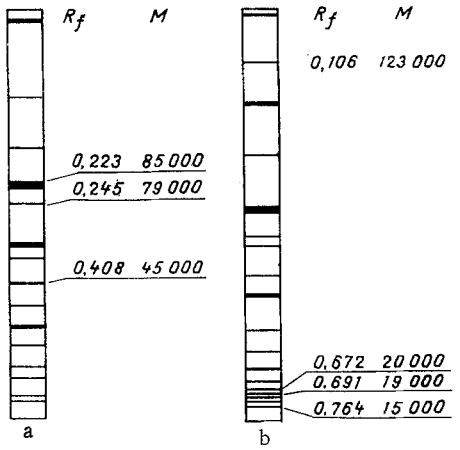

Fig. 1. Electrophoresis of proteins of ARM fragments. R_f) Ratio of mobility of fractions, M) molecular weight of protein (in daltons); a, b) electrophoresis of proteins of groups 1 and 2, respectively.

EXPERIMENTAL RESULTS

Protein spectra and the phospholipid composition of ARM from skeletal muscle cells of intact and sensitized rats were compared.

Before isolation of membrane-bound acetylcholine receptors, the degree of sensitization of the animals was determined. On the 21st-32nd day of sensitization with neisserial allergen the rats exhibited marked cutaneous sensitivity to the specific allegen, the intensity of which in Ovary's active cutaneous anaphylaxis test was expressed as the diameter of the area of erythema, which was 12.5 ± 0.93 mm. Sera of sensitized rats contained homocytotropic antibodies which could be detected by the passive hypersensitivity transfer method in a titer of 1:8. ARM were isolated from skeletal muscle of sensitized and intact animals.

At optimum times of sensitization of the animals, compared with intact rats they exhibited stronger biological activity of ARM in binding with acetylcholine and other choline derivatives. Proteins were isolated from biologically active membrane fragments from intact (group 1) and sensitized (group 2) rats and the composition, mobility, and molecular weights of the individual fractions were studied by PAG electrophoresis. A parallel study was undertaken of the phospholipid composition of ARM isolated from animals of both groups. Corresponding to the two groups of animals, two groups of proteins were studied.

Electrophoresis of proteins of groups 1 and 2 demonstrated their heterogeneous composition and absence of identity in mobility of the individual fractions. Profiles of the group 1 proteins consisted of 12-15 fractions. The spectrum of the group 2 proteins was more varied, consisting of no fewer than 18 components. Fractions appeared which were absent in intact animals (Fig. 1). The spectra also differed in the intensity of staining of individual fractions. Determination of the coefficient of similarity showed that with regard to the general profile of distribution of the components, ARM proteins of groups 1 and 2 had partial similarity: The coefficient of similarity between the spectra was $r = 0.36$.

Analysis of the same groups of proteins by molecular weight showed the presence in both cases of proteins with a high molecular weight of 140,000-142,000 daltons, and also components with a low molecular weight of 18,000-15,000 daltons. The spectra of the group 2 proteins were more saturated with low-molecular-weight fractions. In this case, six lines were observed within the range from 23,000 to 15,000 daltons, compared with only three among the components of group 1. Furthermore, during sensitization a protein with a molecular weight of 123,000 daltons, not present in ARM of intact rats, was observed to appear in ARM during sensitization.

The protein spectra thus obtained included fractions of 47,000, 56,000, 62,000, 110,000, and 112,000 daltons, similar or equal in molecular weight to proteins of warm-blooded animals responsible for cholinotropic activity [10]. In the spectrum of the sensitized animals the fraction with mol. wt. of 45,000 daltons disappeared, and the fraction with mol. wt. of 110,000 daltons in the spectrum of group 2 consisted of a more intensively stained line than the pro-

tein of closely similar mol. wt. of 112,000 daltons in the group 1 spectrum. The abundance of fractions with high electrophoretic mobility and low molecular weight, the presence of only partial similarity between the spectra of groups 1 and 2 according to coefficients of similarity, and the presence of a specific fraction of 123,000 daltons type are all evidence of considerable changes taking place in the protein structures of skeletal muscle cell membranes on sensitization.

The study of the phospholipid composition of groups 1 and 2 showed that in both cases the phospholipids of ARM consisted of phosphatidylcholine, sphingomyelin, phosphatidylinositol, phosphatidylserine, and phosphatidylethanolamine. However, the relative percentages of the individual phospholipids differed. The content of sphingomyelin in ARM of sensitized animals was increased to 24.4 ± 0.5% from 17.2 ± 0.4% in intact rats ($P < 0.01$). The phosphatidylethanolamine content was considerably reduced — from 26.1 ± 0.9 to 16.5 ± 0.7% ($P < 0.01$) and the phosphatidylcholine content was increased from 35.7 ± 1.2 to 43.2 ± 1.4%. The content of phosphatidylserine and phosphatidylinositol in the control and experimental groups was 10.5 ± 0.2 and 95 ± 0.3%, and 10.5 ± 0.5 and 6.4 ± 0.5%, respectively.

At the height of sensitization of the animals with neisserial allergen significant changes were thus found in the phospholipid structures of the membranes. The data showing an increase in the phosphatidylcholine content on sensitization agree with observations by other workers [11] who studied similar changes in target cell membranes during fixation of allergic antibodies (IgE) on their surface. These workers showed a sequence of processes of molecular reorganizations in mast cell membranes under the influence of fixation of IgE, due in particular to deamination of phospholipids, accompanied by an increase in the phosphatidylcholine level.

The results of the present investigation are evidence that in allergic processes, besides activated membranes of target cells, membranes of muscle cells also undergo similar structural changes. Microbial sensitization leads to significant changes in the protein spectrum of muscle cell membranes with increased cholinotropic activity. This phenomenon is combined with profound changes in the phospholipid constructions of the membranes.

Further study of the character and interconnection of protein—lipid structural changes in cell membranes during allergic processes will permit ways of their specific correction to be discovered in the future, and this will be particularly important in the early stages of formation of allergy.

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