THE ANTIGENIC SPECTRUM OF IDENTIFIED *Helix pomatia* NEURONS

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The use of simple cell systems and, primarily, of invertebrate neuron assemblages, as experimental physiological models for the study and analysis of cell mechanisms of function of the nervous system necessitates the development of new methods of approach, including the biochemical analysis of single isolated neurons. In turn, this calls for a choice of methods of sufficient sensitivity to allow analysis of a protein content of the order of 10^{-7} to 10^{-9} g. One such method is unidimensional countercurrent immunodiffusion in a capillary tube [4], which has been suggested for the determination of microquantities of α-antitrypsin.

This paper describes an attempt to use this method to study the antigenic spectrum of isolated *Helix pomatia* neurons.

EXPERIMENTAL METHOD

The principle of the method is as follows: The test antigens react in agarose gel with antibodies and, as a result, precipitation bands are formed. The optical density of the bands, which, over a certain range, is directly proportional to the protein concentration in the sample, is measured on a two-wave microspectrometer. The method of countercurrent immunodiffusion in a capillary tube was described in detail previously [4]. As antigen we used an extract of nerve cells isolated from the subesophageal group of ganglia of *H. pomatia*. For extraction we used 0.01M Tris-HCl buffer, pH 7.4, with the addition of 0.001M EDTA, 0.25M sucrose, and 0.5% Triton X-100. The homogenate was centrifuged for 1 h 30 min at 4°C and 1600 rpm. The supernatant was used as the antigen. An immune rabbit serum, obtained against water-soluble nerve ganglion proteins of *H. pomatia* [1], exhausted beforehand with homologous organs (liver, heart, kidney, intestine, and hemolymph) of the snail in order to neutralize cross-reacting antigens, was used in the experiments. This immune serum revealed only nerve-specific protein antigens.

The relative proportions of antigens and antibodies introduced into the gel were chosen experimentally. To obtain better visualization of rapidly diffusing antigens, the viscosity of the gel was increased by the addition of sucrose to the homogenizing buffer; otherwise the rate of diffusion of the antigen exceeded the rate of diffusion of the antibodies and, as a result, the precipitation bands moved and could not be fixed in the agarose gel.

Water-repellant capillary tubes with a bore of 300-315 μ and a length of 25 mm were used. The length of the agarose thread was 3 mm. Immune serum in a dose of 0.3 μl was layered above the surface of the agarose in the capillary tubes. Antigen was applied in a volume of 0.1-0.3 μl to the opposite end of the gel 2 h later. The capillary tubes were kept for 48 h under water in Petri dishes, after which the agarose gel was expressed from the capillary tube into physiological saline cooled to 4°C and allowed to stand for 2 h for unreacted proteins to be removed. The washed gel was stained with a 0.5% solution of amido black 10B in 7% acetic acid for 1 h. The stained gel was then washed with 7% acetic acid and drawn up into capillary tubes with a bore of 340-360 μ. The capillary tubes were scanned on a two-wave capillary spectrophotometer, made at the Research Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR (Novosibirsk). The densitograms thus obtained were analyzed by determining the area of the peaks.

The protein concentration in the extracts from the subesophageal group of ganglia used in the tests was determined spectrophotometrically at a wavelength of 280 mm. A calibration curve was plotted for human

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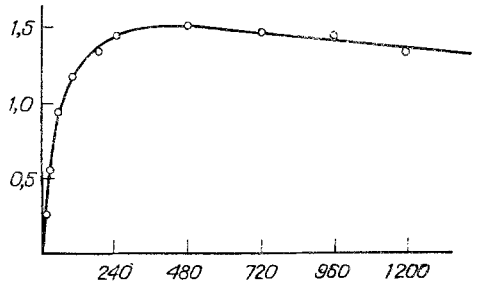

Fig. 1

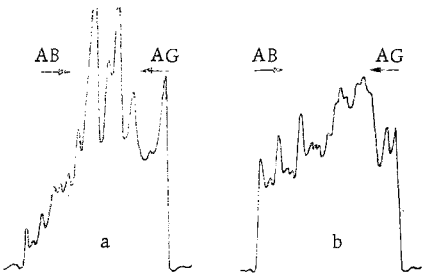

Fig. 2

Fig. 1. Graph of quantity of immunoprecipitate as a function of quantity of antigen. Abscissa, quantity of antigen (in ng) from subesophageal group of ganglia of Helix pomatia. Ordinate, quantity of immunoprecipitate formed, as measured by total optical density in gel (in conventional units).

Fig. 2. Densitograms of antigenic spectra of Helix pomatia neurons: a) RPa-1; b) RPa-2. AG) Antigen of neuron; AB) antibodies of antibrain immune rabbit serum.

serum albumin. Observance of strictly standard conditions for the precipitation test, such as verification of the diameters of the capillary tubes, strict quantitative application of antibodies and antigen, constant volume of homogenizing buffer, and so on, essential for microbiochemical [3, 7] and immunochemical [1, 2, 5, 8] investigations in general, insured the reproducibility of the results.

EXPERIMENTAL RESULTS

Investigations of the relationship between total optical density and quantity of applied protein in the antigen extracts showed that optical density is a linear function of protein concentration within the region up to 120 mg (Fig. 1). An increase in the protein concentration in the test extract led not only to quantitative differences, but also to qualitative redistribution of the precipitation lines in the gel, on account of differences in the relative content of antigens in the samples applied. When protein spectra developed with the aid of exhausted and unexhausted immune sera were compared, virtually identical pictures were obtained, so that thereafter it was possible to work with unexhausted antibrain serum.

The cells studied were: LPl-1, RPl-1, RPa-1, LPa-2, RPa-2, RPa-3, and LPa-3 (according to the classification of Sakharov and Shalanka [6]).

The symmetrical neurons did not differ from each other according to the number of individually detectable antigens. Comparison of individual protein precipitation spectra of different types of neurons revealed both qualitative (different numbers of precipitating lines) and quantitative (differences in optical density of the precipitation bands) differences. For example, RPa-3, RPl-1, LPl-1, and LPl-3 had qualitatively similar antigenic spectra to neuron RPa-1 as regards the number of antigens revealed, but they differ in their quantitative content of individual antigens in these spectra (Fig. 2a). Neurons RPa-2 and LPa-2 (Fig. 2b), on the other hand, have a completely different antigenic spectrum from the spectra of other types of neurons, possibly indicating the presence of individual proteins found in only certain types of nerve cells.

By the method of countercurrent immunodiffusion it is thus possible not only to study the qualitative composition of individual neurons, but also to obtain information on the relative quantitative content of individual nerve-specific protein antigens in isolated nerve cells.

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