In the light of these observations the action of p-CoA on the energy-dependent functions of MCH and on the proton conductance of their inner membrane can be satisfactorily explained. p-CoA is known to inhibit AN transport specifically, like atractyloside [1, 8]. The inhibition constant is very low, namely 0.5 μM, and the action of p-CoA is competitive with respect to ADP and ATP. Carnitine abolishes the effect of p-CoA, since the effective concentration of p-CoA is lowered as a result of the activity of carnitine-palmitoyl transferase, and the palmitoyl carnitine formed had no effect on ANT [11]. The ability of ADP and carnitine to abolish the effect of p-CoA additively is explained by the fact that carnitine lowers the p-CoA concentration in the membrane, and ADP under these conditions competes more effectively with p-CoA for the binding sites on ANT.

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LOCALIZATION OF NERVE-SPECIFIC PROTEIN ANTIGENS ON THE SURFACE MEMBRANE OF NEURONS AND GLIAL CELLS OF Helix pomatia

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UDC 612.017.1

The existence of cross protein antigens common to several species of invertebrates and vertebrates on the membrane of neurons and glial cells of Helix pomatia was demonstrated in vitro by Coons' immunofluorescence method. The presence of nerve-specific protein S-100 on the membrane of these cells was established. The antigenic heterogeneity of membranes of a population of neurons also was observed. Differences were found in the concentrations of antigens on the somatic and axon membranes. The character of distribution of specific fluorescence indicates possible qualitative and (or) quantitative differences in the content of nerve-specific proteins in different areas of the neuron membrane.

KEY WORDS: brain-specific antigen; neurons of invertebrates; immunofluorescence.

The existence of a class of protein antigens specific for nerve tissue can now be accepted as proven. It is considered that these proteins are responsible for conducting and generating the action potential and for synaptic transmission, participate in mechanisms of memory and learning, and so on [5]. Since many of these functions of the nervous system are connected in some way or other with the activity of the neuron mem-

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 9, pp. 299-301, September, 1979. Original article submitted November 19, 1978.

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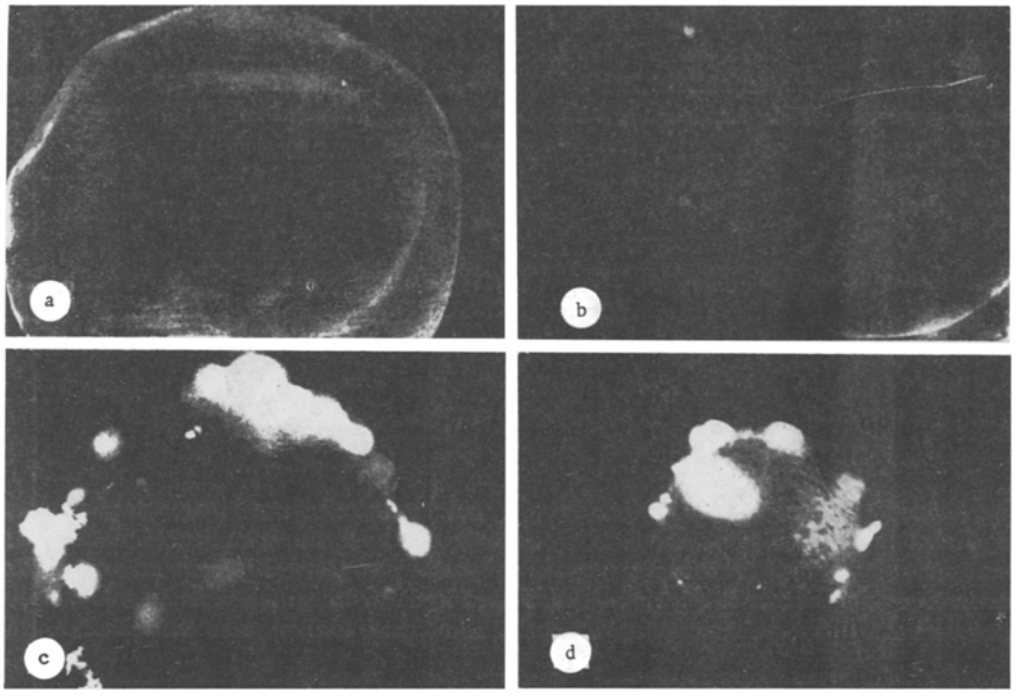

Fig. 1. Isolated nerve cells of Helix pomatia incubated with antisera against nerve tissue of animals of various species (a-d; fluorescent glial cells shown in c and d). Indirect Coons' method. Magnification in a, b, c 400×, in d 200×.

brane, as one aspect of the problem of the physiological role of nerve-specific proteins (NSP) the study of their membrane localization is important on its own account.

Changes observed in the electrical characteristics of intact neurons and of various brain structures under the influence of antibodies against NSP [10] are indirect evidence of the membrane localization of these antigens, but they allow no conclusions to be drawn on the character of their distribution on the cell surface, still less regarding the existence of membrane-bound NSP of glial cells.

It was accordingly decided to study the character of distribution of nerve-specific protein antigens, including protein S-100, on the membrane of isolated nerve and glial cells of Helix pomatia by an immunofluorescence method.

EXPERIMENTAL METHOD

Experiments were carried out on single nerve cells of the isolated subesophageal ganglion complex of Helix pomatia [6]. Cells which according to their morphological features remained viable were removed from the suspension with a micropipet and placed in a drop of physiological saline in a special chamber. The localization of the surface antigens was studied by the indirect Coons' immunofluorescence method in the usual manner [4]: The cells were incubated in a humid chamber with immune serum for 10-15 min; next, after rinsing with physiological saline, they were incubated with fluorescein isothiocyanate-labeled antiserum against rabbit immunoglobulin (prepared by the N. F. Gamaleya Institute of Microbiology and Epidemiology, Academy of Medical Sciences of the USSR). After further washing of the cells to remove unbound antibodies, a preparation for luminescence microscopy was obtained. To verify the immunologic specificity, some of the cells were incubated with nonimmune serum under the same conditions and also with labeled serum by the direct method.

The following immune antisera were used: against snail nerve ganglia, against the crayfish nerve chain, against rat brain, and monospecific antiserum against protein S-100 (AS-100).* The method of obtaining these sera was described previously [7]. It must be pointed out here that according to the hypothesis of species nonspecificity of NSP the use of antisera obtained against nerve tissue of different species of animals

*The antiserum was provided by S. M. Sviridov (Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR), to whom the authors are grateful.

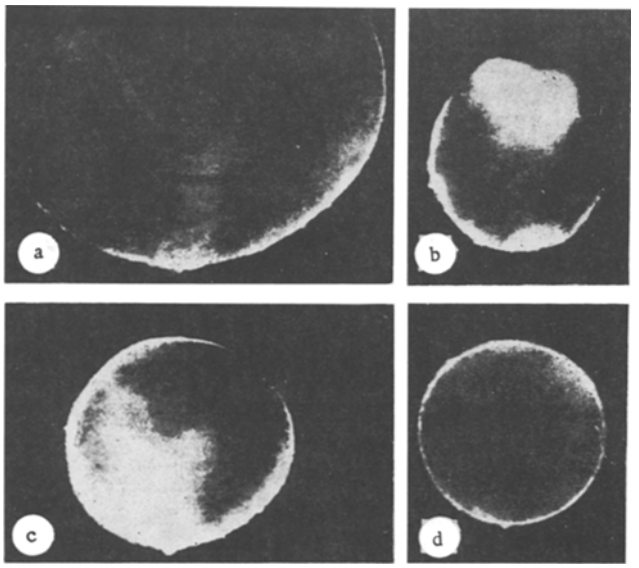

Fig. 2. Localization of nerve-specific protein S-100 on membrane surface of neurons of different sizes (a-d) and glial cells of Helix pomatia (specific fluorescence of glial cells shown in b). Indirect Coons' method, incubation with monospecific antiserum against protein S-100. 200×.

containing cross interspecific antigens can legitimately be expected. Investigations have shown that most NSP possess high evolutionary stability: They are present in nerve tissue of animals of various species phlogenetically very remote from one another [11, 12]. The above-mentioned immune sera were also chosen because, when applied to the surface of neurons, they evoked the corresponding electrophysiological effects [1, 2].

All sera (immune and labeled) were first adsorbed with liver powder and their working dilution was chosen under immunofluorescence control [4]. Since it was necessary to incubate the cells in a physiological medium for molluscan nerve cells, at the first dilution of the antisera a compensating factor was used; its ionic composition was chosen so that after addition to an equal volume of undiluted serum a solution of antibodies was obtained in which the salt concentration corresponded to the ionic composition of hemolymph [9]. For further dilutions physiological saline for snails was used.

The cells were mounted in a drop of physiological saline and the usual method of applying a drop of 50% glycerol to the preparation was rejected. Cells in physiological saline with glycerol shrank, with consequent deformation of their surface and folding. In the region of a fold the intensity of fluorescence was increased, and this could be mistakenly attributed to a local increase in antigen concentration on the cell surface (Fig. 1). The preparations were examined and photographed in the ML-2A luminescence microscope.

EXPERIMENTAL RESULTS

The isolated nerve cells possessed weak yellowish-green autofluorescence, confirming that they remained viable. In control experiments with nonimmune serum and with labeled eluate, the intensity of fluorescence of the isolated neurons was indistinguishable from that of autofluorescence.

During incubation of the nerve cells with antisera against nerve tissue of different species of animals the outer membrane of the nerve and glial cells gave bright specific fluorescence. This fluorescence in the neurons was most frequently in the shape of a ring, probably indicating a uniform distribution of the cross antigens on the surface of the somatic membrane. Fluorescence of the glial cells was stronger and was distributed over the whole surface of the cells (Fig. 1c, d). Differences in the character of fluorescence of the nerve and glial cells were probably atttributable to the greater surface density of distribution of antigens on the membrane of the glial cells and (or) differences in their size. On incubation with antiserum against snail nerve tissue fluorescence of the axon membrane was brighter than that of the somatic membrane (Fig. 1b). This fact suggests that the neuronal membrane is heterogeneous in its antigenic composition. This hetero-

geneity is perhaps connected with functional differences between the axonal and somatic membranes and, in particular, with known differences in the pattern of distribution of chemoreceptors on their surface [3].

In the series of experiments with exhausted serum against rat brain which differed from the unexhausted by containing antibodies against brain-specific antigens only, some cells did not show membrane fluorescence. This fact may perhaps indicate that the neuron population is heterogeneous for the antigenic composition of their membranes. A similar suggestion was put forward by Solntseva [8], who studied the action of antibodies against interspecific cross antigens on the electrical characteristics of snail neurons.

When the cells were incubated with AS-100 the outer membrane of the neurons also showed bright specific fluorescence which, as a rule, was ring-shaped in character (Fig. 2). In an investigation by Hyden and Ronnback [11], who used AS-100 with isolated vertebrate neurons, fluorescence was usually irregular and was concentrated in different zones of the cell surface. It can tentatively be suggested that differences in the character of localization of protein S-100 in vertebrates and invertebrates are connected with differences in the neuron morphology of these species and also, perhaps, with differences in the degree of participation of their somatic membranes in the integrative activity of the nerve cells.

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