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IMMUNOHISTOCHEMICAL INVESTIGATION OF PROTEIN S-100 IN NEURONS AND GLIA OF *Helix pomatia*

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The location of brain-specific protein S-100 was investigated by the indirect Coons' method in neurons and glia of Helix pomatia. This protein was found in the cytoplasm of neurons and glial cells, and in the nucleus and outer member of neurons.

KEY WORDS: brain-specific antigen; neurons of mollusks.

The investigation of the physiological role of brain-specific proteins is a problem of the utmost importance at the present time. These proteins are considered to participate in the regulation of the permeability of neuronal membranes and so to be concerned in the mechanism of electrogenesis. An important place in research in this field is occupied by the identification of nerve-specific proteins and the investigation of their localization in structures of the nervous system.

The study of the properties of protein S-100 is particularly interesting in connection with the known participation of this antigen in the neuronal mechanisms of memory and learning [6]. Data on its distribution in cells of the vertebrate nervous system are contradictory: Some investigations [1, 4-6] have shown that S-100 is localized in the cytoplasm and nuclei of neurons, in tumors of glial nature, and in cell clones from human glia. The results of other investigations have shown that this protein is present in the cytoplasm, nucleus, and axoplasm of neurons [4, 8, 10, 11].

Protein S-100 is known to be species nonspecific. The results of a study of its localization in invertebrate neurons have not been reported in the literature.

The object of this investigation was to study the distribution of protein S-100 in nerve and glial cells of the cerebral structures of Helix pomatia, using the indirect Coons' method.

EXPERIMENTAL METHOD

The visceral ganglion was isolated from the subesophageal complex of Helix pomatia at 4°C and immersed in liquid nitrogen. Sections 5 μ thick were cut through the brain on a freezing microtome at −15°C, their quality was verified under the microscope, and they were dried under a fan and fixed with 96% ethanol for 10 min. Various fixatives (absolute acetone, absolute and 96% ethanol) and different exposures (5, 10, 15, 25, and 30 min) were tested as a preliminary measure. The most distinct morphological picture was obtained by the use of cold 96% ethanol for 10 min. The sections were rinsed with 0.14 M NaCl and then treated with monospecific antiserum (anti-S-100)* for 30 min, followed by labeled serum in the usual way. Labeled eluate of antibodies against rabbit IgG used in the experiments was generously provided by the staff of the Laboratory

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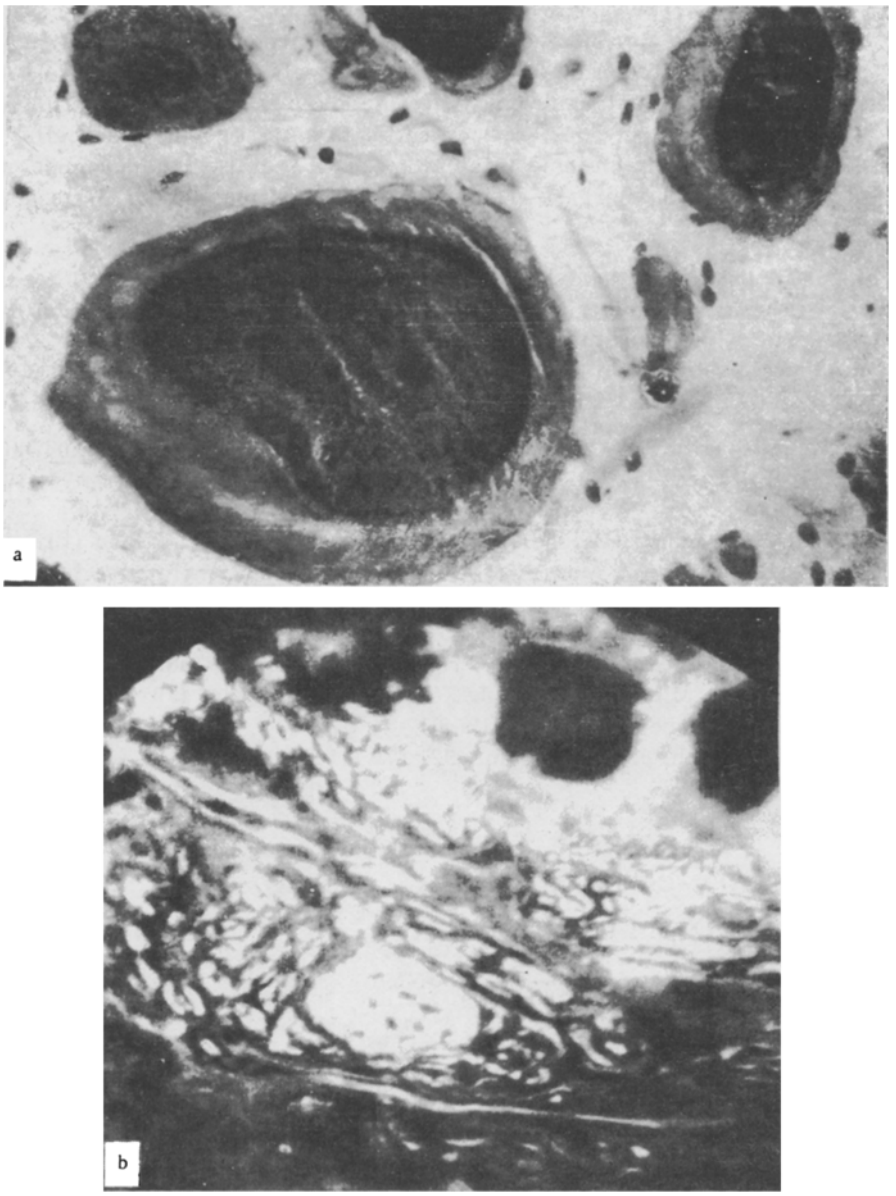

Fig. 1. Immunohistochemical investigation of protein S-100 in neurons and glia of H. pomatia. Section through brain of snail: a) neurons of visceral ganglion of subesophageal complex, stained with gallocyanin and chrome alum; b) immunofluorescence with anti-S-100 in neuron and gliocytes of visceral ganglion.

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As a check on the immunological specificity some of the brain preparations were treated with normal rabbit serum. Sections were cut under similar conditions from other organs of the snail: kidneys, gut, heart To estimate background levels, some sections were incubated with the labeled eluate only. In parallel tests another preparation from the snail was fixed, dehydrated, and embedded in paraffin wax. Sections cut from it were stained with gallocyanin and chrome alum by Einarson's method to study the morphological picture.

EXPERIMENTAL RESULTS

In sections stained with gallocyanin and chrome alum the mollusk neurons were round in shape and fairly large, measuring between 30 × 40 and 80 × 120 μ. The cell nucleus was central in position and measured between 20 × 30 and 40 × 70 μ and contained from 1 to 4 nucleoli (Fig. 1a). The gliocytes were round and measured $4 \times 7\,\mu$; the greater part of the cell was occupied by the nucleus, which was surrounded by a narrow rim

of cytoplasm. Analysis of sections of the snail brain stained by Coons' method showed that the cytoplasm of both nerve and glial cells and the membrane and nucleus of the neurons were characterized by bright specific luminescence (Fig. 1b). A diffuse distribution of S-100, either regular or irregular, was observed in the nuclei of the neurons. Bright luminescence was observed in the nucleolus. Luminescence of the cytoplasmic zone of the gliocytes was very intense, indicating a high concentration of antigen in the glial cells. Experiments in which normal rabbit serum was used as intermediate did not show specific luminescence of the neurons and glia. Luminescence of sections of other organs treated by the Coons' method did not exceed the background level.

The results of this investigation thus show that brain-specific protein S-100 is located in nerve and glial cells of H. pomatia, in which it is distributed in the cytoplasm and in the nucleus of the neurons and gliocytes, in agreement with data in the literature [1, 3, 7, 8].

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POSTMORTEM ELECTRON-CYTOCHEMICAL INVESTIGATION OF BRAIN ATPase ACTIVITY

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The localization of ATPase activity in the rat and human brains at various times after death was determined by a method using lead. This activity was discovered in the cytoplasm of the cells, the chromatin and nucleolus, and also in synaptic terminals. The reaction product in the blood capillaries was localized in the basal layer and on the endothelial cell membranes. The results demonstrate the preservation of a high level of brain ATPase activity after death.

KEY WORDS: postmortem changes; brain; ATPase activity.

The determination of postmortem structural and chemical changes in nerve tissue is an important problem in clinical and experimental neuromorphology. It is particularly important in connection with research in the field of the ultrastructural pathology of the brain, in which the methods of electron cytochemistry are only just starting to be used.

This paper gives data on the ultrastructural localization of ATPase in brain tissue at different times after death. A lead method was used to detect ATPase activity [4].

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