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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EXPERIMENTAL METHOD

The frontal cortex and anterior hypothalamus of albino rats was investigated 0, 4, 8, 12, 24, and 48 h after sacrifice of the animals (3 animals at each time). The same structures also were studied in the human brain (10 autopsies) taken 5, 8, 13, 15, 21, 22, 26, 26, 46, and 47 h after death from physical illnesses. From the time of death until the investigation the brains both of the rats and of the human cadavers remained in situ at room temperature (24°C). Pieces of the corresponding parts of the brain were cut into small pieces measuring not more than 250–300 μ in 0.08 M NaH_2PO_4–NaOH buffer, pH 7.3. For subsequent fixation a 4% solution of formaldehyde was prepared from paraformaldehyde in the same buffer [2] and filtered through a No. 4 glass filter. The pieces of tissue were fixed on melting ice for 5 min, then rinsed once with cold NaH_2PO_4–NaOH buffer and twice with 0.1 M Tris-maleate buffer (pH 7.3), and placed in cold incubation medium of the following composition: 0.1 M Tris-maleate buffer, pH 7.3; 2 mM ATP (disodium salt, from Reanal, Hungary); 2 mM magnesium acetate; 2 mM lead acetate [5]. Incubation was carried out in a dry air thermostat at 37°C with mixing for 15 min. ATPase activity was determined in the presence of Na^+ as well as of Mg^{2+} ions. The Na^+ ions were added to the medium with Tris-maleate buffer. In this investigation, both Mg^{2+}-activated and Na^+-activated Mg^{2+}-dependent ATPase were thus detected. After incubation the fragments were quickly rinsed with physiological saline and postfixed in 2% OsO_4, also in physiological saline, for 20 min at 4°C. The material was then processed for electron microscopy by the rapid method [1] without additional staining. In control experiments for the specificity of the reaction the rat brain was investigated immediately after sacrifice. The following methods of processing the material were used: 1) incubation of the tissue fragments in medium without ATP; 2) incubation of the tissue fragments in medium without ATP but with 10 mM Na β-glycerophosphate; 3) prolonged heating of the tissue fragments in Tris-maleate buffer at 90°C for 5 min; 4) preliminary treatment of the brain tissue fragments with 70% ethanol for 10 min [5].

EXPERIMENTAL RESULTS

Rat Brain

The localization of ATPase activity in the central part of the tissue fragments immediately after sacrifice of the animals conformed to the following pattern: The reaction product, a precipitate of lead phosphate, was detected along the course of the cell membrane of the neurons (but not of the glial cells), on the membranes of the granular cytoplasmic reticulum, and in the ribosomes of both neurons and glial cells. The precipitate also was detected on the outer nuclear membrane, in the chromatin, the nucleolus, and in the fibrous material of the lipofuscin granules. The reaction product was found in "nucleolus-like bodies," which were observed in the cytoplasm of some neurons of the rat hypothalamus. The reaction was clearly defined in the basal layer of the capillary wall but was less intense on the cell membrane of the endothelial cells.

In the peripheral part of the tissue fragments, as well as in the ultrastructures described above, the reaction product also was found on the outer surface of the synaptic membrane, on thickenings of the presynaptic and postsynaptic membranes, in the synaptic spaces, in the matrix of some postsynaptic endings, and also on the synaptic vesicles of some terminals. In control experiments the reaction product was not found in any of the structures mentioned above.

Some decrease in ATPase activity in the rat brain was found for the first time 12 h after death, when the amount of precipitate was reduced in the structures at the edge of the fragment. Synaptic contacts with precipitate located in the postsynaptic matrix or on synaptic vesicles were seen less frequently. In sections taken from the center of the tissue fragments no significant changes in the intensity of precipitate deposition were observed. However, at this period after death the membranes of the cristae of some mitochondria showed ATPase activity (Fig. 1a), evidently due to postmortem destruction of the membrane and liberation of the mitochondrial ATPase [3]. The decrease in the intensity of the reaction in the cells was greater 24 h after death along the course of the membrane of the cytoplasmic reticulum (which were partly destroyed at this time), in the chromatin of the nucleus, and in the nucleolus. The reaction persisted in the cell membrane of some neurons (Fig. 1b). At the edge of the tissue fragment activity remained in some terminals (Fig. 1c). In the basal layer of the capillaries the decrease in activity was not significant. The number of mitochondria with reaction product was a little greater than 12 h after death. The reaction product in sections cut from the marginal zones of the tissue fragments 48 h after death was found in synapses, but only in thickenings of the presynaptic and postsynaptic membranes and in the synaptic space. The distribution of the reaction product in sections from both peripheral and central parts of the fragments showed virtually no precipitate on the fragments of cell structures such as the endoplasmic reticulum. ATPase activity still persisted in the chromatin, the fibrillary parts of the nucleolus, and the "nucleolus-like bodies" (Fig. 1d), but the reaction products

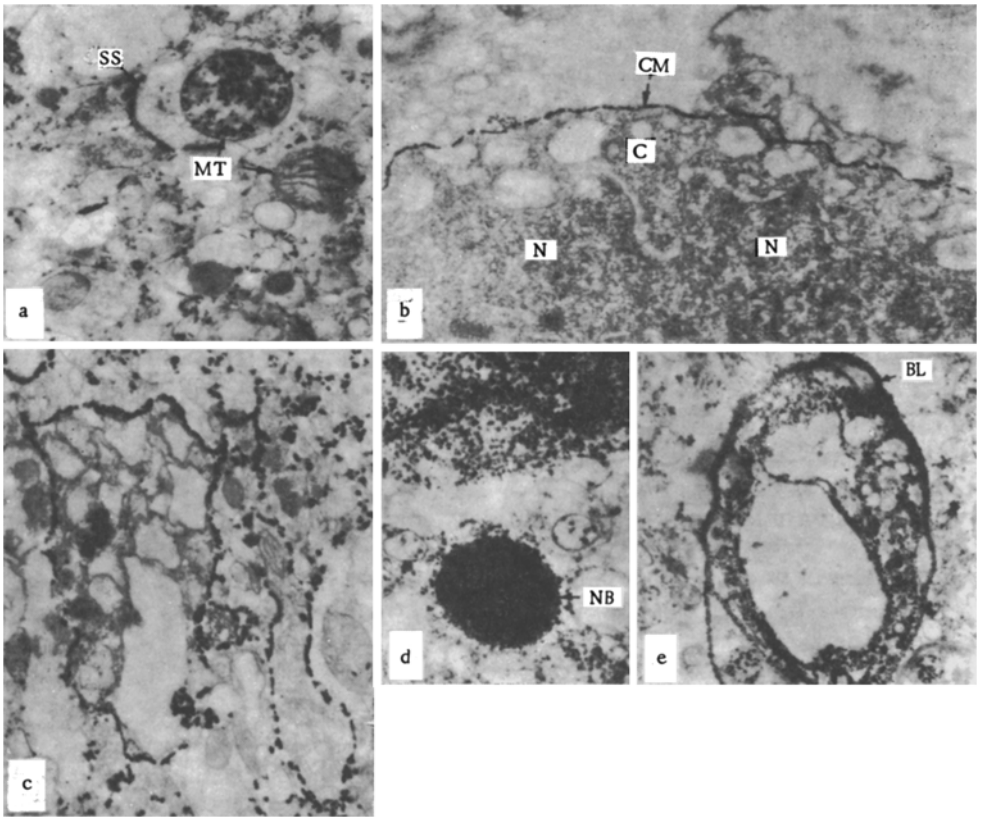

Fig. 1. Postmortem changes in ATPase activity in rat brain: a) neuropil of frontal cortex, 12 h after death. Reaction product observed in some mitochondria: MT) mitochondria; SS) synaptic space, 15,000×; b) hypothalamic neuron, 24 h after death. Reaction product observed on cell membrane: CM) cell membrane; C) cytoplasm; N) nucleus, 14,000×; c) neuropil of hypothalamus, 24 h after death. Precipitate observed in synaptic contacts (arrows), 10,500×; d) hypothalamic neuron, 48 h after death. Reaction product observed in nucleus (N) and in "nucleolus-like body" (NB), 13,000×; e) blood capillary in hypothalamus, 48 h after death. Precipitate mainly localized in basal layer (BL), 7000×.

occurred as an infrequent and coarse-grained precipitate. The intensity of ATPase activity in the basal layer of the capillaries was not less than 24 h after death (Fig. 1e). It must be emphasized that the intensity of the reaction in the lipofuscin did not fall between 0 and 48 h after death. The number of mitochondria with the reaction products was significantly greater than 12 and 24 h after death.

Human Brain

Investigation of the human brain 5 h after death showed reaction products in the same structures as in the rat brain 5 h after death. The intensity and distribution of the precipitate in these structures corresponded with that in the rat brain 12 h after death. In the middle part of the fragments of the human brain some difference was observed in the distribution of the reaction products compared with the rat brain: In the human brain no reaction was found on the cell membrane of the neurons. However, just as in the rat brain, the membrane of the cristae of some mitochondria here already gave a positive reaction for ATPase. The activity of the basal layer of the capillaries in the human brain 5 h after death was appreciably less than in the rat brain even 48 h after death. At the same time, the cell membrane of the capillary endothelial cells of the human brain carried more reaction product than the corresponding membrane in the capillaries of the rat brain. However, there were insufficient grounds for concluding that this cell membrane in the human brain had greater ATPase activity than in the rat brain. The possibility could not be ruled out that the basal layer in the rat brain, with its very strong ATPase reaction, exhausted the incubation medium and so masked the underlying endothelial cells. The intensity of the reaction was not significantly lower in human brain tissue 8 and 13 h after death than 5 h after death. The precipitate of lead phosphate was coarser (clumpy) 15 h after death and some destruction of the cytoplasmic membrane mainly of the glial cells was observed (Fig. 2a). A very slight decrease

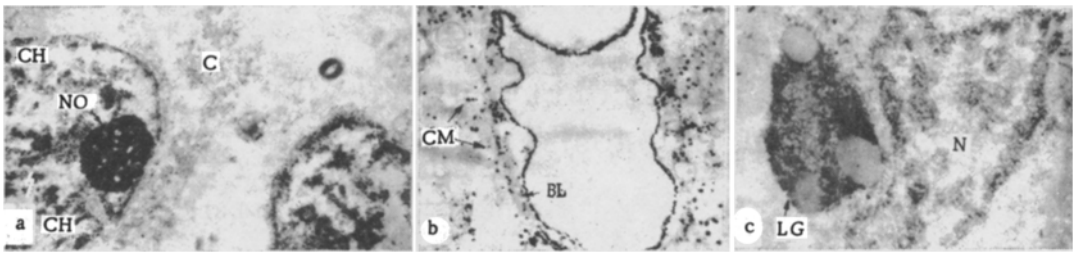

Fig. 2. Postmortem changes in ATPase activity in human brain: a) neuron and glial cell from frontal cortex, 15 h after death. Precipitate localized in cytoplasm (C), chromatin (CH), and nucleolus (NO), 9000×; b) blood capillary in frontal cortex. Precipitate mainly observed along course of cell membrane of endothelial cell (CM) and less marked in basal layer (BL), 10,000×; c) hypothalamus. Lipofuscin granule (LG) in astrocytes adjacent to neuron. Precipitate localized in fibrous part of lipofuscin; N) nucleus of neuron, 12,000×.

in the reaction in chromatin and an appreciable decrease in the cytoplasm were observed in the human brain 21 and 22 h after death. Nevertheless, there was still a sufficiently large number of cells in which the intensity of the reaction corresponded to that in the brain 8 and 13 h after death. By 26 h after death there was a further decrease in ATPase activity in the chromatin and nucleolus and an even greater decrease in the cytoplasm of the human brain.

In the other structures activity remained at the characteristic level of the earlier stages after death. In sections taken from the periphery of the tissue fragments, 46 and 47 h after death the synapses no longer showed ATPase activity. In the cytoplasm of the cells, as a result of extreme destruction of the granular cytoplasmic reticulum, the reaction persisted only on its fragments. The reaction in the chromatin and nucleolus still remained fairly clear. In the lipofuscin and blood capillaries ATPase activity was virtually unchanged compared with the earlier periods after death.

No differences were found in the postmortem decrease in ATPase activity in the frontal cortex and hypothalamus in either the rat or the human brain. On the whole, comparison of the dynamics of the postmortem changes in ATPase activity in the rat and human brain shows that the decrease in enzyme activity took place rather faster in the human than in the rat brain. The important point is that the basic patterns of distribution and intensity of the ATPase reaction in the human brain were preserved until 15-20 h after death, which means that autopsy material can be used for the corresponding tests. As regards the individual ultrastructures, investigations of ATPases in the capillary wall (Fig. 2b) and lipofuscin (Fig. 2c) can be undertaken even later, until 48 h after death. The relative postmortem stability of ATPase activity in the brain tissue revealed by these experiments is in harmony with the results of biochemical and histochemical investigations published previously [6, 7].

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