ELECTRON-CYTOCHEMICAL INVESTIGATION OF ACID PHOSPHATASE IN THE BRAIN AFTER DEATH

V. A. Agafonov

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Acid phosphatase activity was detected by an electron-cytochemical method in the rat and human brain. The lysosomes and lipofuscin granules were found to possess enzyme activity. The use of postmortem material showed that the localization of acid phosphatase is unchanged in the late stages after death.

KEY WORDS: *acid phosphatase*; *postmortem changes*; *brain*.

The use of electron-cytochemical methods for pathomorphological study of the human CNS requires the preliminary solution to the problem of whether autopsy material is suitable for investigations of this type.

Data on changes in the activity and localization of acid phosphatase in the brain tissue of animals and man at different times after death are described below.

In the study of acid phosphatase in the course of postmortem autolysis of the brain, special attention was paid to the lysosomes: They contain a wide range of hydrolytic enzymes and they participate in intracellular processing both of phagocytosed material and of components of the cell itself, if the cell is no longer in need of them [4]. One of the characteristic enzymes of the lysosomes, and one which participates in these processes, is acid phosphatase.

EXPERIMENTAL METHOD

The frontal cortex and anterior hypothalamus of albino rats were investigated immediately after death and 4, 8, 12, 24, and 48 h later. The animals were killed by exsanguination (3 rats at each time). The same regions of the human brain also were studied (10 postmortem cases). Human brains were taken 5, 8, 13, 15, 21, 22, 26, 46, and 47 h after death from physical diseases. Between death and the time of investigation the brains (rat and human) were kept at room temperature (24°C).

Pieces of the corresponding regions of the brain were minced to a size of 250-300 μ in 0.08 mM NaH_2PO_4—NaOH buffer, pH 7.3. For subsequent fixation, a 4% solution of formaldehyde in the same buffer was made up from paraform [3] and filtered through a No. 4 glass filter. The pieces of tissue were fixed on melting ice for 5-10 min. After fixation, the pieces were washed with cold NaH_2PO_4—NaOH buffer once and with 0.1 M Tris-maleate buffer, pH 7.3, twice and transferred into the incubation medium. The incubation medium corresponded in its principal ingredients to the medium used to detect the localization of β-glycerophosphatase in isolated mouse liver nuclei [2]: 0.1 M Tris-maleate buffer, pH 4.0, 10 mM sodium β-glycerophosphate (Merck, West Germany) and 2 mM neutral lead acetate. The medium was prepared by dissolving glycerophosphate in the buffer and then adding the aqueous solution of lead acetate while stirring. The final pH of the medium was 5.5. Incubation was carried out in a dry air thermostat at 37°C for 15 min with stirring. After incubation the pieces were quickly washed with physiological saline and postfixed in 2% osmium tetroxide solution in physiological saline for 20 min at 4°C. The material was then prepared for electron microscopy by the rapid method without further staining [1].

In control experiments for specificity of the reaction the following variants of the

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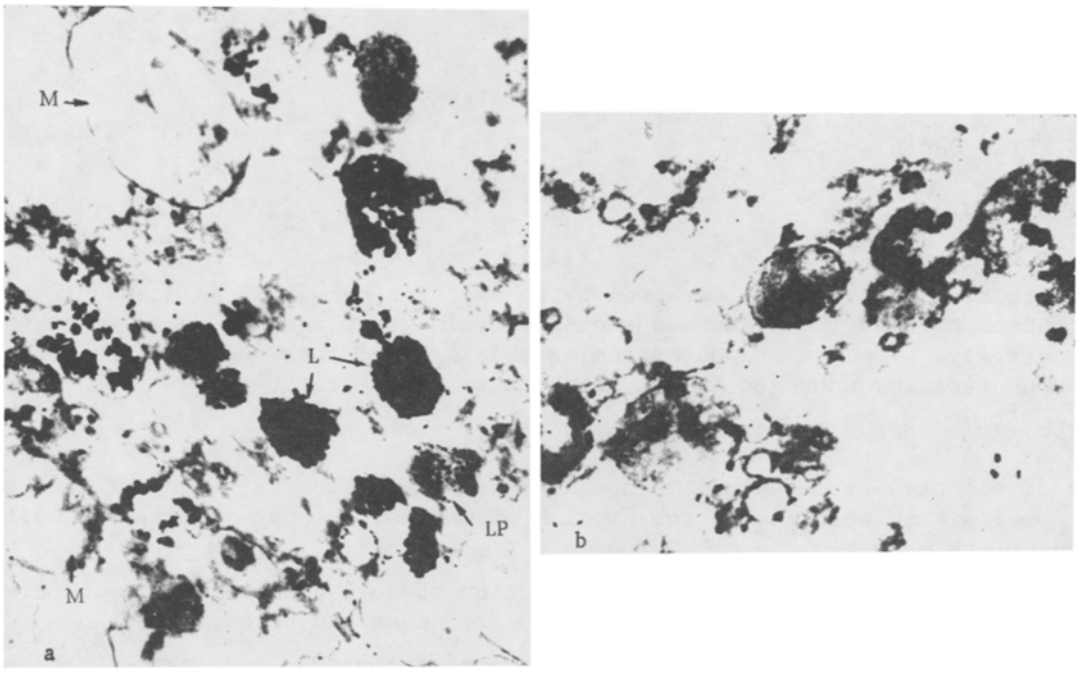

Fig. 1. Localization of acid phosphatase in rat hypothalamus. a) 24 h after death: L) lysosomes; LP) lipofuscin; M) mitochondria (56,000×); b) 48 h after death: mitochondria containing precipitate of lead phosphate (30,000×).

procedure were used: 1) incubation of pieces of tissue in medium without sodium β-glycerophosphate; 2) incubation in complete medium with 0.01 M sodium fluoride; 3) heating the material before incubation to 100°C for 10 min in a 4% solution of formaldehyde in NaH_2PO_4—NaOH buffer, pH 7.3. The hypothalamus of the rat brain was used in these experiments immediately after sacrifice of the animals.

EXPERIMENTAL RESULTS

Investigation of acid phosphatase in the rat brain immediately after sacrifice showed that the reaction product (the lead precipitate) was located only in two types of structures: lysosomes and lipofuscin granules. The finely dispersed residue in the "young" lipofuscin granules was thrown down on membrane fragments, whereas in the "older" granules it occurred in the fibrous material. The reaction product, moreover, was not found in all lipofuscin granules.

In control experiments incubation of the pieces of tissue in medium without substrate or in the presence of the specific inhibitor sodium fluoride [3] led to complete inhibition of the reaction in the lysosomes and lipofuscin. This indicates that the reaction observed is specific for both lysosomes and lipofuscin. Preliminary heating of the tissue completely inhibited the reaction in the lysosomes, although solitary lipofuscin granules with a very slight precipitate were still found. The thermostability of the reaction in lipofuscin in this case can be explained by the different structure of the molecules possessing enzymic activity from that in the lysosomes or by their connection with the structure of the fibrous material of the lipofuscin.

In the first 12 h after death of the animals no changes were found in the distribution or intensity of the reaction for acid phosphatase, although by 12 h some swelling of the lysosomes was observed. On examination 24 h after death the lysosomes appeared not only swollen, but also to have ruptured membranes. The residue of lead phosphate under these circumstances did not go beyond the boundaries of these organelles (Fig. 1a). The reaction in the lipofuscin granules was the same as at the earlier times. The reaction product was visible in the nuclei of individual cells and also in some mitochondria. By 48 h the overwhelming number of lysosomes had tears in their bounding membrane; the intensity of the reaction in them was reduced. In some cases the reaction product could be seen outside the limits of the lysosomes but there was no accompanying lysis of the surrounding structures. Mitochondria with the precipitate were seen more frequently at this time of observation than 24 h after death (Fig. 1b). The activity of the lipofuscin granules was unchanged.

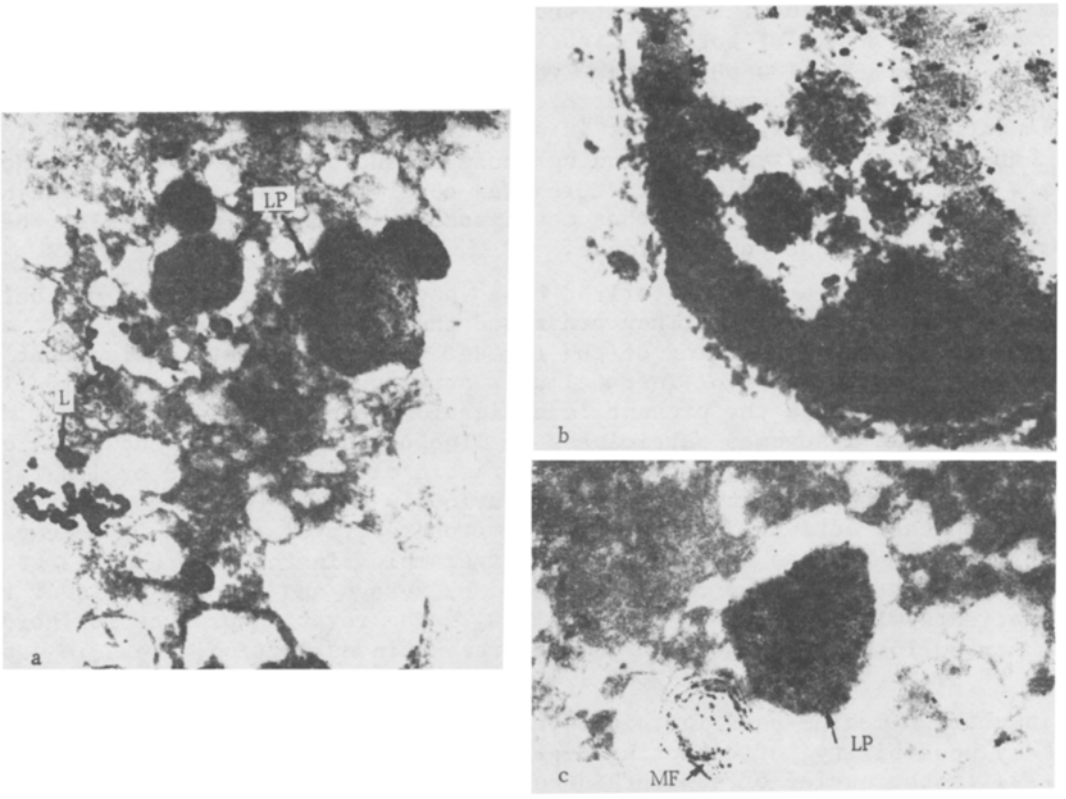

Fig. 2. Demonstration of acid phosphatase activity in human brain 22 h after death. a) hypothalamus: reaction product localized alongside lysosomes (L) and in lipofuscin (LP) (60,000×); b) cortex: chromation of cell nucleus contains reaction product (30,000×); c) hypothalamus: precipitate observed in lipofuscin (LP) and in myelin-like formation (MF) (60,000×).

Investigation of the human brain showed that 5 and 8 h after death the intensity of the reaction for acid phosphatase was the same as in rats 0-24 h after death. The reaction product was observed in the lysosomes (some of them were swollen) and in some lipofuscin granules.

The continuity of the membrane of some lysosomes was disturbed 13 and 15 h after death but the intensity of the reaction and distribution of the product were the same as in rats 24 h after death. Changes analogous to those in the rat brain 48 h after death were observed 21-47 h after death (Fig. 2a). Mitochondria in which the reaction product was found appeared in the human brain 21 h after death and their number increased a little until 47 h. Starting from 22 h the nuclei of some of the destroyed cells also contained the precipitate (Fig. 2b). Myelin-like structures, in which acid phosphatase activity also was demonstrated (Fig. 2c), were found in one of the hypothalamic neurons of the human brains studied 22 h after death. The morphological structure of the lipofuscin granules of the human brain and the intensity of their reaction for acid phosphatase were substantially unchanged at different periods after death.

The results of this investigation justify the following conclusions: 1) acid phosphatase activity in the rat and human brain remains sufficiently stable during the first 12 h after death or it falls insignificantly (this agrees with the result of biochemical [7, 9, 11] and histochemical [6, 8] investigations; 2) whereas immediately after death the typical reaction for acid phosphatase is observed in lysosomes and lipofuscin granules, as autolytic changes develop the products of the reaction for acid phosphatase appear in the mitochondria, the nuclei of individual cells, and also in the cytoplasm close to the lysosomes (especially if rupture of their limiting membrane is observed); 3) in the human brain these cytochemical and structural changes (swelling of the organelles, rupture of the membrane, and so on) develop more rapidly than in the rat brain.

The more rapid fragmentation of the lysosomes in the human brain than the changes in these structures in the rat brain can probably be attributed to the slower postmortem cooling

of the human brain compared with that of the rat. In addition, postmortem destruction of the lysosomes in the brain of both species is intensified on incubation of the material in an aggressive medium with low pH. A few lysosomes remained stable in the late stages after death (48 h), but most of them were destroyed. Considerable diffusion of the enzyme into the cytoplasmic matrix thus took place. It is natural to suggest that not only acid phosphatase diffuses, but also other enzymes, including those of the proteolytic nature. However, no marked lysis of the intracellular structures was observed in the immediate vicinity of the destroyed lysosomes. This suggests that the lysosomes evidently do not play the leading role in postmortem destruction of nerve tissue.

Lipofuscin granules are characterized by a specially high stability of their structure and intensity of their reaction. They preserved their structure not only in the early stages after death, but also after heating of the tissue. This confirms the view that lipofuscin is the final state of degradation of intracellular structures. The presence of acid phosphatase in lipofuscin, confirmed in the present investigation, can be explained as follows: First, the view is held that lysosomes participate in lipofuscin formation. However, on the other hand, the presence of acid phosphatase in lipofuscin may be explained by its formation from mitochondria (this has also been postulated previously [5]). Recent observations have shown that acid phosphatase activity appears in the mitochondria of the CNS in some pathological states. This phenomenon has been observed, for example, in the mitochondria of nerve tissue cells during irradiation of the rat spinal cord by x rays and also in cells of the frontal cortex of patients with Huntington's chorea [10]. The reaction product for acid phosphatase was also observed in the mitochondria in the present investigation, i.e., in postmortem destruction of brain tissue.

The incubation medium used in this investigation has a low pH, which almost completely rules out any possibility of detecting β-glycerophosphatase in the nuclear chromatin [2]. Nevertheless, in the nuclei of some brain cells in the late stages after death, manifestations of acid phosphatase activity were observed. This is in agreement with results obtained previously by light-optical histochemical method in an investigation of the brain taken after death [6].

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