INCREASED ATP-HYDROLASE ACTIVITY OF BRAIN MITOCHONDRIA INDUCED BY CHLORPROMAZINE (ELECTRON-CYTOCHEMICAL STUDY)

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The location of Mg-activated ATPase was determined in mitochondria of the sensomotor cortex of WAG rats 3 h after administration of chlorpromazine (15 mg/kg). Besides normal mitochondria without the reaction product, modified mitochondria also were observed with the product of the ATPase reaction variously localized in them. No precipitate was found in the mitochondria of intact animals. It is suggested that two factors are concerned in the increase in ATP-hydrolase activity of the mitochondria induced by chlorpromazine: depression of glycolytic and respiratory activity of the cells by the neuroleptic and increased permeability of the mitochondrial membranes.

KEY WORDS: chlorpromazine; cerebral cortex; Mg-ATPase; mitochondria; nerve endings.

The effect of chlorpromazine on activity of the Mg-activated ATPases has attracted attention as a topic for research for a long time because of the extensive involvement of enzymes of this group in cell metabolism. Biochemical investigations have yielded contradictory results: Some workers have found an increase, others a decrease in Mg-ATPase activity under the influence of chlorpromazine, while a third group found no change [4]. The contradictory nature of these results can evidently be explained by the choice of different doses of the neuroleptic, different experimental conditions, and different research techniques.

The term Mg-ATPase relates to a series of enzymes with different functions: nuclear enzymes of nucleic acid synthesis [9], mitochondrial ATP-synthetase [3], cytoplasmic ribosomal enzymes [6], ATPase of the blood–brain barrier [5, 10], and so on. For that reason, results obtained with homogenates and even with pure fractions of individual organelles often do not agree when different experimental conditions are used and they are difficult to interpret.

To shed light on the effect of chlorpromazine on brain Mg-ATPase it was decided to use the method of electron cytochemistry. This paper gives data on the effect of chlorpromazine on nerve cell mitochondria.

EXPERIMENTAL METHOD

Male WAG rats weighing 200 g were used. Chlorpromazine was injected intraperitoneally in a dose of 15 mg/kg; physiological saline was injected into the control animals. The rats were decapitated 3 h after injection of the drug and the sensomotor cortex was removed for study.

The localization of Mg-ATPase was determined by the writer's modification [2] of Wachstein and Meisel's method. Tissue samples were dehydrated in ethanol and embedded in Araldite (ultrathin sections were cut on a Reicherdt (Austria) ultratome, stained with lead citrate, and examined with the JEM-100B electron microscope (from Joel, Japan).

EXPERIMENTAL RESULTS

The study of the distribution of Mg-ATPase in the brain of intact animals revealed the reaction product in the nuclei of various cells, the cytoplasm, synapses, and basal layer of the capillaries. It was absent in the mitochondria, although the presence of Mg-ATPase in the mitochondria is well known. The absence of ATPase reaction products can be explained by resistance of the mitochondrial membrane to ATP, and also by the predominance of synthesis over hydrolysis of ATP in these mitochondria. Reaction products began to appear

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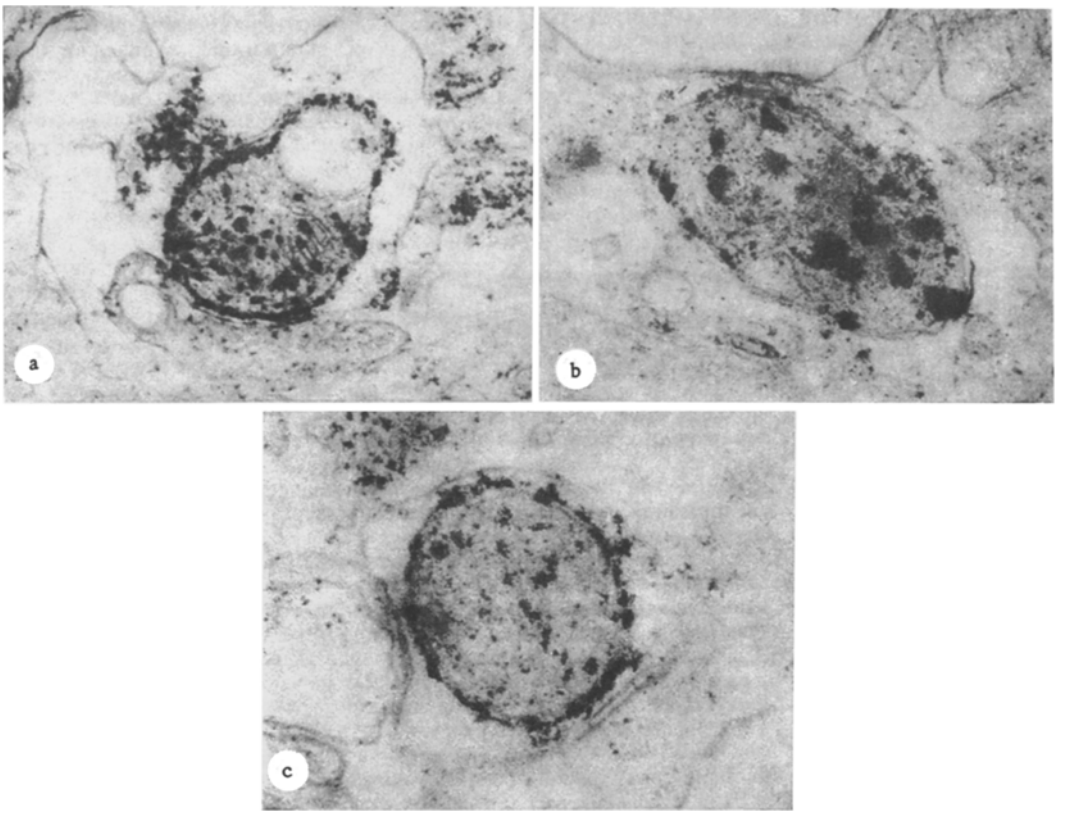

Fig. 1. Location of Mg-ATPase reaction product in mitochondria of sensomotor cortex of rat receiving chlorpromazine (80,000×). a) Reaction product localized between inner and outer membranes and in cristae, structure of mitochondrion clearly visible; b) reaction product localized in mitochondrion consisting of homogeneous and osmiophilic contents; c) reaction product localized mainly at periphery of mitochondrion, structure same as before.

in the mitochondria 3 h after administration of chlorpromazine (Fig. 1a); the space between the inner and outer membranes was filled with precipitate. A small quantity of precipitate also was found in the cristae. The heterogeneity of the reaction of the mitochondria to chlorpromazine and differences in the location of the reaction product in them will be noted. For instance, besides mitochondria with no precipitate, there were others whose internal structure consisted of homogeneous and osmiophilic contents surrounded by a double membrane, with precipitate distributed locally in them (Fig. 1b). In some mitochondria the cristae were difficult to examine, but the reaction product was located mainly on the outer and inner membranes (Fig. 1c). All changes described above relate to mitochondria in the processes of the nerve cells. No product of the cytochemical reaction could be found in the mitochondria of the cell body in either the experimental or the control series.

Large doses of chlorpromazine can inhibit energy metabolism in brain tissue [4]. According to Matsubara and Hagihara [7] there are three possible types of inhibition of respiratory enzymes by neuroleptics: inhibition of electron transport, uncoupling of respiration and phosphorylation, and inhibition of energy transfer. Simultaneously with these, other workers have noted a decrease in the glycolytic and dehydrogenase activity of the brain cells, leading to a reduction in the concentration of substrates for oxidative phosphorylation [4]. A fall in the ATP level in the mitochondrial fraction of nerve endings has also been observed [1]. Interaction between chlorpromazine and biological membranes must also be taken into account. Chlorpromazine is known to form a monomolecular layer on the surface of membranes, thus causing a change in surface tension and the selective permeability of the membrane for organic and inorganic ions [4, 8].

With these considerations in mind the writer has suggested that the appearance of a large quantity of ATPase reaction product after administration of chlorpromazine may be connected with depression of mitochondrial activity as a result of inhibition of the glycolytic and respiratory activity of the cell by chlorpromazine and also with increased permeability of the mitochondrial membrane. When the activity of the mitochon-

dria is reduced, their ATP-synthetase function is known to be depressed, whereas increased permeability of the membrane promotes the passage of ATP through the mitochondrial membrane. Both these factors may thus participate in the increase in ATP-hydrolase activity of the mitochondria under the influence of chlorpromazine, for increasing membrane permeability alone (experiments with preliminary freezing and thawing of brain tissue) led to the deposition of only a small quantity of reaction product in the mitochondria. The heterogeneity of the reaction of the mitochondria can be explained by differences in the initial state of these organelles and also by differences in their "age."

LITERATURE CITED

1. E. L. Dovedova and R. P. Porfir'eva, Farmakol. Toksikol., No. 1, 14 (1974).
2. V. N. Kleshchinov, in: Proceedings of the 10th All-Union Conference on Electron Microscopy [in Russian], Tashkent (1976), pp. 192-193.
3. A. Lehninger, The Mitochondrion, W. A. Benjamin, Reading, Mass. (1965).
4. K. S. Raevskii, The Pharmacology of Neuroleptics [in Russian], Moscow (1976).
5. V. A. Shakhlamov, Capillaries [in Russian], Moscow (1971).
6. F. Grumt, I. Grumt, and V. A. Erdmann, Eur. J. Biochem., 43, 343 (1974).
7. T. Matsubara and B. Hagihara, J. Biochem. (Tokyo), 63, 156 (1968).
8. S. Silbert, Natl. Cancer Inst. Monogr., 23, 285 (1966).
9. R. M. Torack, J. Cytochem. Histochem., 13, 191 (1965).

ULTRASTRUCTURE OF PARIETAL CELLS IN SUMMER AND DURING HIBERNATION

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The parietal cells of red-cheeked susliks were studied in summer and during hibernation. Electron-microscopic investigation and morphometric analysis revealed definite changes in ultrastructure during hibernation, reflecting the absence of secretory activity of the cells. No marked dystrophic changes were found in the parietal cells in this period. Structural differences determined by stages in the life cycle were preserved.

KEY WORDS: parietal cells; ultrastructure; hibernation; morphometry.

The state of hibernation in mammals is characterized, along with other features, by a fall in body temperature to 4-5°C and, in the species studied in this investigation (the red-cheeked suslik, Citellus erythrogenus Br.), by absence of exogenous feeding for a long period (up to 7-8 months). The study of the morphology of the stomach during hibernation, when specific secretory function is absent, is therefore of considerable interest, more especially because no research of this kind at the electron-microscopic level could be found in the accessible literature.

The object of this investigation was to compare the ultrastruture of parietal cells of the red-cheeked suslik killed in summer (i.e., in the active period) after starvation for 24 h, and during hibernation (December, January, or early February).

EXPERIMENTAL METHOD

The tissue was fixed with 4% paraformaldehyde solution, postfixed with 1% osmic acid solution, and embedded in a mixture of Epon and Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the JEM-100C electron microscope. Parietal cells along the whole length of the fundal glands in the region of the body of the stomach were studied. A comparative morphometric analysis was made

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