LITERATURE CITED

1. F. I. Ershov et al., Acta Virol., 23, 32 (1979).
2. T. M. Sokolova and U. Kiessling, Antibiotiki, No. 12, 1112 (1978).
3. T. Hunt et al., J. Mol. Biol., 66, 471 (1972).
4. B. Lebleu et al., Biochem. Biophys. Res. Commun., 82, 665 (1978).
5. H. R. B. Pelham et al., Eur. J. Biochem., 67, 247 (1976).
6. H. Suzuki, J. Biochem. (Tokyo), 83, 1061 (1978).
7. J. Vilcek et al., J. Virol., 10, 614 (1972).

EFFECT OF ACETYLCHOLINE ON Na,K-ATPase OF BRAIN MICROSOMES FROM RATS OF DIFFERENT AGES

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KEY WORDS: aging; brain; ATPase; acetylcholine.

The process of aging is characterized by limitation of the functional capacity of various organs and tissues, including the brain [8]. There is reason to suppose that an important role in the origin of these disturbances is played by the energy deficiency which develops with age.

To study the metabolic mechanisms of age changes in the brain, an important contribution could be made by an examination of the state of transport Na,K-ATPase in old age and changes in its activity under the influence of acetylcholine (ACh). It has been shown that ACh inhibits Na,K-ATPase in brain microsomes [3] and synaptosomes [2, 4], and thus helps to regulate excitation processes in the neuron. There have been isolated studies of brain ATPase activity in the late stages of ontogeny [5-7], but these were conducted on the whole brain without differentiation of its parts. The results of these investigations have proved contradictory.

The object of this investigation was to determine Na,K-ATPase activity in microsomes of the cerebral cortex and the effect of ACh on it in animals of different ages.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats aged 6-7 months (mature) and 25-26 months (old). The animals were decapitated, the brain was removed as quickly as possible, and the cortex was separated from the white matter of the hemispheres. Cortical tissue was homogenized in 0.32 M sucrose with 0.01M Tris-HCl buffer, pH 7.4, for 30-40 sec in a glass homogenizer with Teflon pestle. Microsomes were obtained by differential centrifugation (40,000g, 1 h) of the supernatant obtained after removal of nuclei and mitochondria. The fraction thus isolated was resuspended in isolation medium and used after a single freezing and thawing.

Total ATPase activity was determined in medium (final volume 2 ml) containing (in mM): NaCl 100, KCl 20, ATP-Na_2 2, $MgCl_2$ 2, and 150-170 μg microsomal protein or 250-300 μg protein of homogenate. Mg-ATPase activity was determined in an identical solution in the presence of strophanthin K (0.1 mM). Experimental samples contained ACh in a concentration of 6 mM. The reaction (15 min, 37°C) was started by addition of the substrate. It was stopped by the addition of an equal volume of cold 10% TCA. Inorganic phosphorus was determined in the supernatant [11]. Protein was determined by Lowry's method [12], after preliminary destruction of the membrane with 2% sodium deoxycholate.

Na,K-ATPase activity was calculated as the difference between total and Mg-ATPase activity.

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TABLE 1. ATPase Activity (in μmoles P_i/mg protein/h) of Cerebral Cortical Microsomes of Rats of Different Ages

Age of animals	Na,K-ATPase	Mg-ATPase
6—7 months	5,46±0,48 (10)	18,78±1,01 (10)
24—26 months	3,81±0,47* (8)	21,50±0,86* (8)

Legend. Number of experiments indicated in parentheses. Asterisk indicates significant difference ($P < 0.05$) from first age group.

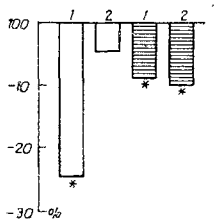

Fig. 1. Changes in cerebral cortical microsomal ATPase activity in rats of different ages under the influence of ACh (activity in control taken as 100%). Unshaded columns — mature, shaded columns — old rats; 1) Na,K-ATPase, 2) Mg-ATPase.

EXPERIMENTAL RESULTS

Table 1 shows that Na,K-ATPase activity in the brain microsomes fell during aging by 30% whereas Mg-ATPase activity increased. A similar pattern of age dynamics of ATPase activity was found when a homogenate of the cerebral cortex of animals of these ages was studied. However, investigations of the homogenate, unlike that of the microsomal fraction, revealed no differences in specific activity of Mg-ATPase in the mature and old rats.

In mature animals ACh (6 mM) inhibited Na,K-ATPase activity much more strongly (by 25%, $P < 0.001$) than in old animals (by 8%; $P < 0.05$). Meanwhile, marked inhibition of Mg-ATPase activity (by 10%; $P < 0.05$) was found in the old rats, but not in the mature animals (Fig. 1).

The redistribution of ATPase activity during aging may be due to several causes, above all to an age modification of the environment of the enzymes on the membrane. It has been shown [1] that a deciding factor in the modulation of Na,K-ATPase activity is the physical state of the membrane and, in particular, the physicochemical state of the lipids. There is information in the literature on changes in the fatty acid composition of total lipids and phospholipids of brain tissue in old rats [10] and, in particular, of the brain microsomes [9], with a decrease in the content of unsaturated fatty acids and an increase in the content of lipid peroxides [13]. These age changes in the cell membranes may themselves lead to a reduction in the activity of transport Na,K-ATPase and a change in the effect of ACh on the activity of this enzyme in old age. However, they do not rule out the possibility that during aging the number of Na,K-centers on the membrane may be reduced and the structure of the enzyme molecule itself changed.

LITERATURE CITED

1. A. A. Boldyrev and Ko Che-chung, Byull. Éksp. Biol. Med., No. 12, 672 (1977).
2. T. K. Vysochina, Zh. Évol. Biokhim. Fiziol., 14, No. 1, 88 (1978).
3. N. N. Demin and E. M. Makarova, Dokl. Akad. Nauk SSSR, 185, 730 (1967).
4. Z. P. Kometiani, T. Ya. Dzariashvili, and L. G. Tsakadze, Biokhimiya, No. 5, 1039 (1975).
5. M. M. Korobenkova, in: Abstracts of Sectional Proceedings of the Second All-Union Biochemical Congress [in Russian], Section 3, Tashkent (1969), p. 121.

6. V. N. Nikitin and R. I. Golubitskaya, Tr. Nauch.-Issled. Inst. Biol. (Khar'kov), 21, 143 (1954).
7. A. A. Sarkisyan and G. G. Adamyan, Zh. Éksp. Klin. Med., No. 2, 67 (1970).
8. V. V. Frol'kis, Regulation, Adaptation, and Aging [in Russian], Leningrad (1970).
9. M. Brunetti et al., Ital. J. Biochem., 26, 365 (1977).
10. D. Eddy and D. Harman, J. Geront., 30, 647 (1975).
11. C. Fiske and J. Subbarow, Science, 65, 401 (1927).
12. O. H. Lowry et al., J. Biol. Chem., 193, 365 (1951).
13. M. Yoshihawa and S. Hirai, J. Geront., 22, 162 (1967).

CHANGES IN THE PHOSPHOLIPID SPECTRUM AND ACTIVITY OF SOME ENZYME SYSTEMS OF PHOSPHOLIPID SYNTHESIS IN THE BRAIN AND LIVER OF ALBINO RATS WITH ALLOXAN DIABETES

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KEY WORDS: phospholipids; rat brain and liver; alloxan diabetes.

The physiological role of phospholipids (PL) as essential components of biological systems in living organisms is evident. PL are known to participate in the regulation of activity of membrane-bound enzymes [12], of insulin secretion [5], limitation of glycolytic reactions in the brain [9], the adjustment of tissue sensitivity to the action of hormones [8], and compensation of a deficiency of the main energy substrate – glucose [2].

In the investigation described below changes in the qualitative and quantitative composition of PL in brain and liver tissue were studied in albino rats with severe metabolic disturbances associated with alloxan diabetes, in the course of conversions of certain products of lipogenesis – free glycerol and L-α-glycerophosphate (GP), and of the activity of the corresponding enzyme systems – glycerokinase (GK), L-α-glycerophosphate dehydrogenase (GPD) in NAD-dependent (GPD-1) and NADH-dependent (GPD-2) systems.

EXPERIMENTAL METHOD

Diabetes was induced in noninbred albino rats of both sexes weighing 170-200 g by intraperitoneal injection of alloxan in a dose of 15 mg/100 g body weight. Animals with a blood glucose higher than 180 mg% were killed on the 20th day of the disease, and chloroform-methanol extracts of PL from acetone powders of brain and liver were fractionated by linear ascending chromatography on FN-11-Filtrak (East Germany) paper, soaked in silicic acid [10]. The quantity of free glycerol [4], activity of GK and GPD-1, and the GP level were determined in the fraction obtained at 1700g by a microspectrophotometric method [7], GPD-2 activity was determined as in [6], and the blood glucose was estimated by the orthotoluidine method. A mixture of phosphotrioses was obtained by Meyerhof's method [11].

EXPERIMENTAL RESULTS

An increase in the total acid PL (APL) by about 62% was found in the brain tissue of albino rats on the 20th day of alloxan diabetes, accompanied by a relatively stable level of total and neutral PL (NPL), mainly on account of a twofold increase (by 121%) in the content of cardiolipins (CL), which play an important role in regulation of the activity of respiratory chain enzymes [3]. The possibility cannot be ruled out that the observed increase in the CL level may be a compensatory-adaptive reaction of the body due to inhibition of

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