The investigations thus showed that insulin can influence Na,K-ATPase activity of the microsomal fraction of rat brain *in vitro*. The results depend both on the dose of hormone added to the incubation medium and on the experimental conditions.

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PHOSPHOLIPIDS, CEREBROSIDE, AND CEREBROSIDE SULFATE LEVELS IN THE CNS OF MICE WITH ACUTE EXPERIMENTAL VIRAL DEMYELINATION

Kh. N. Annanepesov, M. V. Levitina, and E. V. Sergienko

UDC 616.832-002-091.934-022:578.833.26']-07:616.83-008.939.52/53

KEY WORDS: acute viral encephalomyelitis; phospholipidis; cerebrosides; cerebroside sulfates.

One of the most urgent problems in modern neurology is that of the demyelinating diseases. The etiology and pathogenesis of this group of diseaaes, which includes multiple sclerosis, multifocal leukoencephalopath, etc., have not yet been completely explained.

However, opportunities for the intravital study of pathological changes in the nerve tissue of human patients are limited. Because of this, it is particularly valuable to study experimental models of the demyelinating process in animals. Accumulation of data on the possible role of viral infection in the genesis of demyelination [1, 2, 10] had led to the development of adequate experimental viral models.

One such model is encephalomyelitis caused by the neurotropic ghm strain of murine hepatitis coronaviruses (EMH). These viruses, it has been suggested, cause destruction of oligodendrocytes and the appearance of demyelinated regions in the white matter of the brain and spinal cord [2, 10]. Considering that lipids are the main structural component of myelin sheaths, which are the main structure destroyed during demyelination, their study in EMH would seem to be particularly important. There are no data in the literature on biochemical changes taking place in nerve tissue in this disease.

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR L. N. Klimov.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 1, pp. 33-34, January, 1985. Original article submitted March 26, 1984.

0007-4888/85/9901-0040$09.50

TABLE 1. Content of Phospholipids (in % of total lipid phosphorus) in Brain and Spinal Cord of Mice with EMH (M ± m, n = 8-10)

Phospholipids	Brain		Spinal cord	
	control	EMH	control	EMH
Phosphatidylserine	11,99±0,28	11,82±0,33	12,89±0,30	13,42±0,41
Phosphatidylinositol	3,65±0,17	2,14±0,12*	2,95±0,22	2,82±0,20
Sphingomyelin	3,98±0,20	5,02±0,24*	6,67±0,34	6,05±0,25
Phosphatidylcholine	40,33±0,60	40,97±1,17	31,59±0,71	31,83±0,90
Ethanolamine-plasmalogen	20,05±0,74	20,50±1,23	27,04±0,53	25,94±0,49
Phosphatidylethanolamine	18,39±0,44	18,15±0,36	17,32±0,62	18,21±0,53
Diphosphatidylglycerol	1,27±0,26	1,20±0,30	1,24±0,18	1,26±0,20

Legend. Here and in Table 2: *P < 0.05.

TABLE 2. Content of Cerebrosides and Cerebroside Sulfate (in mg/g wet weight of brain) in CNS or Mice with EMH (M ± m)

Fractions studied	Brain		Spinal cord	
	control (n=8)	EMH (n=10)	control (n=10)	EMH (n=11)
Total cerebrosides	10,51±0,24	9,17±0,33*	28,28±0,45	19,60±0,60*
Cerebrosides with hydroxyacids	6,08±0,24	5,23±0,17*	17,38±0,67	12,40±0,46*
Cerebrosides with normal fatty acids	4,43±0,06	3,94±0,26*	10,45±0,26	7,20±0,18*
Cerebroside sulfates	0,82±0,08	0,73±0,024	2,27±0,06	1,61±0,11*

The aim of the present investigation was to study phospholipids, cerebrosides, and cerebroside sulfate in the CNS of mice with acute EMH.

EXPERIMENTAL METHOD

Material was obtained from C3H mice infected intracerebrally at the age of 4 weeks with the ghm strain of murine hepatitis viruses. On the 5th-7th days the disease began to develop in the animals and was accompanied by pareses and spastic convulsions. Animals in which EMH ran a severe course was investigated. The animals were decapitated and total lipid extract prepared from the brain and spinal cord separately by Folch's method [5]. Phospholipids were fractionated by two-dimensional thin-layer chromatography on silica-gel KSK [6, 8] and their relative quantities determined. Cerebrosides and cerebroside sulfates were isolated from the lipid extract by one-dimensional thin-layer chromatography on silica-gel KSK. The cerebrosides were determined quantitatively as sugar by the reaction with orcine [9] with certain modifications [4]. The content of cerebroside sulfate was determined as the sulfate group with azure I [7]. Normal healthy animals of the same age served as the control.

EXPERIMENTAL RESULTS

Investigation of phospholipid fractions in the CNS of mice with EMH revealed no dramatic changes (Table 1). Only a small increase in the sphingomyelin content and a decrease in the phosphatidylinositol content were found in the brain of the affected animals. It can be postulated that not only does demyelination take place in the CNS in EMH, but regenerative changes also develop, with accumulation of sphingomyelin, giving rise to changes in the fractional composition of the phospholipids.

The content of cerebrosides (Table 2) in the brain of the affected animals was 12.8% lower than in the controls (the fraction with hydroxyacids was reduced by 14%, cerebroside content was more marked at 30.7% (fraction with hydroxyacids reduced by 28.7%, that with normal fatty acids by 31.1%). A significant fall in the cerebroside sulfate level also was noted in the spinal cord of the affected mice, where it reached 29.1%, whereas in the brain of the affected animals the decrease was only 11%.

The marked decrease in the content of myelin lipids (cerebrosides and cerebroside sulfates) in the spinal cord of animals with EMH was evidently the result of breakdown of myelin, in which the spinal cord is rich. Investigations [10] have shown that oligodendrocytes, which are the site of synthesis of cerebrosides and cerebroside sulfate in nerve tissue [3], are injured by the virus. Degeneration of oligodendrocytes in such cases may lead to disturbance of synthesis of galactosphingolipids, and this is probably reflected in the content of these substances.

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ATP-DEPENDENT PROTON TRANSLOCATION ACROSS THE RAT BRAIN SYNAPTIC VESICLE MEMBRANE

V. I. Mel'nik, R. N. Glebov, and G. N. Kryzhanovskii*

UDC 612.8.015

KEY WORDS: synaptic vesicles; rat brain; proton pump; H^+-ATPase; acridine orange; valinomycin

Synaptic vesicles (SV) of brain nerve endings possess ATPase activity, which is connected with the storage, uptake, and release of neurotransmitters [1]. It has been suggested that neurotransmitter transport is coupled with the electrochemical H^+ potential, the two components of which, namely pH difference and inside-positive electrical potential, are created through the activity of H^+-ATPase, located in the SV membrane, which accumulates H^+ on account of hydrolysis of ATP. This concept is confirmed by data obtained on various secretory granules: chromaffin granules of the adrenals [9], peptide-containing granules of the neurohypophysis [17], and insulin-containing granules of the pancreas [8].

There is weighty evidence in support of this concept, which has been obtained on SV preparations. For instance, there is a distinct parallel between the action of inhibitors and proton-carrying uncouples on ATPase activity and catecholamine transport in brain SV [11, 18], and acetylcholine transport in SV of the electric organ of the skate [3]. The pH measured in isolated SV of the skate electric organ is low (5.3-5.6) [4, 12]. Meanwhile, some particular features of the ATPase of SV in particular, is weak stimulation by proton-carrying uncouples [2, 18, 19], indicate that H^+-ATPase is not present in the SV membrane [19].

An important contribution to the solution of this problem must be made by direct investigation of the ability of brain SV to undertake ATP-dependent proton translocation. To investigate this problem the method of continuous recording of transmembrane H^+ gradients by means of the dye acridine orange (AO) was used [5, 7, 10]. In the undissociated form, this weak base passes readily across the membrane and, because of the high pK of its NH_2-group, it is distributed in accordance with the H^+ concentration gradient on both sides of the membrane [10]. Accumulating inside the vesicles, as its concentration increases the dye undergoes oligomerization and its optical properties are changed [7]. Accumulation of the dye, which is proportional to the H^+ gradient, can be recorded either as a decrease in its extinction using the two-wavelength method [5, 7] or as quenching of fluorescence [10], which reflects a decrease in content of the monomer form in the incubation medium. A similar AO probe has been used to investigated ATP-dependent H^+ gradients in chromaffin granules of the adrenals [15].

*Corresponding Member, Academy of Medical Sciences of the USSR.

Laboratory of Molecular Pathology and Biochemistry, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 99, No. 1, pp. 35-38, January, 1985. Original article submitted June 15, 1984.

 0007-4888/85/9901-0042$09.50