as well as considerable changes in lymphocyte functional activity influenced by activating agents were found as compared to healthy persons.

The reduction of MP of blood cells and, in particular, the MP of lymphocytes in patients with HD is in accordance with the disturbances revealed in the cation-transport systems of these patients. [1,3].

It is reasonable to assume that the decrease of lymphocyte Ca^{2+}-ionophore sensitivity in HD patients is associated with an increased intracellular Ca^{2+} content due to both the enhancement of the passive Ca^{2+} permeability of the cell membranes and the disturbances in the Na^{+}-Ca^{2+} exchange [3,8,9].

These changes are undoubtedly of great importance in HD pathogenesis. The drop of blood cell MP and the increased intracellular Ca^{2+} content in patients with HD are assumed to be one of the essential factors, leading to an activation of the sympathetic, serotonic, and GABA-ergic systems as well as to an increased vascular reactivity [4].

The decrease of lymphocyte sensitivity to T-cell mitogens (PHA and Con A) attests to disturbances in the T-dependent immunity system in HD patients. The results obtained are in accordance with published data on a T-cell deficiency in animals with experimental arterial hypertension and, in particular, in spontaneously hypertensive rats [10].

Thus, the results of the investigations point to appreciable disturbances in the lymphocyte membranes and confirm the systemic nature of the membrane defects in this pathology.

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Detection of Cytoplasmic PCK BB in Nerve Cell Nuclei of Normal, Schizophrenic and Alzheimer Patients

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UDC 616.895.8+616.894−053.9]−07:616.8−018.1−008.931:577.152.273

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 2, pp. 153−155, February, 1993
Original article submitted July 7, 1992

Key Words: *phosphocreatine kinase BB; brain; nuclear fraction; schizophrenia; Alzheimer's disease*

It has been demonstrated in our previous reports that in patients with mental diseases a depression of the activity of phosphocreatine kinase (PCK BB), which is one of the main enzymes of the brain's energy metabolism, is accompanied by a decrease of its content in extracts of cytoplasmic proteins from nervous tissue [1,2,6].

Additionally, it has been shown by the immunocytochemical method that normally PCK BB is located in the astrocyte cytoplasm and in indi-

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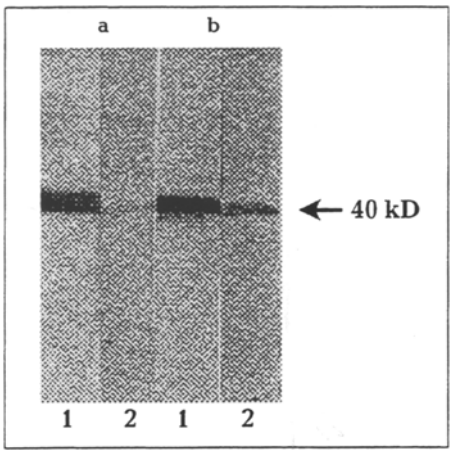

Fig. 1. Immunoelectroblots of brain proteins. *a*) immunoelectroblot of water–soluble extract of brain proteins in the norm (1) and in Alzheimer's disease (2); *b*) immunoelectroblot of membrane protein fraction separated with 10 M carbamide and 1% Triton X–100 from isolated nerve cell nuclei in the norm (1) and in Alzheimer's disease (2).

vidual neurons [4,6]. In patients with mental pathology (schizophrenia and, especially, Alzheimer's disease) the number of PCK BB-immunoreactive astrocytes has been found to be vanishingly small. At the same time, in several cases of Alzheimer's disease and schizophrenia PCK BB has been found in glial cell nuclei in the white substance of the brain sections; here, the nuclear membrane stained the most intensively. In the norm, the cell nuclei apparently also stain, but the presence of the isozyme in the nuclei is masked by the intensive staining of the cytoplasm.

In the present paper we study the localization of cytoplasmic PCK BB in the nuclear fraction of nerve cells in the norm and in patients with schizophrenia and Alzheimer's disease.

MATERIALS AND METHODS

Samples of frontal cortex (area 10) from patients with schizophrenia (4 cases) and Alzheimer's disease (4 cases) who had suddenly died, as well as from a control group without any mental pathology (3 cases) were used in the experiments. All test groups correspond to one another in age (50-65 years) as well as in the time from the moment of death to the sample taking (5-8 h). The samples were immediately frozen in liquid nitrogen.

For isolation of the nuclear fraction, samples of brain tissue were homogenized in 0.32 M sucrose, and isolation was carried out by a sucrose density gradient after Osadchaya [5]. The nuclear fraction was then suspended in a buffer containing 1% Triton X-100 and 10 M carbamide and dialyzed against buffered physiological solution, and the PCK BB content was determined by immunoblotting. Other fractions obtained by centrifugation were treated and analyzed in the same manner. PCK BB in the protein fractions was determined by immunoblotting [12] with the use of both monoclonal antibodies and polyclonal antiserum to PCK BB. The techniques for obtaining antiserum and for performing immunoblotting have been described previously [2,6].

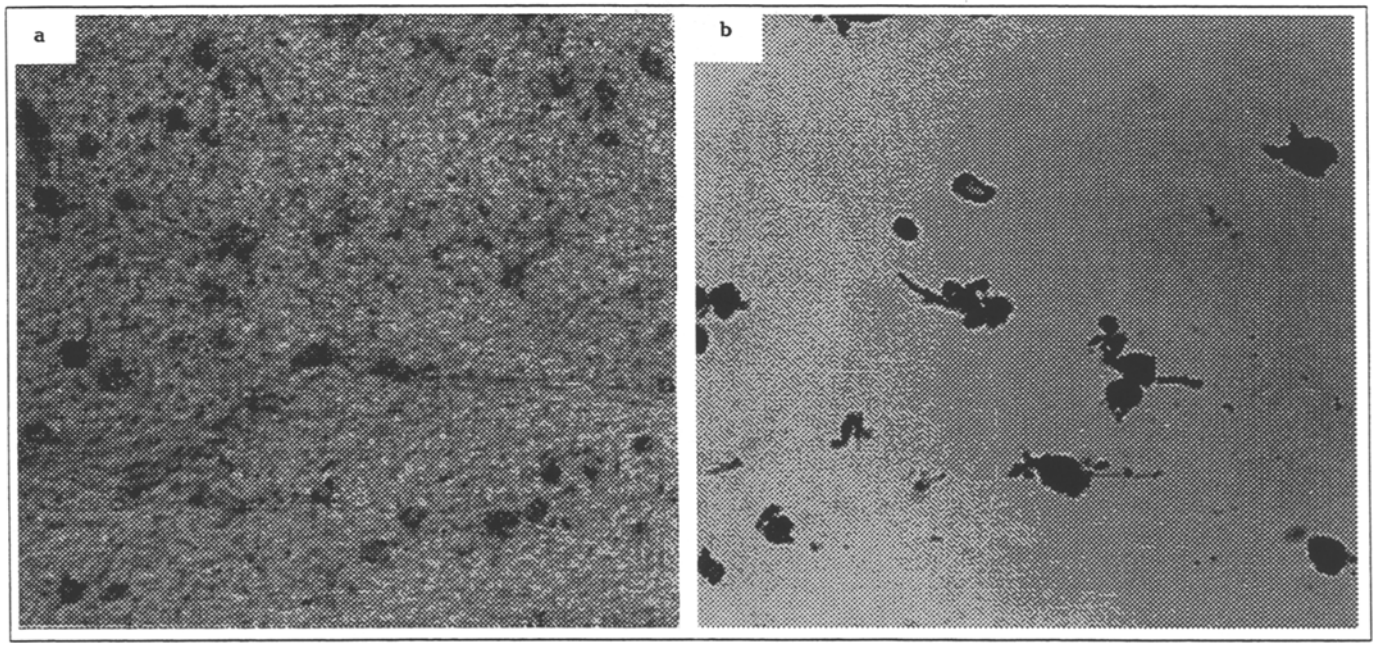

Fig. 2. Immunocytochemical location of PCK BB in white substance (*a*) and in nuclei isolated from brain tissue (*b*) from a patient with Alzheimer's disease, ×400.

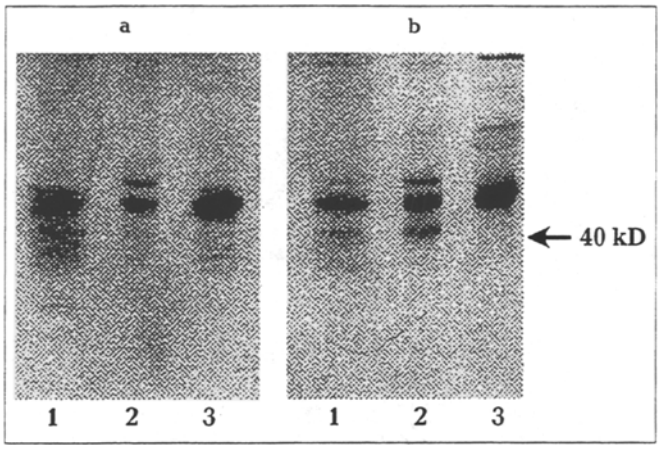

Fig. 3. Immunoelectroblot of proteins of some Subcellular fractions obtained by sucrose density gradient centrifugation of brain tissue homogenate from a healthy subject. 1) proteins solubilized with detergents from nuclear membrane fraction; 2) proteins solubilized with detergents from mitochondrial membrane fraction; 3) extract of water–soluble cytoplasmic proteins; *a*) developed by monoclonal antibodies to PCK BB; *b*) developed by polyclonal antiserum to PCK BB.

For the immunocytochemical analysis of the nuclear fractions of brain tissue from healthy persons and from patients with mental pathologies (schizophrenia and Alzheimer's disease), the smears of the nuclear fraction material were fixed for 10 min with 10% formalin, and then, after washing with running water, the PCK BB content was determined immunochemically by the peroxidase-antiperoxidase method [11]. The control samples were incubated in the absence of antiserum to PCK BB.

RESULTS

For a study of the possible PCK BB presence in nerve cell nuclei, the nuclear fraction was isolated from the samples of brain tissue of normal, schizophrenic, and Alzheimer patients. As described above, a significant reduction of PCK BB content in patients with mental diseases has been found in the water-soluble extract of cytoplasmic proteins of brain tissue [2,6]. No PCK BB content in the brain extracts from patients with schizophrenia and Alzheimer's disease was found in the present study either.

The PCK BB content in all nuclear fractions isolated was determined by immunoblotting with the use of polyclonal antiserum and monoclonal antibodies. The results obtained suggest a high PCK BB content in the fractions of nerve cell nuclei of the brain tissue from the control group. In patients with schizophrenia and Alzheimer's disease the PCK BB content in the nuclear fraction was found to be lower than normal (Fig. 1).

As described above, in addition to the disappearance of immunopositive astrocytes, the astrocyte nuclear membrane was detected on the brain sections in several cases of Alzheimer's disease and schizophrenia (Fig. 2, *a*). The immunocytochemical study of the nuclear fractions of brain tissue from healthy persons and patients with mental diseases revealed a positive reaction to PCK BB in the nuclei in all cases examined (Fig. 2, *b*). The reaction to PCK BB in single capillaries was hardly expressed.

Thus, we demonstrated the presence of PCK BB in the nuclear fraction of the human brain, despite its being considered a cytoplasmic isozyme. In patients with mental pathologies the disappearance of PCK BB from the nerve cell cytoplasm was found not to be accompanied by an increase of this enzyme in the nuclear membrane fraction, in contrast to protein kinase C, which can be easily translocated from the cytoplasm to the membrane [7].

During the isolation of the nuclear fraction in a sucrose density gradient we also obtained other subcellular fractions; in some of them, produced by fractionation of normal brain extract, the PCK BB content was determined by immunoblotting. The results testify the presence of PCK BB not only in the nuclei but also in the fraction of synaptosomes, microsomes, and mitochondria of normal brain cells (Fig. 3). Mitochondria are known to contain one of the PCK isozymes, namely mitochondrial PCK (MIT-PCK), and consequently the presence of a band corresponding to 40 kD in the blot of the mitochondrial extract may be ascribed to the crossing-over of polyclonal antibodies to PCK BB with mit-PCK. However, the near-total identity of the blots developed by poly- and monoclonal antibodies to PCK BB suggested the presence of this isozyme in the mitochondrial membranes, since the monoclonal antibodies used did not have crossing-over with mit-PCK. In addition, there are reports that PCK BB is associated with synaptosomal brain membranes [3,8] as well as with postsynaptic electrocyte membranes and "Torpedo" synaptic vesicles [13]; PCK has been found in sarcoplasmatic reticular membranes [9], in plasmatic membranes, and in myocardial cell nuclei [10]. Therefore, cytoplasmic PCK BB seems to be associated with a variety of nerve cell membranes. In patients with mental pathologies (schizophrenia and Alzheimer's disease) a lowered PCK BB content was detected not only in the cell cytoplasm, but also in the fraction of nuclear membranes. Nevertheless, the question remains open about changes in the amount of PCK BB associated with other subcellular membranes.

The authors wish to thank Dr.Morris (University of Wales, U.K.) for supplying monoclonal antibodies to PCK BB.

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PHARMACOLOGY

Effect of Amiridin and Piracetam on Memory Disturbances Induced by Experimental Stress

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UDC 615.214.015.4.612.821.1/.3]:613.863].07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol.115, № 2, pp.155–157, February, 1993
Original article submitted September 1, 1992

Key Words: *amiridin; piracetam; avoidance response; memory; stress*

The preparation amiridin was developed for the treatment of nervous and mental diseases [2,15]. In particular, it has proved highly effective in the treatment of senile dementia. However, experimental studies performed so far on the effects of amiridin on higher nervous activity have been confined to the methodology of passive avoidance [2,4]. The influence of the drug on more complex behavior has not yet been investigated.

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The present study was aimed at a comparative investigation of the effect of amiridin and piracetam on the formation of the avoidance response (AR) in the norm and after its functional impairment.

MATERIALS AND METHODS

The study was performed on 94 mongrel male rats with a body weight of 180-220 g in 4 series of experiments. In each series 3 groups of animals were used. Animals of group 1 received amiridin injections (1 mg/kg), while those of the second group received piracetam injections (300 μg/kg). The preparations

0007–4888/93/0002–0150$12.50