Changes in the Levels of Neurospecific Proteins and in Behavioral Phenomena in Rats with Hepatic Encephalopathy

G. A. Ushakova[1], O. Z. Fomenko[1], and S. G. Pierzynowski[2,3]

Neirofiziologiya/Neurophysiology, Vol. 43, No. 3, pp. 231-234, May-June, 2011.

Received May 10, 2011.

We showed that under conditions of experimental chronic hepatitis, excessive increases in the levels of a Ca-binding protein, S-100b, and a neuronal cell adhesion molecule, NCAM, occur in all parts of the brain of Wistar rats. This is accompanied by suppression of locomotor and orientational/research activity of the animals and increase in their stress sensitivity. Treatment by α ketoglutarate and cytoflavin in chronic hepatitis provides a clear neuroprotective effect.

Keywords: hepatic encephalopathy, S-100b, NCAM, behavior.

INTRODUCTION

The number of subjects suffering from pathologies of the liver, to great regret, has noticeably increased within the last time period. It was found that diseases of the liver can be accompanied by considerable disorders in the functioning of the brain. A complex of significant neurological and mental disorders qualified as hepatic encephalopathy (HEP) frequently develops in such patients [1]. A number of studies demonstrated clear interrelations between chronic hepatitis C and such manifestations as chronic fatigue, depression, attention disorders, decrease in the ability to concentrate, and drop in the rate of information processing [2]. Patients with minimum HEP symptoms or their clear manifestation demonstrate cognitive disorders of different intensities. Hyperammonemia manifested to one extent or another is believed to be the main source of hepatic pathology-associated neurological changes in the CNS. When HEP has developed, the action of neurotoxins is directed, first of all, toward cerebral astrogliocytes. Astrocytes in this case undergo functional overloading (because of long-lasting intoxication by short-chain fatty acids, ammonium, manganese, and GABA), and noticeable morphological modifications develop in these cells. As a result, astrocytes lose their ability to adequately eliminate neurotransmitters and astrocyte-neuronal interaction. The data obtained earlier dealt mostly with changes in the morphological characteristics of the nerve tissue and disorders in the neurotransmission process [3, 4]. At the same time, there was practically no information about the effects of chronic diseases of the liver on the distribution of astrocyte- and neuron-specific proteins in different cerebral structures and on behavioral phenomena.

We studied changes in the levels of an astrocyte-specific Ca-binding protein, S-100b, and a neuron-specific protein, neuronal cell adhesion molecule (NCAM) in rats with experimental chronic hepatitis, ChH. In the same animals, ChH-related changes in the behavioral phenomena and possibilities for correction of the pathological process using α ketoglutarate and cytoflavin were examined.

METHODS

Reagents from Sigma (USA) and Serva (USA) were used in the experiments.

The development of ChH in Wistar rats (adult animals weighing 160 to 180 g) was induced using a technique described in the patent u2006004614 [5].

[1] Oles' Gonchar Dnipropetrovsk National University, Dnipropetrovsk, Ukraine.
[2] Lund University, Lund, Sweden.
[3] Institute of Agricultural Medicine, Lyublin, Poland.

Thirty-two rats divided into 4 groups, 8 animals each, were used in the experiments. Group 1 served as the control; ChH with no additional curative measures was induced in animals of group 2. Rats of group 3, after the development of ChH, were treated with α ketoglutarate (0.228% in drinking water, for 10 days). Animals of group 4, after the development of the above-mentioned pathology, were injected with cytoflavin (0.5 ml/100 g of body mass, i.p., for 10 days). All procedures with the rats were carried out in accordance with the ethical rules for manipulations with experimental animals.

After formation of the ChH model, animals were decapitated under light anesthesia (isofuran). Different regions of the brain (hippocampus, thalamus/hypothalamus, cerebellum, and cerebral cortex) were separated. Tissues of these structures were homogenized in buffer A containing (mM): tris-HCl, 25, EDTA, 1.0, β mercapthoethanol, 2.0, FMSF, 0.2, and merthiolate, 0.01% (pH 7.4) at a 1:10 ratio. In the course of sequential phases of centrifugation, fractions containing soluble and membrane-bound (extracted using Triton X-100, 2%) proteins were separated. The amounts of NCAM and S-100b were estimated using a competition inhibitory IFA technique; monospecific antibodies and corresponding purified protein standards were used [6].

The behavioral activity of animals was subjected to the open-field test (by Bureš) [7].

Statistical calculations were carried out using Excel software. Intergroup comparisons were performed according to Student's t-test; correlations were estimated using Pearson's criterion.

RESULTS AND DISCUSSION

As is known, there are rather clear correlations between the functional activity of one cerebral region or another and the type of functional activity of the animal's organism controlled by this region. We studied the distribution of neurospecific proteins in the regions of the brain mostly responsible for motor activity, cognitive activity, and sensory sensitivity.

Changes in the distributions of S-100b and NCAM were described in detail earlier for the conditions of acute neurological disorders (global hypoxia, ischemic and hemorrhagic insults, and craniocerebral traumas) [8, 9]. At the same time, estimations of the amounts of these neurospecific proteins under conditions of chronic diseases, especially visceral ones, are extremely limited.

According to our measurements, the levels of both S-100b and NCAM exceeded considerably normal values under conditions of the experimental ChH in all regions of the brains of group-2 rats. The greatest rise of the S-100b level (to about 1210%) was observed in the cerebellum, and in the thalamus/hypothalamus it was only slightly smaller (to 1060%). In the sensorimotor cortex and hippocampus, the respective figures for the S-100b level were 450 and 470%, as compared with the control.

The astroglial Ca-binding protein S-100b is involved in the control of calcium homeostasis in both astrocytes and neurons. Depending on its concentration (nanomolar and micromolar levels, respectively), this protein acts as either a neurotrophic or neurotoxic factor [10]. Increases in the S-100b level to micromolar values lead to intensification of expression of β amyloid proteins and to apoptosis of brain cells. The development of HEP under ChH conditions leads to increase in the permeability of the blood-brain barrier (BBB). We found that hyperproduction of S-100b in the brain and weakening of the barrier function of the BBB result in increase in the level of the above protein in the blood serum [11]: this phenomenon can be used as a marker of the development of HEP.

The NCAM concentration in membrane fractions of the rat brain under ChH conditions was found to be the highest in the somatosensory cerebral cortex (group 2, 128.6 ± 15.3 μg/ml vs. 24.9 ± 1.5 μg/ml in the control group 1) and in the thalamus/hypothalamus (140.2 ± 22.8 vs. 46.4 ± 4.5 μg/ml, respectively). In the cerebellum and hippocampus, the corresponding values for NCAM were 97.3 ± 13.9 vs. 38.9 ± 5.9 μg/ml and 116.5 ± 9.4 vs. 50.1 ± 4.0 μg/ml, respectively, for the former and latter structures.

Due to its adhesive properties, NCAM is capable of regulating the dimension of synaptic clefts, and this, to a considerable extent, modulates the efficacy of synaptic transmission. This agent is involved in the regulation of the learning process and of the emotional and mental state of the organism [12, 13].

Our data demonstrated that the development of HEP significantly suppresses locomotor and cognitive activities of rats under open field conditions (Fig. 1) and increases their sensitivity to stress. The latter statement was confirmed by worsening of the emotional state of the animals (increased number of defecation boluses was indicative of such a shift). The analysis demonstrated the existence of negative correlations between the levels of the above-mentioned proteins and behavioral indices of the rats. The correlation coefficient r for the levels of S-100b and intensity

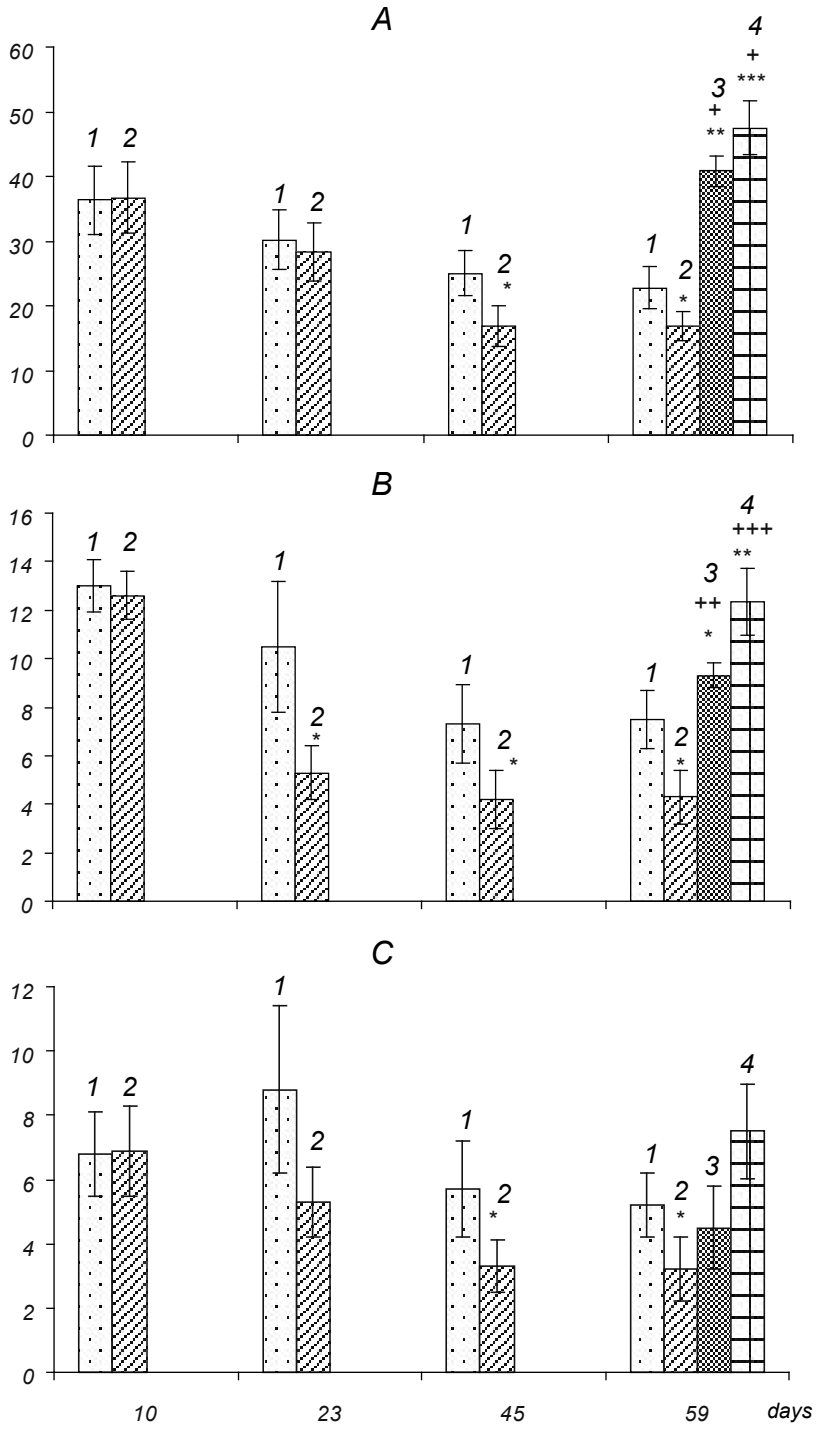

Fig. 1. Indices of locomotor (A) and orientation/research (B, C) activities of rats under open field conditions in the norm (1) and after the development of experimental chronic hepatitis, ChH (2), under ChH conditions with introduction of α ketoglutarate (0.228% in drinking water for 10 days, 3) and under analogous conditions with introduction of cytoflavin (50 mg/kg daily for 10 days, 4). Means and s.d. of the numbers of crossed squares (A), numbers of vertical stands (B), and numbers of looking into test holes (C) within the observation period are shown. Horizontal axis) Days of the experiment. Numbers of animals in each of the experimental groups, n = 8; one, two and three asterisks indicate cases of significant differences from values in the control group 1 with $p < 0.05$, < 0.01 and < 0.001; one, two, and three crosses show the same for comparison with values in group 2 (ChH).

of locomotor activity was –0.33, and the respective value for NCAM was –0.53. In other words, abnormal excessive increases in the levels of these proteins correlated rather clearly with decreases in the indices of locomotor and orientation/research activity of the animals.

We also examined pharmaceutical correction-induced changes in the relative levels of the above proteins and characteristics of the behavioral phenomena manifested by the animals. For such correction of the ChH state, cytoflavin (Polysan, Russia) was tested. Succinic acid (100 mg/ml), nicotinamide (10 mg/ml), inosine (20 mg/ml), and riboflavin mononucleotide (2mg/ml) are the active components of this drug. Cytoflavin was i.p. injected daily in doses of 0.5 ml/100g of body mass during 10

days. We also tested an intermediate metabolite of the citric acid cycle, α ketoglutarate, which intensified the process of detoxication of ammonia and products of oxidative stress. It should be mentioned that, a present preclinical testing of this preparation has been performed (Essentis, Sweden). Ketoglutarate was introduced as 0.228% solution in drinking water for 10 days. The treatment with both α ketoglutarate and cytoflavin positively influenced the levels of examined neurospecific proteins and normalized behavioral phenomena in experimental rats; the respective indices became closer to the indices in the norm.

The results obtained show that chronic impairment of the detoxication function of the liver results in the development of intense encephalopathy with significant suppression of the locomotor and orientation/research functions of the animals and a rise in the stress sensitivity of their organisms. The treatment by cytoflavin and α ketoglutarate in the case of ChH-related encephalopathy provides rather clearly manifested neuroprotective effects.

REFERENCES

1. D. Häussinger, "Hepatic encephalopathy," *Acta Gastroenterol. Belg.*, **73**, No. 4, 457-464 (2010).
2. S. Bengmark, "Bio-ecological control of chronic liver disease and encephalopathy," *Met. Brain Dis.*, **24**, No. 1, 223-236 (2009).
3. M. J. McPhail and S. D. Taylor-Robinson, "The role of magnetic resonance imaging and spectroscopy in hepatic encephalopathy," *Met. Brain Dis.*, **25**, No. 1, 65-72 (2010).
4. A. Lemberg and M. A. Fernández, "Hepatic encephalopathy, ammonia, glutamate, glutamine and oxidative stress," *Ann. Hepatol.*, **8**, No. 2, 95-102 (2009).
5. Patent 15752 of Ukraine, MPK G 09 B 23/28, A technique of modeling chronic hepatitis C, V. Yu. Nikolenko, Yu. I. Nikolenko, and O. Yu. Nikolenko; declarant and owner Donetsk State Medical University, № u2006004614, publ. 17.07.2006/
6. G. Ushakova, O. Fed'kiv, O. Prykhod'ko, et al., "The effect of long-term lactobacilli (lactic acid bacteria) enteral treatment on the central nervous system of growing rats," *J. Nutr. Biochem.*, **20**, No. 9, 677-684 (2009).
7. J. Bureš, O. Burešova, D. P. Hewston, Methods and main experiments for studying the brain and behavior [in Russian], Vyssh. shkola, Moscow (1991).
8. J. Steiner, B. Bogerts, M. L. Schroeter, et al., "S100B protein in neurodegenerative disorders," *Clin. Chem. Lab. Med.*, **49**, No. 3, 409-424 (2011).
9. L. H. Brennaman and P. F. Maness, "NCAM in neuropsychiatric and neurodegenerative disorders," *Adv. Exp. Med. Biol.*, **663**, 299-317 (2010).