Neurophysiology, Vol. 46, No. 2, April 2014

Redistribution of Cell Adhesion Proteins in the Brain and the Peculiarities of Behavioral Phenomena in Rats with Chronic Pancreatitis

V. A. Makarchouk[1] and G. O. Ushakova[2]

Received March 1, 2014.

We demonstrated that, under conditions of the development of experimental pancreatitis accompanied by a decrease in locomotor and orientational-research activity of animals and also by the development of the stress state, concentrations of the soluble and membrane isoforms of neuronal cell adhesion molecule (NCAM) change in the cerebellum and thalamus.

Keywords: NCAM isoforms, chronic pancreatitis, behavioral phenomena.

INTRODUCTION

Over the past years, the number of patients with chronic pancreatitis (ChP) has increased in the entire world and in Ukraine, in particular [1]. Chronic pancreatitis is a progressive inflammatory disease of the pancreas that leads to gradual destruction of parenchyma and fibrous degeneration of this gland. As a result, exocrine and endocrine insufficiency of this organ develops [2].

Disturbance of the processes of free-radical oxidation is an early nonspecific negative factor underlying the development of different diseases, including ChP. The level of antioxidant activity in pancreatic tissue is one of the lowest in the organism; this is why the release of products of free-radical oxidation and endotoxins from this gland in circulation contributes to the formation of an endogenous intoxication syndrome [3]. Endotoxins cause changes in the blood-brain barrier permeability. The main part of such endotoxins belongs to intermediate-mass molecules capable of manifesting clearly pronounced neurotoxic activity and binding to specific cellular receptors. Therefore, blocking of such receptors results in inadequate modifications of metabolism and functioning of the nerve cells [4].

An early and severe complication of acute pancreatitis is pancreatic encephalopathy; its pathogenesis and mechanisms of development remain poorly studied [5]. The mechanism underlying the development of such encephalopathy in ChP remains also unknown in detail and is, therefore, intriguing for researchers. Data on the effects of ChP on the distribution of nonspecific proteins in different cerebral structures and also on the behavior of the animals with this pathology are absent.

In our study, we examined changes in the levels of fractions of neuronal cell adhesion molecule (NCAM) in various structures of the brain and modifications of behavioral phenomena in rats observed within the fibrous stage of ChP.

METHODS

Experiments were carried out on male rats (6 months old) weighing 190-220 g. To model ChP, the animals were subjected, after laparotomy, to occlusion of the pancreatic duct by ligation (Prolene 5/0) [6]. One group (n = 6) consisted of sham-operated rats subjected only to laparotomy (control), while another group (n = 6) included rats in which ChP (induced by the above occlusion) corresponded, on the 30th day, to the fibrous stage of this pathology.

Thirty days after surgery, the brains of the rats were removed and cleaned form the membranes and capillaries; the cerebellum, hippocampus, and thalamus were isolated. Tissue samples were homogenized at +4°C in buffer A containing 25 mM Tris-HCl (pH 7.4), 1 mM EDTA, 2 mM β-mercaptoethanol, 0.2 mM PMCP, and 0.01% merthiolate (ratio 1:10). In the course of serial stages of centrifugation, we obtained fractions that

[1] Institute of Gastroenterology, National Academy of Medical Sciences, Dnipropetrovsk, Ukraine.
[2] Oles' Gonchar Dnipropetrovsk National University, Dnipropetrovsk, Ukraine.
Correspondence should be addressed to
V. A. Makarchouk (e-mail: viktoriam7@gmail.com).

contained water-soluble (sNCAM) and membrane (mNCAM) fractions of cell adhesion protein; extraction was performed using Triton X-100 (2%).

In the obtained protein fractions, we estimated quantitatively the contents of NCAM with the help of an inhibitory IFA technique using monospecific antibodies against NCAM and the corresponding purification of the protein used [7]. The content of NCAM was characterized by the ratio of its amount in the sample and the content of total protein (TP) in the given extract (μg NCAM/mg TP). The amount of TP in the cerebral fractions was estimated using the Bradford technique [8].

The behavioral activity of the animals was examined using the standard open-field test [9].

Statistical processing of numerical data was performed using standard software (SPSS for Windows 9.0). For indices, we calculated the mean values and error of the mean . Intergroup differences were considered significant at $P < 0.05$. Correlation analysis was also performed (by Pearson) [10].

RESULTS AND DISCUSSION

To study the distribution of NCAM, we chose cerebral structures responsible mostly for the control of motor activity, sensory sensitivity (including algesic one), and processes of learning and memory.

Changes in the distribution of NCAM under conditions of acute neurological disorders (global hypoxia, ischemic and hemorrhagic insult, and craniocerebral injuries) were described earlier in detail [11]. However, information on the redistribution of NCAM in different cerebral structures in chronic diseases of the internal organs, in ChP in particular, remains rather limited.

According to the data of quantitative analysis of NCAM fractions in the cerebellum, we found that the amount of sNCAM in this cerebral structure was higher by 19.7% than in the control (2.18 ± ± 0.14 *vs* 1.75 ± 0.14 μg/mg TP; $P < 0.05$). At the same time, the concentration of mNCAM was lower by 46.0% (212.52 ± 29.19 and 114.83 ± 9.48 μg/mg TP, respectively; $P < 0.01$). Such shifts were related, perhaps, to "cutting" of the extracellular domain of mNCAM from the cell membranes. Correlation analysis demonstrated the presence of strong inverse correlation between the concentrations of sNCAM and mNCAM in fractions extracted from the cerebellum ($r = –0.886$, $P < 0.05$). As is known, sNCAM is significantly involved in the control of extracellular signaling [12]. NCAM regulates cell-to-cell adhesion and migration of neurons [13]. Transmembrane adhesive molecules not only function as messengers in the process of cell-to-cell recognition but also can transform signals inside the cell and induce cellular responses that regulate synaptic plasticity [14]. Redistribution of water-soluble and membrane NCAM isoforms in the cerebellum results from endotoxication in ChP and

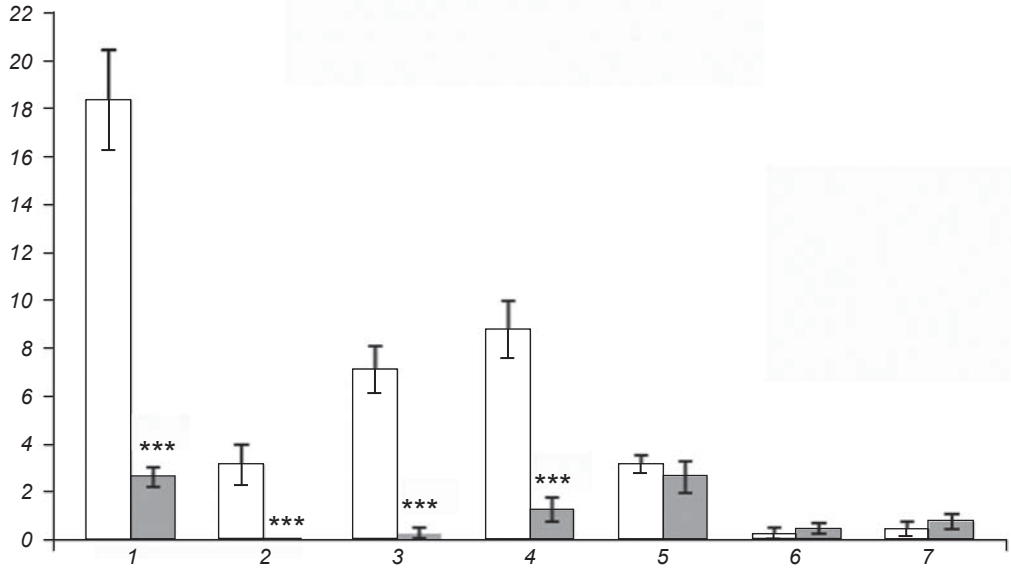

Fig. 1. Behavioral characteristics of control rats and animals with chronic pancreatitis, ChP (in the fibrous stage) in the open-field test. Open and gray columns show indices in the control animal group and in rats with ChP, respectively. Vertical scale) Numbers of behavioral phenomena within the observation period. 1-4) Numbers of crossed external and internal squares, numbers of rearing, and realizations of the burrow reflex; 5-7) numbers of acts of grooming, urination, and defecation, respectively. Three asterisks show the cases of significant intergroup differences with $P < 0.001$. Each group consisted of six animals.

influences the functional activity of the mentioned cerebral structure of the studied animals.

In the thalamus, we found an opposite trend of NCAM redistribution; decreases in the concentration of sNCAM and increases in mNCAM amount were quite obvious (by 34.2%, on average, from 153.82 ± ± 7.55 to 206.45 ± 21.74 μg/mg TP). In the hippocampus, no significant differences in the concentrations of NCAM forms in experimental ChP were observed (which, apparently, is indicative of relatively normal functioning of this cerebral structure responsible for cognitive activity) during 30 days of the experiment.

A certain imbalance between the concentrations of NCAM isoforms in the cerebellum and thalamus is indicative of specific redistribution of this protein between the above-mentioned cerebral structures; because of this phenomenon, the plasticity in the nervous system in the case of endotoxication under conditions of the development of ChP is, most likely, partly disturbed. The obtained data indicate that redistribution of NCAM isoforms in the brain of experimental animals is accompanied by a decrease in the intensity of locomotor and orientational/ cognitive activity of rats (Fig. 1), as well as by manifestations of the stress state (some increases in the number of acts of urination and defecation). Correlations between the levels of NCAM fractions and indices of the behavior of rats were different. For the number of crossed external squares and the level of sNCAM in the rat cerebellum, the r value was –0.638 ($P < 0.05$), while the analogous coefficient for the level of sNCAM in the thalamus was 0.638 ($P < 0.05$). Between the level of mNCAM in the cerebellum and the numbers of rearing, correlation was strong and positive (r = 0.828, $P < 0.05$).

As our data show, the development of experimental ChP in rats during 30 days significantly influences functioning of the cerebellum and thalamus; redistribution of NCAM isoforms is one of the aspects of these modifications. Changes in the characteristics of cell-to-cell adhesion in the above-mentioned cerebral structures can be a factor that leads to the development of pancreatic encephalopathy; this is accompanied by depression of motor activity of the animals and the development of the stress state.

Experiments were carried out in accordance with the International Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985), as well as with the Law of Ukraine "On Protection of Animals from Inhumane Treatment" (Kyiv, 2006).

The authors, V. A. Makarchouk and G. O. Ushakova, confirm that they have no conflict of interest.

REFERENCES

1. Yu. M. Stepanov and N. G. Zaichenko, "Chronic pancreatitis: biliary mechanism, factors, and time course," *Zaporozh. Med. Zh.*, **70**, No. 1, 46-50 (2012).
2. R. K. Tandon and P. K. Garg, "Oxidative stress in chronic pancreatitis: pathophysiological relevance and management," *Antioxidants Redox Signal.*, **15**, No. 10, 2757-2766 (2011).
3. C. Çöl, K. Dinler, O. Hasdemir, et al., "Oxidative stress and lipid peroxidation products: effect of pinealectomy or exogenous melatonin injections on biomarkers of tissue damage during acute pancreatitis," *Hepatobiliary Pancreat. Dis. Int.*, **9**, No. 1, 78-82 (2010).
4. A. Catalá, "Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions," *Chem. Phys. Lipids*, **157**, 1-11 (2009).
5. D. Zhen, L. Jun, L. Rong, and H. Xiao Hua, "Experimental pancreatitis results in increased blood-brain barrier permeability in rats: A potential role of MCP-1," *J. Digest. Dis.*, **13**, 179-185 (2012).
6. B. J. Page, D. F. Toit, C. Muller, et al., "An immunocytochemical profile of the endocrine pancreas using an occlusive duct ligation model," *J. Pancreas*, **4**, No. 1, 191-203 (2000).
7. T. T Ngo, H. M. Lenhoff, and A. Yaklich, *Enzyme-Mediated Immunoassay* [Russian translation], Mir, Moscow (1988).
8. M. Bradford, "Rapid and sensitive methods for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Anal. Biochem.*, **72**, 248-254 (1985).
9. J. Bureš, O. Burešova, and J. Hewstone, *Technique and Basic Experiments for Studying the Brain and Behavior* [Russian translation], Vyssh. Shkola, Moscow (1991).
10. A. Petrie and C. Sabin, *Medical Statistics at a Glance* [Russian translation], Geotar-Med, Moscow (2003).
11. L. H. Brennaman and P. F. Maness, "NCAM in neuropsychiatric and neurodegenerative disorders," *Adv. Exp. Med. Biol.*, **663**, 299-317 (2010).
12. M. Hagiyama, N. Ichiyanagi, K. B. Kimura, et al., "Expression of a soluble isoform of cell adhesion molecule 1 in the brain and its involvement in directional neurite outgrowth," *Am. J. Pathol.*, **174**, No. 6, 2278-2289 (2009).
13. S. Zecchini, L. Bombardelli, A. Decio, et al., "The adhesion molecule NCAM promotes ovarian cancer progression via FGFR signaling," *EMBO Mol. Med.*, **3**, 480-494 (2011).
14. R. Kleene, M. Mzoughi, G. Joshi, et al., "NCAM-induced neurite outgrowth depends on binding of calmodulin to NCAM and on nuclear import of NCAM and fak fragments," *J. Neurosci.*, **32**, No. 30, 10784-10798 (2010).