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Expression of HSP70 in the Brain of Rats During Experimental Cerebral Ischemia Modeling and on the Background of Neuroprotection

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Abstract

Experimental research has found that acute cerebral ischemia modeling in rats is accompanied by a significant increase in the main marker of oxidative degradation of proteins – nitrotyrosine beginning from the first day of the experiment. Concurrently there has been a decline in stress-protein HSP70 in brain tissue. Administration of Cerebrocurin for 4 days has led to a decrease in the levels of nitrotyrosine, as well as to increase in the expression of stress-responsive protein HSP70 in the acute period of cerebral ischemia.

Keywords: HSP70, cerebral ischemia, neuroprotection

Introduction

Studies conducted in the last decade have shown a critical role of heat shock protein 70 (HSP70) in protecting cells from irreversible ischemic damage to the highly aerobic tissues of the brain and heart [1, 2]. The increased expression and neuroprotective action of HSP70 has been shown under conditions of oxidative and nitrosative stresses, glutamate excitotoxicity, during deprivation of oxygen and glucose, as well as in different experimental animal models of focal and global cerebral ischemia [2, 3]. HSPs are a family of proteins produced by virtually all living organisms in response to exposure to stressful factors including heat, hypoxia, ischemia, metabolic disturbances, viral infection, and pharmacological agents [3-5]. The genes of these proteins are activated not only under conditions of stress, but also wound healing, and tissue remodeling during numerous processes of cellular proliferation, differentiation and apoptosis [6]. HSPs take part in all processes of the vital activity of cells, tissues and organs. Apparently, many members of HSPs perform chaperone functions by stabilizing new proteins to ensure correct folding or by recognizing damaged or newly synthesized polypeptides and correcting their structure by ATP-depended way, or remove irreversibly damaged proteins through the proteasome apparatus [6]. Furthermore, it is assumed that interaction between HSP70 and membrane phospholipids can play a dramatic role in folding of membrane polypeptides and processes of their translocation through the membrane that contributes to the protective action of HSPs [7].

Currently the search for the neuroprotective and energy tropic agents with HSP-mediated mechanism of action is conducted among compounds neuropeptides, capable of raising the concentration of HSP70 due to modulation of the expression factors of global transcription. The discovery of neuroprotective peptide factors impelled to develop a new strategy for pharmacotherapy – peptidergic, or neurotrophic therapy for the treatment of neurodegenerative diseases [8-9]. On this basis Cerebrolysin was developed and successfully used in the therapy of many neurologic disorders. However, it should be noted that Cerebrolysin is not always effective in acute cerebral ischemia, and frequently produces GOBA-like action on the functional state of the brain retarding the recovery processes [10]. In this connection the search for new effective drugs with neuroprotective and modulating properties remains topical. Recently new domestic neuropeptide agent Cerebrocurin appeared in clinic. It contains free amino acids, neuropeptides and low-molecular compounds obtained from controlled proteolysis of low-molecular proteins and peptides from cattle embryos [11]. Experimental studies revealed the ability of Cerebrocurin to prevent hyperactivation of microglia and to decrease production of IL-1α and other inflammatory cytokines that reflects the influence of the drug on a local inflammatory response and processes of oxidative stress in the region of cerebral ischemia [7].

The purpose of the present research was to investigate the ability Cerebrocurin to modulate HSP70 levels in the cerebral tissue of rats with acute cerebral ischemia.

Materials and Methods

The experimental part was performed on white nonlinear rats weighing 180-200 g collected from the breeding center of Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. Animals were kept on a standard food ration in natural alternation of day and night. The experiments were conducted in compliance with Commission on Bioethics and Pharmacy "General ethical animal experimentation" (Kyiv, 2001), consistent with the provisions of the European Convention "On Protection of Vertebrate Animals used for experimental or other scientific purposes" (Strasbourg, 1986) [13]. Acute cerebral ischemia was modeled by irreversible bilateral common carotid artery ligation under general anaesthesia with sodium thiopental (40 mg/kg) [13.14].

Cerebrocurin (***ingredients***: 2 ml of solution for injection contains a complex of free amino acids, peptides and low molecular products of controlled proteolysis of proteins from the brain of cattle embryos; **manufacturer**: Scientific Production Enterprise "NIR", Ukraine, Kiev) was introduced intraperitoneally in a dose of 0.001 ml/100 g once a day for the entire acute period of acute cerebral ischemia (4 days). At the end of the specified period animals were removed from experiment by means of decapitation under thiopental sodium anesthesia. The removed ischemic cerebral hemisphere was properly washed in the cool 0.9% KCl solution (4^0C), ground and homogenized in a 10-fold volume of medium: 250 mM sucrose; 20mM Tris HCl-buffer, and 1 mM EDTA (pH 7,4).

Activity of free radical oxidation processes was analyzed by the cerebral levels of the oxidative stress marker 3-nitrotyrosine (3-NT), which were determined by the immunoenzyme method [14]. The cerebral HSP70 expression was determined by Western blot analysis.

Tissue homogenate obtained by the methodology described above was centrifuged at 13000g and 4^0C.

The supernatant containing cytosol proteins was analyzed using electrophoresis and Western blotting. Proteins were divided in 10% polyacrylamide gel (PAG).

Transfer of proteins from PAG on PVDF-membrane was carried out by electroelution for 45 min. Preincubation of Western blots were conducted in a solution of TBST with addition of 1% Tween-20 for 1 hour. Then Western-blots were incubated in in the presence of the primary monoclonal antibody (Santa Cruz Biotechnology) against HSP70 used 1:1000 ratio for 1 hour. After washing the blots were incubated in the presence of secondary antibodies conjugated to horseradish peroxidase (1:2000) for 1 hour. Detection of HSP70 protein was carried out by computerized densitometric analysis using Adobe Photoshop Program [14].

The results of the study have been processed with application of Statistical Software Package «STATISTICA® for Windows 6.0» (StatSoft Inc., № AXXR712D833214FAN5), а также «SPSS 16.0», «Microsoft Excel 2003».

Some statistical procedures and algorithms were realized in the form the specially written macros in the appropriate programs. The results for all forms of analysis were statistically significant at a significance level of $p<0.05$.

Results and Their Discussion

Acute cerebral ischemia modeling led to the intensification of the processes of free-radical oxidation in the brain tissue that was testified by a significant increase in the marker of oxidative degradation of proteins 3-nitrotyrosine (3-NT) beginning from the first day of model pathology, and reaching maximum values on the 4th day (Table 1).

Table 1. Action of Cerebrocurin (0.001 ml/100 g of weight) on the levels of 3-nitrotyrosine in plasma and cerebral tissue of rats in the acute period of cerebral ischemia

Groups of animals	3-nitrotyrosine in plasma, nM/g of protein	3-nitrotyrosine in brain tissue, nM/g of protein
Intact	6.5 ± 0.57	15.3 ± 1.1
Control, ischemia 1 day	12.6 ± 1.3	31.7 ± 2.7
Control, ischemia 4 day	23.7 ± 2.2	77.5 ± 7.0
«Cerebrocurin», 1 day	8.2 ± 0.71*	18.1 ± 2.0*
«Cerebrocurin», 4 day	11.3 ± 0.8*	21.2 ± 2.3*,

Note: * - statistically significant differences between parameters in the two groups ($p\leq0.05$).

Increase in the concentration of 3-NT was observed along with the dynamic changes in HSP70 levels in the brain. The results of densitometric analysis showed the gradual decrease of HSP70 concentration. Thus, on the first day of the experiment there was a reduction in the area of HSP70-positively painted complex by 40% in comparison to intact group, and on the 4^{th} day – by more than 74% (Table 1).

A fall in protein HSP70 levels, in our opinion, is caused by the development of the oxidative and nitrosative stress, disruption of the compensation mechanisms of the organism. Besides, under the action AFO, HSP70 itself is exposed to oxidative modification, which destroys its functional activity and limits protective functions under the conditions of ischemia [2, 12].

Таблица 2. Action of Cerebrocurin (0.001 ml/100 g of weight) on the levels of HSP70 in the brain tissue of rats in the acute period of cerebral ischemia.

Groups of animals	Area +- painted complex, conventional units. (M±m)	Optical concentration+ - painted complex, conventional units, (M±m)
Intact	63.5 ± 2.14	0,37 ± 0,039
Control, ischemia 1 day	37.6 ± 1.48	0,19 ± 0,025
Control, ischemia 4 day	16.8 ± 1.18	0,07 ± 0,022
«Cerebrocurin», 1 day	51.6 ± 1.69*	0,3 ± 0,024*
«Cerebrocurin», 4 day	44.6 ± 1.37*	0,23 ± 0,041*

Note: * - statistically significant differences between parameters in the two groups ($p \leq 0.05$).

Course administration of “Cerebrocurin” at a dose of 0.001 ml/100g of weight has led to a statistically significant reduction in the concentration of 3-NT in the brain, and these alterations were observed beginning from the first day of the experiment. On the background of reduction in cytotoxic 3-NT levels there was an increase in HSP70 levels by 27% in comparison to the control group of animals on the first day of the experiment; and by 62% on the 4^{th} day.

Identified property of Cerebrocurin to activate the synthesis of HSPs may be explained, first of all, by its ability to modulate genome response by activation of global transcription factors triggering HSP synthesis that was shown by our early research [12, 15]. Secondly, a number of works have been shown the ability of neuropeptides to bind directly with HSPs and present them in this form to dendritic cells [4, 9, 12]. Besides, Cerebrocurin, like a number of other neuropeptide drugs, has significant antioxidant activity due to its ability to positively affect the expression of genes, which code the synthesis of some of the major enzymes of antioxidant system catalase and superoxide dismutase [8, 12, 15]. This property of the investigational drug allows to inhibit oxidative degradation of HSP70 itself under conditions of intensification of free radical oxidation processes that prolongs its protective action.

Thus, the determined effect of preparation Cerebrocurin to influence on HSP70 levels is, in our opinion, the key point in the mechanism of its neuroprotective action. Currently the search of effective agents with neuro- and cardioprotective action among functionally active endogenous protein-chaperone,

to which HSP70 belongs to, is one of the most topical and perspective areas in pharmacology. The ability of Cerebrocurin to increase the synthesis of protective HSP70 is of considerable interest for its use in the treatment of ischemic brain damage.

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