Effect of Cattle Brain Tissue Hydrolysates on Protein Synthesis in Mouse Organs and Cell Culture

O. Yu. Abakumova, A. V. Karyakin, I. A. Skvortsov, T. A. Tsvetkova, I. V. Yakusheva, G. S. Vasil'ev, and L. I. Kondakova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 126, No. 8, pp. 171-174, August, 1998
Original article submitted January 17, 1997

Hydrolysates of cattle cerebral cortex, cerebellum, and stem (cerebrolysates) stimulate protein synthesis in mouse brain hemispheres and cerebellum for 7 days after a single intramuscular injection. Cerebrolysate-stimulated protein synthesis is tissue- and organ-specific; cerebrolysates stimulate protein production in cultured transformed HGUK1 glial cells.

Key Words: *cerebrolysates; brain hemispheres; cerebellum; protein production; organ specificity*

Nerve cells possess a highly active protein-producing system comparable to that of endocrine cells [11]. Protein production and catabolism are disturbed in disease (craniocerebral injuries, demyelinizing diseases, epilepsy, etc.) and in health during aging [5]. Normalization of impaired protein metabolism in nerve cells is important for preventing and treating diseases of the central nervous system (CNS).

Enzymatic brain tissue hydrolysates free from protein and other high-molecular compounds (cerebrolysin and cerebrolysate, CL) have been widely used for the treatment of various CNS diseases [3]. The mechanism of their action is not clear; they stimulate protein production and oxidative processes in neurons, increase the CNS resistance to hypoxia, and inhibit the production of free radicals in nerve cells in hypoglycemia.

In this study the ability of CL to stimulate protein production was used to characterize the tissue and organ specificity of the effects of hydrolysates prepared from various brain compartments on protein metabolism in brain hemispheres, cerebellum, and other organs of mouse *in vivo*. Direct effect of CL on protein production in cells was studied *in vitro* in cultured glial cells.

MATERIALS AND METHODS

Experiments were carried out on male albino mice weighing 25 g. Two preparations were used: CL, an enzymatic hydrolysate of cattle cerebral cortex, from which lipids and proteins were removed, and cerebrolysate-M (CL-M), a similar preparation of the cerebellum and brain stem. CL and CL-M contained 6 mg/ml amine nitrogen. For studies of protein-producing activity of the preparations, they were intramuscularly injected in a dose of 25 μl/mouse and the animals were decapitated after 6, 24, 96, and 168 h, 4 animals per term.

Protein production was assessed by incorporation of ^{14}C-leucin (Amersham) in internal organs. The label was injected intraperitoneally in a dose of 0.2 mBq/mice 2 h before decapitation. The hemispheres, cerebellum, liver, kidneys, and spleen were frozen immediately after removal and stored at -70°C. Then all organs except the liver were hydro-

Research and Therapeutic Center for Preventing and Treating Psychoneurological Disability; Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Moscow

0007-4888/98/0008-0794$20.00

lyzed at 37°C in 0.6 N KOH, precipitated with 1.0 N perchloric acid, and radioactivity of the precipitate was measured. The liver was homogenized, and the homogenate was processed as described previously [1]. The tissue content of DNA was measured by a method described previously [6]. The intensity of protein production was assessed in cpm/mg DNA.

For quantitative assessment of organ-specific effects of CL and CL-M, the specificity coefficient K was calculated by the formulae:

$$K_{CL}=(A_{hem}/A_{or}-1)\times 100;$$
$$K_{CL\text{-}M}=(A_{cer}/A_{or}-1)\times 100,$$

where A_{hem} and A_{cer} are the ratios of ^{14}C-leucin incorporation in cell proteins of brain hemispheres and cerebellum, respectively, after injection of the preparations, to the value of ^{14}C-leucin incorporation in cell proteins of brain hemispheres in the control, and A_{or} is a similarly derived ratio for the other organs (for K_{CL} this group includes the cerebellum and for $K_{CL\text{-}M}$ it includes brain hemispheres). The specificity coefficient ranged from -100 *ad infinitum*. A negative value of the coefficient indicates the absence of specific effect of the agent on the studied organ, while a positive value indicates the degree of CL or CL-M stimulation of protein production in the studied organ.

An NGUK1 neurinoma cells of the rat Gasser's node [2] was used *in vitro*. The cells were cultured at 37°C in RPMI-1640 with fetal serum (10%) and gentamicin (40 μg/ml). CL and CL-M in different concentrations were added to a confluent monolayer of cells on the third day of culturing. Protein production was assessed by incorporation of ^{14}C protein hydrolysate (0.08 mBq/ml) for 5 h. The label was added 20 h after the preparations. The experiment was stopped by fixing in ice-cold ethanol:CH_3COOH (9:1), after which the cells were stained with 0.2% crystal violet in 2% ethanol. After washing in water, the stain was eluted with 10% CH_3COOH and optic density (E) was measured at 590 nm. The number of cells was estimated, assuming that E_{590}=0.1 corresponds to 32.5×10^3 NGUK1 cells. Standard radiometry with Bray's liquid [1] was carried out after cell lysis with 0.3 N KOH. The results were expressed in cpm/10^5 cells.

RESULTS

Six hours after injection, CL significantly ($p<0.05$) decreased the level of protein production in hemispheral and cerebellar cells (83 and 80% of the control, respectively). CL-M virtually did not change protein production in hemispheral and cerebellar cells during the same period (91 and 96% of control, respectively). After 24 h, CL significantly and more actively than CL-M increased protein production in hemispheral cells, and this increase persisted for up to 168 h (Fig. 1, *a*). CL-M more actively than CL stimulated protein production in cerebellar cells 24 and 96 h after injection (Fig. 1, *b*). Thus, we can speak about tissue specificity of the studied agents. The highest stimulation of protein production in brain hemispheres and cerebellum was observed on day 4 after injection.

Figure 1 demonstrates organ specificity of CL and CL-M which more actively stimulated protein production in the hemispheral and cerebellar cells

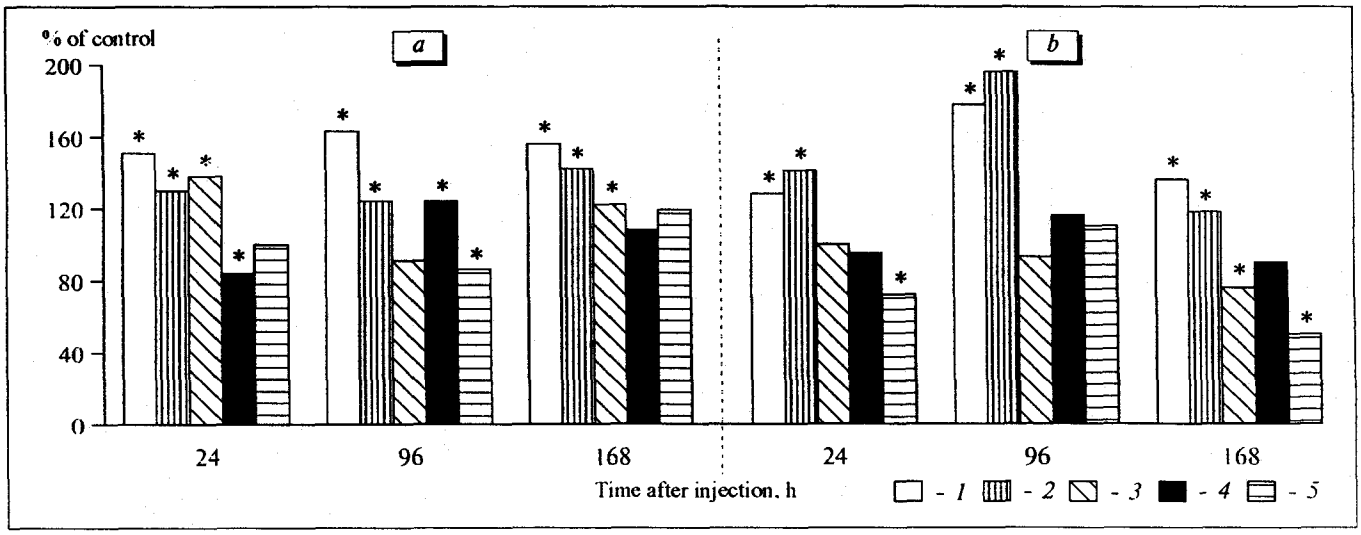

Fig. 1. Protein production in mouse organs after injection of 25 μl/mice cerebrolysate (*a*) and cerebrolysate-M (*b*). *1*) brain hemispheres; *2*) cerebellum; *3*) liver; *4*) spleen; *5*) kidneys. In the control incorporation of ^{14}C-leucin in hemispheric, cerebellar, hepatic, spleenic, and renal proteins was 7126, 3423, 45,071, 4179, and 18,127 cpm/mg DNA, respectively. *$p<0.05$ *vs.* the control.

TABLE 1. Coefficients of Specificity of CL and CL-M Effects on Protein Production in Various Organs at Different Periods after Injection

Organ	Period after injection, h							
	6		24		96		168	
	CL	CL-M	CL	CL-M	CL	CL-M	CL	CL-M
Brain hemispheres	3.7	-26.7	16.8	-9.6	31.8	-9.1	9.7	14.9
Cerebellum	-27.0	36.9	-14.1	10.7	-24.1	9.9	-8.8	-12.9
Spleen	—	—	-44.3	-33.0	-23.9	-41.0	-34.1	-23.9
Liver	—	—	-8.8	-29.0	-44.0	-52.7	-21.8	-35.9
Kidneys	—	—	-23.0	-49.2	-30.2	-43.9	-16.8	-57.6

than in the liver and spleen, where protein production increased only at some terms. By the present time, it is difficult to explain the inhibitory effect of CL on protein production in the kidneys.

The specificity coefficients for CL and CL-M stimulation of the protein producing system of cells confirm high tropism of these preparations to cerebral hemispheres and cerebellum, respectively (Table 1).

Labeled amino acids are incorporated in brain cortex and cerebellar proteins at the same rate [12]. Therefore, different levels of protein production in brain hemispheres and cerebellum after injection of CL and CL-M can be explained only by its specific induction with these preparations.

Amino acids in the studied hydrolysates cannot modulate the rate of protein production in brain cells, because infusion of such amino acids as valine, tyrosine, or phenylalanine into blood plasma did not change the protein production in this organ [8].

Besides insulin [4], insulin-like growth factor-1 participates in the regulation of protein production in the cells of various brain compartments; the activity of insulin-like growth factor-1 limits the expression of receptors on cell surface and modifies signal transduction through receptors [7,9]. The mechanism of CL stimulation of protein production in the cells is little known; we believe that short peptides capable of modulating the growth factor signal transduction through cellular receptors are the active substances in these preparations. The ability of cerebrolysin to stimulate compact groups of cells forming processes in a culture of neurons may be due to the presence of active short peptides participating in the transduction of the nerve growth factor signal.

TABLE 2. CL and CL-M Effects on Protein Production in NGUK1 Cells

Concentration of agent, μl/ml medium	CL	CL-M
	% of control	
0.2	112	117
0.5	136	97
1.0	163	113
2.0	141	103
5.0	148	105

Note. In the control the level of ^{14}C protein hydrolysate incorporation is 32,880 cpm/10^6 cells.

Direct stimulating effect of CL on transformed glial cells of the rat Gasser's node (Table 2) confirms the ability of CL to stimulate protein production in nerve cells and suggests that brain hydrolysates can be effective in various neuronal disorders, since glial cells have an important role in CNS cell regeneration [13].

The authors are grateful to Dr. L. N. Titova for qualified assistance in the study.

REFERENCES

1. O. Yu. Abakumova, in: *Modern Methods in Biochemistry* [in Russian], Moscow (1977), pp. 285-292.
2. A. P. Avtsyn, L. I. Kondakova, A. S. Khalanskii, *et al.*, *Tsitologiya*, **31**, No. 1, 97-101 (1989).
3. T. N. Osipenko, I. A. Skvortsov, and S. V. Korshunov, *Zh. Nevropatol. Psikhiatr.*, **91**, No. 8, 100-103 (1991).
4. T. M. Tret'yak and A. V. Kulikov, *Uspekhi Sovrem. Biol.*, **109**, No. 2, 252-262 (1990).
5. E. Einshtein, in: *Brain and Cerebrospinal Fluid Proteins* [in Russian], Moscow (1988), p. 278.
6. G. Blobel and V. R. Potter, *Biochim. Biophys. Acta*, **166**, 48-57 (1969).
7. A. R. Dcosta, X. Xu, R. L. Ingram, and W. E. Sonntag, *Neuroscience*, **65**, 805-813 (1995).
8. D. S. Dunlop, X.-R. Yang, and A. Lajtha, *Biochem. J.*, **302**, 601-610 (1994).
9. K. Hayase and H. Yokogoshi, *J. Nutr.*, **124**, 683-688 (1994).
10. W. Keung, E. Silber, and U. Eppenberger, *Anal. Biochem.*, **182**, 16-19 (1989).
11. A. Lajtha and D. Dunlop, *Life Sci.*, **29**, 755-767 (1981).
12. H. Nakamichi, M. Murakami, S. Miura, *et al.*, *Nucl. Med. Biol.*, **21**, 959-962 (1994).
13. T. Sivron and M. Schwartz, *Glia*, **13**, 157-165 (1995).