УДК 577.112

PROTEIN SYNTHESIS IN WHITE RATS BRAIN AND MYOCARDIUM ULTRASTRUCTURE COMPONENTS AT EXPERIMENTAL CRUSH SYNDROME

G.A. Kevorkian, A.S. Kanayan, H.F. Khachatryan,
L.H. Voskanian, A.G. Guevorkian, S.A. Avanesyan, L.A. Manukian

H.Buniatian Institute of Biochemistry of NAS
P.Sevak str., 5/1, 375014, Yerevan, Armenia.

Key words: crush syndrome, protein synthesis, brain, myocardium, endoplasmic reticulum, mitochondria, cytosol

Crush syndrome (CS) is a special type of traumatic pathology accompanied by a shock and intoxication of organism with a heavy and specific clinical course and high lethality. The CS problem has been studied since the beginning of 1930, and the distructing earthquakes in Armenia gave a new impulse for a wider study of this problem.

The level of protein synthesis in the brain and heart mitochondria (M), endoplasmic reticulum (ER) and cytosol (C) has been studied by incorporation of radioactive amino acid in protein molecules at CS experimentally modeled in white rats. The compression of soft tissue is accompanied by an acute hemodynamic shock and myoglobinuria with acute renal failure [9].

The main reason is that the cells of the damaged muscle seize a considerable amount of noncellular water. There begins a development of NO-dependent vasodilation, hypokalemia in tissues and hyperkalemia in blood (50-65% increase in blood), hypercalcemia (in kidney, myocardium, brain tissues) which induce a development of cardiovascular insufficiency, kidney and brain injury [1,5,10,11,13,14]. There are a data indicating that the main intoxication of organism begins at decompression and the organism is protected only due to some long-lasting arrangements, such as dialysis, arterio-venous hemofiltration, estimation of α-amilase, lactate dehydrogenase, aspartate transaminase, activity of enzymes of adenyline compounds and use of preparations which decrease their activity [3,4,12].

Thus, we put an aim to study the changes of the level of *in vivo* protein synthesis in brain and myocardium M, ER and C in white rats with experimental CS.

Material and Methods

In the experiments 72 Wistar male rats weighing 160-200*g* were used. CS was induced by a compression of femoral soft tissues using a special press with force of 100*kg/1kg* of animal weight for 2 and 5*hrs*. The animals were divided into the following groups: intact, control (2 and 5 *hrs* compression) and experimental (2, 4, 24 and 48 *hrs* decompression). During 60 *min* before decapitation the animals were injected ^{14}C- leucine with specific radioactivity of 240 $mCi \bullet mmol^{-1}$ in a quantity of 50 *mcCi*/100*g* of the mass. After decapitation the investigated organs were perfused in solution (0.15*M* KCL), homogenized in 0.44*M* sucrose, 1*M* EDTA, and by the method of differential centrifugation cellular fractions were obtained. The purity of the fractions of M and ER vesicles was checked morphologically and enzymatically [2,7]. The levels of incorporation of the radioactive precursor to the membrane proteins of M, ER and C were determined after membrane fractions solubilization in hyamine-10X (0.5N, NEN, USA). The level of radioactivity was counted on scintillation spectrometer SL- 4221 (Roche Bioelectronique Kontron, France) where liquid scintillator on dioxan base was used. The efficiency of evaluation made 80-90% counted by ^{14}C, $\delta_i = 92\%$. Data were obtained in desintegration$\bullet min^{-1} \bullet mg^{-1}$ of protein. Absolute radioactivity was evaluated by the method of external standardization. The protein was determined by Lowry (test-set of "Sigma" Chem.Co., USA). Statistical reliability was evaluated with the help of Student's t-test and Sigma Plot program..

Results and Discussion

It has been already shown that at CS the protein contents seriously change in the liver and kidneys cell subcellular particles and soluble fraction [8]. Later those investigations were extended and the following experimental data have been obtained. At CS the following regularity has been observed referring to the activity of protein synthesis of cardiomyocytes. In soluble fraction of cardiomyocytes the level of protein synthesis decreases (47.9%) during 2 *hrs* compression. At 2 *hrs* decompression the level of synthesis increases (81.9%) remaining lower of that (22%) of the intact group (tab.1). It remains the same at 4, 24 and 48 *hrs* decompression and after 2 *hrs* compression in comparison with the control group.

During the compression the synthesis of ER membrane proteins does not change. At 2 *hrs* decompression no reliable changes are observed. At 4 *hrs* decompression an acute activation is observed which increases (51%) and remains the same at 24 *hrs* decompression. At 48 *hrs* decompression the activity decreases to 44% of that of the intact and control levels (table 1).

During 2 *hrs* compression the synthesis of membrane proteins of M decreases more than twice. At 2 and 24 *hrs* decompression the synthesis does not reliably change, only at 48 *hrs* decompression the synthesis decreases (32.5%) in comparison with the intact group,though it is higher than that of the control group (42.5%).

During 5 *hrs* compression no reliable changes are observed in C if compared with the periods of decompression (tab. 2). At 2 *hrs* decompression after 5 *hrs* compression the level of protein synthesis decreases to 29.8% of that of the control group. Investigations of the next periods of

decompression have shown that at 4 *hrs* decompression the activity increases thrice and at 24 and 48 *hrs* decompression – twice compared with the control group.

Table 1

The level of protein synthesis in myocardium subcellular particles after 2 *hrs* compression and 2, 4, 24, 48 *hrs* decompression

Subcellular particles	Intact group	Control group, 2 *hrs* compression	Experimental groups (decompression)			
			2 hrs	4 hrs	24 *hrs*	48 *hrs*
Cytosol	9206 ± 761	4800 ±267 $p_1 < 0,005$	7181 ±380 $p_1 > 0,05$ $p_2 < 0,005$	13850 ±154 $p_1 < 0,05$ $p_2 < 0,005$	8749 ±361 $p_1 > 0,05$ $p_2 < 0,001$	6217 ±95 $p_1 < 0,01$ $p_2 < 0,005$
Endoplasmic reticulum	5653 ± 156	5693±346 $p_1 > 0,5$	5942 ±446 $p_1 > 0,5$ $p_2 > 0,5$	8972± 1091 $p_1 > 0,05$ $p_2 > 0,05$	7853 ±423 $p_1 < 0,05$ $p_2 < 0,01$	3142 ±84 $p_1 < 0,001$ $p_2 < 0,001$
Mitochondria	7615 ± 347	3608 ±152 $p_1 < 0,001$	8409 ±844 $p_1 > 0,4$ $p_2 < 0,005$	7191 ±935 $p_1 > 0,5$ $p_2 < 0,005$	6916 ±798 $p_1 > 0,4$ $p_2 < 0,01$	5142 ± 170 $p_1 < 0,001$ $p_2 < 0,001$

During 5 *hrs* compression the membrane proteins of ER have undergone the following changes: in the control group the level of synthesis decreases (26.8% compared with the intact group). At 2 *hrs* decompression it decreases making 41.3% of the control group. At 4 *hrs* decompression an acute activation is observed (26.8% in comparison with the control, and 16.9% with the intact group). At 24 *hrs* decompression the activity begins to decrease. At 48 *hrs* decompression the activity decreased thrice reaching the level of the intact group.

During 5 *hrs* compression the synthesis of myocardium membrane proteins decreases (27.6%). At 2 *hrs* decompression it decreases to 42.7% of that of the intact group. At 4 *hrs* decompression the protein synthesis increases (15.8% in comparison with the control group). At 24 *hrs* decompression the activity began to decrease and reached the level of the control group. At 48 *hrs* decompression it decreased twice (tab. 2).

Some significant changes have been observed in the brain tissue in the level of protein synthesis. During 2 *hrs* compression the level decreases in C (32.7%). At 2 *hrs* decompression after 2 *hrs* compression the level of synthesis decreases to 78.8% of that of the intact group, then begins to increase. At 4 *hrs* decompression it reaches the level of the intact group . At 24 and 48 *hrs* decompressions the level of protein synthesis decreases, respectively to 20% and 47.3% of that of the intact group (tab. 3).

Table 2

The level of protein synthesis in myocardium subcellular particles after 5 *hrs* compression and 2, 4, 24, 48 *hrs* decompression

Subcellular particles	Intact group	Control group, 2 *hrs* compression	Experimental groups (decompression)			
			2 hrs	4 hrs	24 hrs	48 hrs
Cytosol	9206±761	9410 ±154 $p_1 > 0,5$	6606 ±274 $p_1 < 0,025$ $p_2 < 0,001$	19961±1464 $p_1 < 0,001$ $p_2 < 0,001$	14174 ±89 $p_1 < 0,001$ $p_2 < 0,001$	11741 ±168 $p_1 < 0,025$ $p_2 < 0,001$
Endoplasmic reticulum	5653 ±56	4138 ±749 $p_1 > 0,05$	3320 ±113 $p_1 < 0,001$ $p_2 > 0,2$	15216±1142 $p_1 < 0,001$ $p_2 < 0,001$	5253 ±76 $p_1 > 0,05$ $p_2 > 0.1$	4615 ±82 $p_1 < 0,005$ $p_2 > 0,5$
Mitochondria	7615±347	5514 ±366 $p_1 < 0,01$	4362 ±912 $p_1 < 0,025$ $p_2 > 0,2$	14235± 682 $p_1 < 0,001$ $p_2 < 0,001$	6026 ±140 $p_1 < 0,01$ $p_2 > 0,2$	3928 ±87 $p_1 < 0,001$ $p_2 < 0,01$

At crash syndrome no reliable changes have been observed in the brain ER, however at 2 *hrs* decompression the level of synthesis of membrane proteins decreases (51.9%). At 4 and 24 *hrs* decompression the activity increases twice compared with 2 *hrs* decompression. At 48 *hrs* decompression it begins to decrease insignificantly.

Table 3

The level of protein synthesis in brain subcellular particles after 2 *hrs* compression and 2, 4, 24, 48 *hrs* decompression

Subcellular particles	Intact group	Control group, 2 *hrs* compression	Experimental groups (decompression)			
			2 hrs	4 hrs	24 *hrs*	48 *hrs*
Cytosol	10378±320	6987 ± 25 $p_1 < 0,001$	3239 ±124 $p_1 < 0,001$ $p_2 < 0,001$	11324±812 $p_1 > 0,2$ $p_2 < 0,005$	8094 ±699 $p_1 < 0,025$ $p_2 > 0,05$	5470 ±68 $p_1 < 0,001$ $p_2 < 0,001$
Endoplasmic reticulum	8223±92	8190 ±592 $p_1 > 0,5$	3953 ±318 $p_1 < 0,001$ $p_2 < 0,001$	6869 ±762 $p_1 > 0,1$ $p_2 > 0,05$	6728 ±165 $p_1 < 0,001$ $p_2 > 0,05$	5261 ±233 $p_1 < 0,001$ $p_2 < 0,005$
Mitochondria	6922 ±352	3537±185 $p_1 < 0,001$	2956 ±153 $p_1 < 0,001$ $p_2 > 0,05$	4768 ±555 $p_1 < 0,025$ $p_2 > 0,05$	6224±1013 $p_1 > 0,5$ $p_1 < 0,05$	5614 ±354 $p_1 < 0,05$ $p_2 < 0,005$

More expressed changes have been observed in the M membrane protein of brain. During 2 *hrs* compression the synthesis decreases twice. At 2 *hrs* decompression it decreases to 42.7% of that of the intact group. At 4, 24 and 48 *hrs* decompression the level of protein synthesis increases reaching the level of the intact group (tab. 3).

During 5 *hrs* compression the level of protein synthesis in the soluble fraction of brain does not change. At 2 *hrs* decompression it decreases a little (16%). At 4 *hrs* decompression it increases twice if compared with the control group, then again decreases always remaining at a high level (tab. 4). At CS in brain cells an insignificant activation of the level of protein synthesis is observed (16%), referring to the membrane fraction of ER. At 2 *hrs* decompression the level of protein synthesis decreases (56%), at 4 *hrs* decompression it increases (77.4% if compared with the intact group, and 30.1% if compared with 2 *hrs* decompression). At 24 *hrs* decompression the protein synthesis reaches the level of the intact group. At 48 *hrs* decompression it begins to decrease (tab.4).

Table 4.

The level of protein synthesis in brain subcellular particles after 5 *hrs* compression and 2, 4, 24, 48 *hrs* of decompression

Subcellular particles	Intact group	Control group, 2 *hrs* compression	Experimental groups (decompression)			
			2 hrs	4 hrs	24 hrs	48 hrs
Cytosol	10378±320	11119±180 $p_1 > 0,05$	7668 ±291 $p_1 < 0,001$ $p_2 < 0,001$	22419±1212 $p_1 < 0,001$ $p_2 < 0,001$	9665 ±311 $p_1 > 0,05$ $p_2 < 0,01$	8514 ±181 $p_1 < 0,005$ $p_2 < 0,001$
Endoplasmic reticulum	8223 ±92	9537 ±346 $p_1 < 0,025$	3638 ±118 $p_1 < 0,001$ $p_2 < 0,001$	14585± 132 $p_1 < 0,005$ $p_2 < 0,025$	8899 ±47 $p_1 < 0,001$ $p_2 > 0,1$	5789 ±23 $p_1 < 0,001$ $p_2 < 0,001$
Mitochondria	6922 ±352	7385±1671 $p_1 > 0,5$	2597 ±396 $p_1 < 0,001$ $p_2 < 0,05$	11715±1112 $p_1 < 0,01$ $p_2 > 0,05$	5035 ±49 $p_1 < 0,005$ $p_2 > 0,2$	2339± 69 $p_1 < 0,001$ $p_2 < 0,05$

The data in the tables are given in desintegration•min-1•mg protein-1, n=6

p_1 – in comparison with intact and control (2 hrs compression) groups;

p_2– in comparison with control and experimental (2, 4, 24 and 48 hrs decompression) groups

At crush syndrome the level of protein synthesis in the membrane of M does not change during compression period. At 2 *hrs* decompression it decreases as in ER membrane fraction (72.5%). At 4 *hrs* decompression the M protein synthesis increases (4.5 times if compared with 2 *hrs* decompression, and 1.7 times, if compared with the intact group). At 24 *hrs* decompression it

again begins to decrease. At 48 *hrs* decompression it decreases (3 times, if compared with the intact group).

Thus, it is concluded CS is accompanied by numerous damages, the most significant of which are stress, shock, pain syndrome, violation of the neurohumoral system involving the mediators of the sympathic part of vegetative nervous system. The morphological study of brain tissue during different terms of decompression after 2 and 5 *hrs* compression has revealed a contraction of the vessels of the cerebral shell, perivascular edema, and hypoxia. Global cerebral ischemia involves the entire brain [5]. A spreaded glial edema is observed.

Reduced structural changes in the brain are observed at all investigation periods. Undoubtedly, at crush syndrome the major place is given to the involvement of the immune system in pathogenesis. Disorders of blood–brain barrier and intoxication of the central nervous system by infiltration of toxins through the peripheral nerves are confirmed experimentally. The experimental data show that during 2 *hrs* compression the main change is the decrease in the level of protein synthesis in C of the investigated organs. Membrane proteins of ER give different reactions to compression.

During 2 *hrs* compression more serious damages of protein synthesis are observed in brain and myocardium membranes of M. The levels of protein synthesis in brain and myocardium decrease twice. During 5 *hrs* compression this process is restored, probably because the white rats switch on the adaptation protective mechanisms very quickly due to the high level of their plastic exchange. That helps to restore protein synthesis, one of the most important processes in the organism. It is important to note that at 2 *hrs* decompression after 2 *hrs* compression a very acute decrease in the level of protein synthesis is observed in all the investigated organs and subcellular structures, except M. An acute activation of synthesis of membrane proteins takes place. The decrease of K^+ concentration is supposed to make the protein synthesis apparatus in M switch on for the accomplishment of protein synthesis. K^+ is very important at CS, as it deteriorates myocardial activity which results in infarction.

At 2 *hrs* compression the protein synthesis increases in cardiomyocytes C, and the synthesis of ER membrane does not change. By means of this activation the deficiency of M protein is completed. The decrease of protein synthesis, which is observed at 2 *hrs* decompression, probably has a protecting trigger mechanism, because at 4 *hrs* decompression an excessive activation of protein synthesis is observed, after which all the supplies of macroerges and free pool of amino acids are exhausted [6]. That is why the activity of protein synthesis continues to decrease during 48 *hrs* decompression. Undoubtedly, general intoxication of organism at decompression periods has a special influence upon decrease of protein synthesis.

Поступила 25.06.01

СИНТЕЗ БЕЛКОВ УЛЬТРАСТРУКТУРНЫХ КОМПОНЕНТОВ КЛЕТОК МОЗГА И СЕРДЦА БЕЛЫХ КРЫС ПРИ СИНДРОМЕ ДЛИТЕЛЬНОГО РАЗДАВЛИВАНИЯ

Г.А.Геворкян, А.С.Канаян, Г.Ф. Хачатрян, Л.О.Восканян,
А.Г.Геворкян, С.А. Аванесян, Манукян Л.А.

Синдром длительного раздавливания (СДР) характеризуется общей интоксикацией организма, нарушениями сердечно-сосудистой деятельности, гипоксией, острой ишемией мозга и многими другими серьезными нарушениями.

Экспериментальная модель СДР представляет собой раздавливание бедренной мышцы белых крыс в течение 2- и 5-ти часов с последующим посткомпрессионным периодом 2, 4, 24 и 48 часов.

Изучение белоксинтезирующей активности субклеточных образований мозга и сердца – митохондрий, эндоплазматического ретикулума и цитозоля выявили определенные сдвиги в активности синтеза белка, как при компрессии, так и в посткомпрессионном периоде. Наиболее значительные изменения отмечались в посткомпрессионном периоде, характеризующемся распространением токсичных продуктов из поврежденных почек по всему организму.

ՍՊԻՏԱԿ ԱՌՆԵՏՆԵՐԻ ՈՒՂԵՂԻ ԵՎ ՍՐՏԱՄԿԱՆԻ ԵՆԹԱԲՋՋԱՅԻՆ ԿԱՌՈՒՑՎԱԾՔՆԵՐԻ ՍՊԻՏԱԿՈՒՑՆԵՐԻ ՍԻՆԹԵԶԸ ԵՐԿԱՐԱՏՎ ՃՆՇՄԱՆ ՀԱՄԱԽՏԱՆԻՇԻ ԺԱՄԱՆԱԿ

Գ.Ա. Գևորգյան, Ա.Ս. Կանայան, Հ.Ֆ. Խաչատրյան, Լ.Հ. Ոսկանյան,
Ա.Գ. Գևորգյան, Ս.Ա.Ավանեսյան, Լ.Ա.Մանուկյան

Երկարատև ճնշման համախտանիշը /ԵՃՀ/ բնութագրվում է օրգանիզմի ընդհանուր թունավորմամբ, սրտանոթային համակարգի գործունեության խանգարմամբ, ուղեղի թթվածնային քաղցով և սուր իշեմիայով և շատ այլ լուրջ խախտումներով:

ԵՃՀ փորձնական մոդելը իրենից ներկայացնում է սպիտակ առնետների ապդրամկանի 2- և 5-ժամյա ճնշում՝ 2, 4, 24 և 48ժ հետճնշման ժամանակահատվածով:

Ուղեղի և սրտի ենթաբջջային կառուցվածքների՝ միտոքոնդրիումների, էնդոպլազմատիկ ցանցի և ցիտոզոլի սպիտակուց սինթեզող ակտիվության ուսումնասիրմամբ հայտնաբերվել են սպիտակուցի սինթեզի տեղաշարժեր ինչպես ճնշման, այնպես էլ հետճնշման ժամանակ: Առավել զգալի փոփոխություններ տեղի են ունենում հետճնշման ժամանակահատվածում, որը բնորոշվում է վնասված երիկամներից թունավոր արգասիքների տարածմամբ ամբողջ օրգանիզմով:

REFERENCES

1. *Adachi J., Morita S., Yasuda H., Miwa A. et al.* Clin. Chim. Acta, 1998; 269: 137.
2. *Galoyan A.A., Kevorkian G.A., Voskanyan L.H. et al.* Neurochem. Res., 1988, 13, p. 493.
3. Hayrapetyan *H.L., Khachatrian H.F., Mardanyan S.S., Kevorkian G.A.* Med. Sci. Mon., 2000, 5, 845.
4. *Hayrapetyan H.L., Khachatrian H.F., Mardanyan S.S. et al.* Med. Sci. Mon., 2000, p. 6,1068.
5. *Kevorkian G.A., Kanayan A.S., Hayrapetyan H.L., Guevorkian A.G. et al.* Encyclopedia Armenica. Yerevan, 2001, p. 86.
6. *Kevorkian G.A., Marukhyan G.L., Arakelyan L.N., Galoyan A.A.* Neurochem. Res., 2001, 26, p. 827.
7. *Kevorkian G.A., Simonyan A.A., Abramyan K.S., Badalyan R.B., Shatverova L.A.* Armenian Biol. J., 1977, 30, 18.
8. *Khachatrian H.F., Guevorkian A.G., Barsegyan D.L., Kevorkian G.A.* Med. Sci. Armenia, NAS RA, 1999, 3, p. 37.
9. *Nakata Y., Hiraide A., Shimazu T. et al.* Am. J. Emerg. Med., 1999; 52: 617.
10. *Oda Y., Shindoh M., Yukioka H., Nishi S. et al.* Ann. Emerg. Med., 1997, 30, p. 507.
11. *Rubinstein I., Abassi Z., Coleman R. et al.* J. Clin. Invest., 1998, 101, p. 1325.
12. *Shigemoto T., Rinka H., Matsuo Y., Kaji A. et al.* Ren. Fail., 1997, 30, p. 711.
13. *Walikonis R.S., Poduslo J.F.* J. Biol. Chem., 1998a, 273, p. 9070.
14. *Walikonis R.S., Poduslo J.F.* Neurochem. Int., 1998b, 203, p. 457.