CYSTATHIONASE ACTIVITY OF ALBINO RATS FED ON RICH PROTEIN DIET IN EXPERIMENTAL MANGANESE POISONING

Vl. Boyadjiev, L. Halacheva

The cystathionase (cysteine desulfhydrase, E. C., 4, 4, 1, 1,) is a key enzyme in the processes of transulfuration. The paramount importance of these processes for the metabolism of sulfur-containing amino acids and for the detoxication of the organism substantiates the interest in studies of the enzymes regulating them, shown by numerous authors in the past few years (5, 9, 13, 14, 14).

Cystathionase activity is influenced by hormonal and alimentary factors (6, 9, 12). F. Chatagner (6, 7) succeeded in demonstrating that the high methionine content in rations, rich in caseine, stimulates the cystathionase activity. The results have been confirmed experimentally by addition of pure methionine to the physiological alimentary ration (18% protein according to cal). Analogical data were also obtained during investigation of the influence of rations with varying protein content on the activity of cystathionase, succinic dehydrogenase and cytochrome oxidase in control animals, after experimental poisoning with lead and manganese (1, 2, 3).

The cystathionase activity is inhibited by experimentally induced lead and manganese poisoning (2, 4).

The literature data referred to and our own studies as well show the complexity of the interrelationships nutritive ration+metal ion — enzymic synthesis+regulation. On the other hand, the thorough understanding of these mechanisms has an essential practical bearing on the goals of biological prophylaxis, respectively, on the prophylactic occupational nutrition during work with heavy metals. The uncertainty and the significance of the facts and considerations outlined above justified the undertaking of an experimental study with the aim to ascertain the influence exerted by rich protein rations upon the activity of cystathionase in experimental manganese poisoning.

Materials and Methods

To accomplish the task thus outlined experiments were carried out on 280 male albino rats with average weight 130 ± 20 grams. The animals were divided in two groups depending on the alimentary ration. Each group was composed of 60 control and 80 poisoned rats.

Table 1

Cystathionase activity

Sample	Ration	Groups	Nutrition		Poisoning			Restoration	P	
			0	15	20	40	60			
Brain homogenate 50%	I	c	15,8±0,25	16,5±0,30	15,2±0,72	16,0±1,15	14,8±0,72	15,3±0,80		>0,05
		p			12,3±0,29	10,5±0,48	8,0±0,50	13,9±1,50	0,99	0,001
	P 20/40/60	pc/p			0,02	0,01	0,001	<0,05		
					<0,05	0,02				
	II	c	15,2±0,42	17,8±0,51	20,3±0,65	21,9±0,72	23,0±0,72	25,2±0,85		0,01
		p			18,1±0,57	16,0±0,39	14,9±0,23	20,9±1,45	0,94	0,001
	P 20/40/60	pc/p			<0,05	0,02	0,001	<0,05	0,99	
					<0,05	<0,05				
Hepatic homogenate 50%	I	c	28,2±0,54	27,5±1,12	27,3±0,85	26,9±0,90	27,3±0,40	26,8±0,75		>0,05
		p			23,2±1,18	20,0±0,55	16,8±0,90	25,4±1,30	0,99	0,001
	P 20/40/60	pc/p			0,02	0,001	0,001	<0,05		
					<0,05	0,02				
	II	c	29,5±0,81	33,4±0,45	34,1±0,30	35,2±0,80	37,3±0,50	39,2±0,45	0,92	0,02
		p			29,2±1,21	27,0±0,60	25,0±0,48	32,7±0,99	0,98	0,001
	P 20/40/60	pc/p			<0,05	0,01	0,001	<0,05		
					<0,05	<0,05				
50% blood hemolysate	I	c	2,9±0,75	2,3±0,40	3,2±0,72	3,1±0,38	2,9±0,95	2,8±0,85		>0,05
		p			2,1±0,09	1,4±0,07	0,7±0,05	2,7±0,48	0,99	0,001
	P 20/40/60	pc/p			0,05	0,01	0,001	<0,05		
					<0,05	<0,05				
	II	c	2,8±0,05	3,0±0,45	4,1±0,30	4,9±0,25	5,0±0,15	6,2±0,20	0,95	0,02
		p			3,5±0,32	2,9±0,16	2,0±0,15	4,5±0,80	0,98	0,01
	P 20/40/60	pc/p			<0,05	0,02	0,01	<0,05		
					<0,05	<0,05				

The animals were fed on synthetic protein rations, accordingly foe group I — with 18% and group II — with 35% protein caloricity. Thr nutritive rations' composition is described in earlier works by the same authors (3).

The animals were poisoned with $MnCl_2$ per os, according to a method described in the report already referred to (3), following preliminary 15-day adaptation to the nutritive ration.

The activity of the enzyme was followed up in dynamics: in animals sacrificed prior to adaptation, at 15 days, every 20 days in the process of poisoning and after 60-day recovery period.

Determination of the activity was performed after the methods of Bergeret (10) and Brüggemann (4) as modified by Halacheva (9).

Cerebral and hepatic homogenate — 50% and blood hemolysate — 50% were employed in the work. The enzyme activity is calculated in mcg reacting cysteine in 1 cm^3 homogenate, respectively hemolysate for 30 min at 37° C. The limit concentration of cysteine in the incubation mixture used is 10^{-1M} at pH 7.6, which proved to be optimal for the specific conditions (9) available.

Results and Discussion

The rich in proteins ration activies the cystathionase of the control animals. The increase of the cerebral and hepatic enzyme is substantial in the course of the 15-day adaptation period. Subsequently, practically rectilinear course is observed of the curve for the liver and slightly ascen-

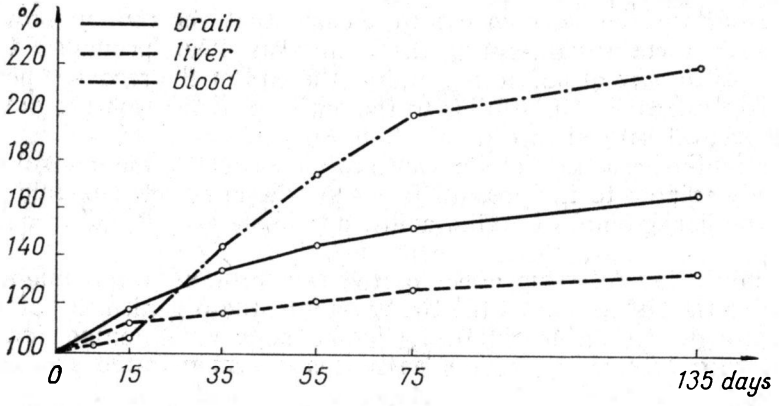

Fig. 1. Percentual increase during nutrition with rich 35% protein caloricity ration

dent — for the brain. The changes in the activity of the blood cystathionase reveal sinusoid course — insignificant up to the 15th day, rapidly rising up to the 75th day and setting down towards the end of the experiment (Fig. 1).

The manganese inhibits the activity of the enzyme in the organs investigated of both groups of animals (Table 1).

Expressed in percentuals at the 60th day of poisoning, the activity of cystathionase in the animals of group I shows the most pronounced fall in the blood, next ranking the brain and liver. In the rats fed on rich in proteins ration, the percentual decrease is less pronounced at the same ratio of organs investigated (Fig. 2).

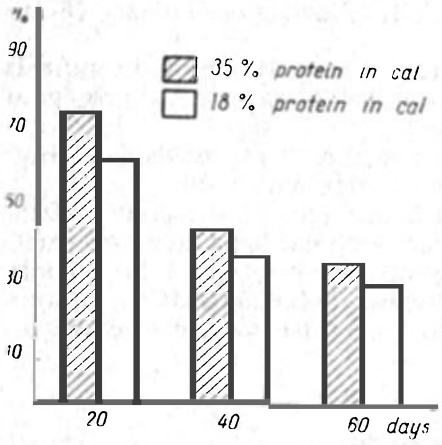

Fig. 2. Percentual decrease in the organs

The analysis of Table 1 shows that during the period of restoration, the activity of cystathionase in the poisoned rats, receiving 18% protein ration, virtually reaches the starting level with relatively small, statistically unreliable differences between the values of control and poisoned animals. At first glance, the restoration in the poisoned rats, fed on rich in proteins diet is not full — the difference for the activity of cystathionase between controls and poisoned animals is higher for all the organs under investigation. The thorough analysis of the data in the table shows that the level of enzyme activity of the group, fed on rich protein ration, is considerably higher than the initial one at termination of the recovery, levelling or exceeding the activity at the 15th day of adaptation, that is at the time the poisoning commences.

The results presented demonstrate clearly that the rich in proteins ration exerts a certain maintaining the inhibition effect, pronounced plainly up to the 20th day of poisoning, and at the end of the recovery period.

The fact established is similar to the activity of the rich protein rations in experimental saturnism (2).

The stimulating action of the rich protein ration in the control animals is probably related to the possibility for synthesis of new enzymic proteins against the background of amino acids, administered in a sufficient amount (7, 12).

The inhibition-deterring effect of the rich protein ration might be attributed to the regulation of the SH-groups in the organism by alimentary route, through the sulfur-containing amino acids (methionine and others) supplied with caseine. Lenz and Matseo have demonstrated the formation of biological chelates between SH-groups and manganese, which favour its deposition within the organism. Thus, the concentration of circulating manganese is reduced, its binding with the apoenzyme is rendered more difficult and its effect on the co-factor pyridoxalphosphate is decreased.

Inference

The rich protein ration (35% protein caloricity) increases the activity of cystathionase in the blood, brain and liver of white rats, most significantly for the blood enzyme.

The experimental manganese poisoning causes reduction of the cystathionase activity in the animal organs investigated, regardless of the quantitative composition of the protein diet. Stronger inhibition of the activity of the enzyme is observed in the animals fed on 18% protein caloricity diets. The activity of the blood enzyme shows strongest reduction.

The rich protein diet conditions a certain maintaining effect on the inhibitory action of manganese.

REFERENCES

1. Бояджиев Вл., Л. Халачева. Активност на цистатионазата у бели плъхове при експериментално манганово отравяне. — *ЖЕТ,* 1969, 2, 85—90.
2. Бояджиев, Вл., Л. Халачева. Промени в активността на сукцин-дехидрогеназата у бели плъхове, хранени с богата белтъчна дажба при експериментално манганово отравяне. — *Jnt. Arch. Nutr. Aliment,* под печат.
3. Бояджиев, Вл., Л. Халачева. Промени в цитохромоксидазната активност на бели плъхове, хранени с богата белтъчна дажба при експериментално манганово отравяне. — *Хигиена и здравеопазване,* под печат.
4. Brüggemann, J., M. Waldschmidt. Die serinsulfhydrase aus Hühaerleber Rückreation und vergleich mit der Cysteindesulfurase. — *Biochem. Z.,* 335, 1962, 408—422.
5. Chatagner, F. Adaptation métabolique chez les animaux. Influence de la e teneur en proteins du regime sur la décarboxylase de l'acide l'cysteinsulfinique dans le foie du rat. — *Biochim. Biophys. Acta,* 81, 1964, 400—405.
6. Chatagner, F., O. Trautmann. Effect of d,l-ethionine on the level of cystathionase in rat liver. — *Nature,* 1963, 75—78.
7. Chatagner, F., O. Trautmann. Soluble cysteine desulohurase of rat liver. — *Nature,* 194, 1962, 1281—83.
8. Eldjarn, L. Role of the sulfur compaunde. — *Scand. J. Clin. Invest. Suppl.,* 86, 1965, 7—20.
9. Khalacheva, L. Studies over the cystathionase reaction by the production of alanin. — *God. Trud. VMI — Varna,* 5, 1966, 24—29.
10. Jollée — Bergeret, B., J. Labouesse, F. Chatagner. Influence des hormones thyroidiennes sur la désulfuration de la cystein par le foie du rat. — *Bull. Soc. Chem. Biol.,* 42, 1960, 51—63.
11. Lenz, G. R., A. E. Martell. Metal chelates of some sulfur containing amino acids. — *Biochemistry,* 3, 1964, 745—50.
12. Loiselet, G., F. Chatagner. Amino acides de la cystathionase du foie des rats. — *Bull. Soc. Chem. Biol.,* 47, 1965, 33—46.
13. Matsuo, Y. Metal chelates of Schiff bases of pyridoxal phosphate and amino acids. — *J. Amer. Chem. Soc.,* 79, 1957, 2011—14.
14. Matsuo, Y., D. M. Greenberg. A crystalline enzyme thet cleaves homoserine and cystathionine. — *J. Biol. Chem.,* 230, 1958, 545—a61; 234, 1959, 507—516
15. Mudd, S. N., J. D. Finkelstein, F. Irreverre, L. Laster. Transsulfuration in mammals. — *J. Biol. Chem.,* 240, 1965, 4382—92.

АКТИВНОСТЬ ЦИСТАТИОНИНА У БЕЛЫХ КРЫС, ПОЛУЧАВШИХ БОГАТЫЙ БЕЛКОМ КОРМ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ОТРАВЛЕНИИ МАРГАНЦЕМ

В. Бояджиев, Л. Халачева

РЕЗЮМЕ

Сообщается об исследовании воздействия богатой белком пищи (35% белка по кал) на активность цистатионазы в крови, мозге и печени крыс при экспериментальном отравлении марганцем.

Было установлено, что она повышает активность цистатионазы в исследованных органах, причем это наиболее заметно в энзиме крови.

Экспериментальное отравление марганцем вызывает снижение цистатионазной активности в исследованных органах животных, независимо от количественного состава белкового корма. В большей степени угнетается активность энзима у животных, получавших корм, содержащий 18% белка по кал. Заметнее всего снижается активность энзима крови.

Богатая белком пища обусловливает известное заторможивающее ингибирующее действие, вызываемое марганцем.