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The effectiveness of the bonding of amino acid by tRNA of livers of rats experimentally exposed to cadmium and barium

Zdolność wiązania aminokwasów przez tRNA wątroby szczurów doświadczalnie narażonych na kadm i bar

The process of amino acid activaction takes place in the presence of specific aminoacyl-tRNA synthetases. These enzymes, together with ATP, catalyse the bonding of tRNA and amino acids (5). The effectiveness of binding amino acid by tRNA has a direct influence on the intensity of protein biosynthesis. This effectiveness varies in various metabolic states of the cell (16). Heavy metals influence cellular metabolism. They usually reduce enzyme activity, block chemical reactions, bind with proteins, interact with other elements and even break certain bonds (1, 8, 9, 11, 14). Experiments in the present work were conducted in order to determine changes in the effectiveness of amino acid binding by tRNA of the livers of rats receiving heavy metals such as cadmium and barium in their feed.

EXPERIMENTAL PROCEDURES

The experiment was conducted on white Wistar rats of both sexes. The animals were divided into groups of ten rats each. They received cadmium or barium in their drinking water for six weeks. The first group received 100 mg/l of cadmium (cadmium chloride solution), the second received 100 mg/l barium (barium chloride solution), the third was the control and received redistilled and deionized water. All animals were fed the same LSM feed and watered *ad libitum*. After the experiment the animals were killed using ketamine and the livers were harvested for further testing.

Preparations of tRNA were obtained by phenol extraction by the method of Sein and Zubay. The tRNAs used by this method were cleaned by chromatography

on DEAE–52 column (18, 20). Additionally, the tRNA was deaminoacylated by the method of Denney (6). The concentrations of tRNA were determined spectrophotometrically at $\lambda = 260$ nm and next were used to test the effectiveness of bonding for ten amino acids. They were: arginine, threonine, aspartic acid, asparagine, serine, isoleucine, lysine and phenylalanine.

Preparations of aminoacyl–tRNA synthetases necessary for aminoacylation were obtained from the rabbit liver. These preparations were obtained from the salted out protein fraction in the range of 40–70% ammonium sulphate after the salt was removed by dialysis (4). Protein was determined by Bradford method in these enzyme preparations (3).

The effectiveness of the bonding of amino acids by tRNA was tested by using marked amino acids (Dupont Company, Boston, USA). The incorporating system consisted of the following components in total of 200 µl: 100 mM Tris/HCL buffer, pH 7.5, 10 mM $MgCl_2$, 10 mM ATP, 10 mM KCl, 0.4 mM dithiothreitol, 0.1 mM phenylmethylsulfonylfluoride, 1.0 A_{260} units of tRNA, 50 µl of enzymes, and ^{14}C–amino acids (18.5 kBq). In controls the tRNAs were omitted. The incubation was carried out at 37°C for 20 min. Next, the samples of 100 µl were applied onto Whatman 3 MM discs which were rinsed four times in cold trichloroacetic acid and then in Hokin fluid (0.8 ml 10 M NaOH + 62.4 ml glacial acetic acid + ethanol to 1 liter) and ether, and dried. Radioactivity was measured in a Beckman scintillation counter. The effectiveness of the bonding of amino acids by tRNA was determined by the binding of labelled ^{14}C–amino acids by tRNA. The effectiveness of the bonding of amino acids was expressed by quantity of impulses per minute (cpm) in a tested sample per 1.0 ODU of tRNA.

RESULTS

The experiment showed that the addition of heavy metals influence the effectiveness of binding amino acids by tRNA (Table 1).

Tab. 1. The influence of cadmium and barium on the effectiveness of binding amino acid by tRNA in rat's liver

	Binding of amino acids by tRNA pmole/ 1 ODU		
Amino acids	Cd $\bar{x} \pm SD$	Ba $\bar{x} \pm SD$	Control $\bar{x} \pm SD$
Arg	23.84 ± 4.15	29.46 ± 5.51	31.76 ± 6.14
Thr	14.32 ± 3.11	15.67 ± 3.61	17.19 ± 3.95
Asp	9.12 ± 2.52	10.82 ± 2.80	12.35 ± 2.38
Asn	14.39 ± 4.20	14.65 ± 3.13	19.55 ± 3.46
Ser	21.41 ± 5.45	27.46 ± 5.82	30.35 ± 5.91
Ile	14.58 ± 3.64	16.65 ± 4.10	18.50 ± 3.45
Ala	11.42 ± 2.56	16.14 ± 3.56	16.58 ± 4.10
Leu	6.54 ± 1.98	8.75 ± 2.12	10.44 ± 2.45
Lys	14.52 ± 3.68	15.40 ± 3.54	18.48 ± 3.35
Phe	14.32 ± 4.10	15.20 ± 4.82	23.18 ± 4.18

$\bar{x}$ – average from ten animals, SD – standard deviation

tRNA of the livers of rats receiving cadmium and barium displayed a reduction in effectiveness of binding amino acids compared to control animals (Fig. 1).

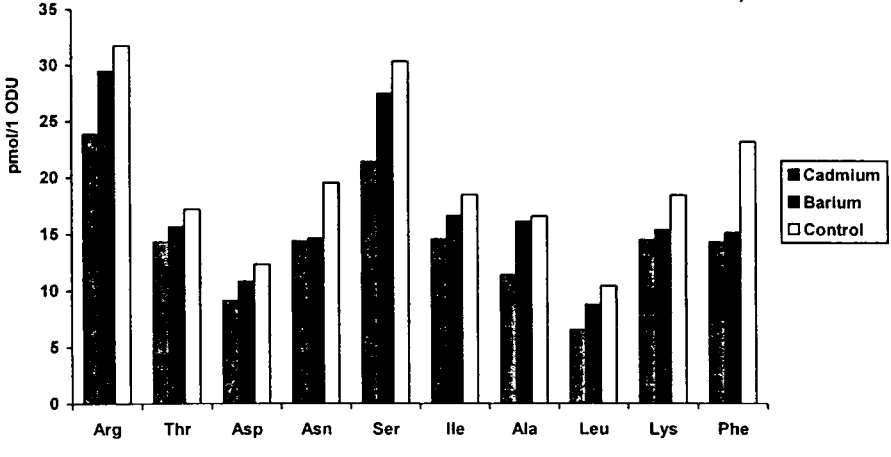

Fig. 1 The influence of Cd and Ba on the effectiveness of binding amino acids by tRNA in rat's liver

DISCUSSION

The effectiveness of amino acid binding by tRNA has a direct influence on the process of amino acid activation and an indirect influence on the process of translation. The influence of cadmium and barium added to the feed varies the effectiveness of amino acid binding by rat liver tRNA. In the case of cadmium the reductions were greater. Exposure to heavy metals is greater in the contemporary world (2, 10, 17). The reduction in binding effectiveness of amino acids by tRNA caused by heavy metals will lead to a reduction in protein synthesis. It is known that the biological effects of certain factors, including heavy metals, on the organism vary (11, 13, 19). In large part they are dependent upon the state and age of the organism (7, 12, 15). Of capital importance is the type of metal and the dose and the possibility of influence on tRNA.

CONCLUSION

1. Heavy metals such as cadmium and barium added to the feed influence the effectivenes of binding amino acids by tRNA of rat livers.

2. The influence of tested heavy metals varied according to the various amino acids and also depended on the type of metal.

REFERENCES

1. Badiello R., Feroci G. et al.: Interaction between trace elements: Selenium and cadmium ions. J. Trace Elem. in Med. Biol., 10, 3, 156, 1996.
2. Bluhm R., Branch R.A.: Clinical problems interpreting mercury levels – Experience from mercury exposed chloralkali workers. Int. Arch. Occup. Envir. Health, 68, 6, 421, 1996.
3. Bradford M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72, 248, 1976.
4. Charęziński M., Borkowski T.: Occurrence of aminoacyl–tRNA synthetases complex in calf brain. Arch. Biochem. Biophys., 207, 2, 241, 1981.
5. Delure M.: Aminoacyl–tRNA synthetases. Curr. Opin. Struct. Biol., 5, 48, 1995.
6. Denney R.M.: Detection and partial purification of rapidly sedimenting forms of aminoacyl––transfer ribonucleic acid synthetases from human placenta. Arch. Biochem. Biophys., 183, 156, 1977.
7. Factorlitvak P., Kline J.K. et al.: Blood lead and blood pressure in young children. Epidemiology, 7, 6, 633, 1996.
8. Garte S.J., Fulton H. et al.: Kinetics of metallothionein gene inductional by cadmium in human lymphocytes. Biochem. Molec. Biol. Inter., 37, 3, 459, 1995.

9. Hechtenberg S., Schafer T. et al.: Effects of cadmium on cellular calcium and protooncogene expression. Ann. Clin. Lab. Sci., 26, 6, 512, 1996.
10. Kolev S.T., House I. et al.: Concentration of blood lead and ethnicity in the United Kongdom. Occup. Envir. Med., 53, 12, 841, 1996.
11. Koizumi T., Shirakura H. et al.: Mechanism of cadmium–induced cytotoxicity in rat hepatocytes: Cadmium–induced active oxygen–related permeability changes of the plasma membrane. Toxicology, 114, 2, 125, 1996.
12. Lanphear B.P., Weitzman M. et al.: Racial differences in urban children's environmental exposures to lead. Am. J. Public Health, 86, 10, 1460, 1996.
13. Min K.S., Onosaka S. et al.: Renal accumulation of cadmium and nephropathy following long–term administration of cadmium–metallothionein. Toxicol. Appl. Pharm., 141, 1, 102, 1996.
14. Naarala J., Loikkanen J. et al.: The combination of lead wiith a protein kinase C inhibitor cause oxidative stress in human neuroblastoma cells. Neurosci. Res. Commun., 19, 3, 135, 1996.
15. Pelech L., Malina L. et al.: Screening of the gasoline lead in school children in the Czech Republic. Toxicol Letters, 88, 1–3, 233, 1996.
16. Pasternak K.: Activity of aminoacyl–tRNA synthetases in myelogenesis and leucemia. Appl. Bol. Commun., 1/3, 143–145, 1991.
17. Ratcliffe H.E., Swanson G.M. et al.: Human exposure to mercury: A critical assessment of the evidence of adverse health effects. J. Toxic. Envir. Health, 49, 3, 221, 1996.
18. Sein K.T., Becarevic A., Kanazir D.: A simple modified method for the extraction of rat liver sRNA. Anal. Biochem., 28, 65, 1969.
19. Wilson A.K., Cerny E.A. et al.: Effects of cadmium on osteoclast formation and activity *in vitro*. Toxic. Appl. Pharm., 140, 2, 451, 1996.
20. Zubay G.: The isolation and fractionation of soluble ribonucleic acid. J. Mol. Biol., 65, 375, 1972.

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STRESZCZENIE

W procesie biosyntezy białka ważną rolę pełni tRNA, który bierze udział w aktywacji aminokwasów przy udziale specyficznych enzymów. Zdolność wiązania aminokwasów przez tRNA zmienia się w różnych stanach metabolicznych komórki związanych z procesami fizjologicznymi i patologicznymi. Celem pracy było określenie zmian zdolności wiązania aminokwasów przez tRNA wątroby szczurów narażonych doświadczalnie na kadm i bar. Z wątroby szczurów doświadczalnych i kontrolnych preparowano tRNA, a następnie *in vitro* badano zdolność wiązania aminokwasów. Wykazano, że pod wpływem podawanego kadmu lub baru następowały zmiany zdolności wiązania aminokwasów przez tRNA wątroby szczurów. Działanie badanych metali ciężkich było zróżnicowane dla poszczególnych aminokwasów, jak również zależało od rodzaju metalu. Kadm powodował znaczniejsze niż bar obniżenie zdolności wiązania przez tRNA wszystkich badanych aminokwasów.