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## PECULIARITIES OF METABOLISM OF FREE PLASMA AMINO ACIDS IN THE BLOOD OF RATS UNDER THE EXPOSURE TO SODIUM FLUORIDE

***I.L. Kolesnyk***

Kharkiv Medical Academy of Postgraduate Education, Kharkiv

*Для оцінки і обґрунтування структурно-метаболічних механізмів, що лежать в основі формування інтоксикації вивчено стан пулу вільних плазмових амінокислот крові щурів під впливом фториду натрію в умовах формування хронічної фторидної інтоксикації на білих щурах популяції Вістар, яким на протязі 1,5 місяця перорально вводився розчин фториду натрію в дозі 20 мг / кг маси. Оціка стану білкового обміну здійснювалася по спектру вільних плазмових амінокислот методом іонообмінної хроматографії на ионитах. Наші дослідження показали зниження в плазмі крові концентрації таких свободних амінокіслот як аланина, а-аміномасляної кислоти, треоніну, серину, проліну, валіну, лізину і підвищення рівнів таурину, аспарагінової, глутамінової кислот, гліцину, цистеїну. Динаміка цистину, метіоніну, лейцину, ізолейцину, цістіаніна, тирозину, фенілаланіну, орнитина, гістидину, аргініну, аміаку не порушувалася порівняно з контрольною групою спостереження. Зниження білоксинтезуючої функції і превалювання катаболічних процесів над анаболічними також підтверджувалося пригніченням процесів інкорпорації $^{3}$Н-тимідину, $^{3}$Н-уридину і $^{14}$С-лейцину в гепатоцитах і спленоцитах, що ще раз свідчить про уповільнення синтетичних процесів.*

**Ключові слова:** інтоксикація, плазма крові, спектр вільних плазмових амінокислот, фторид натрію, щури популяції Вістар.

*To assess and substantiate the structural and metabolic mechanisms underlying the formation of intoxication, we studied the state of the pool of free plasma amino acids under the influence of sodium fluoride in the context of chronic fluoride intoxication in white rats of the Wistar population, which were orally administered sodium fluoride for 1.5 months at a dose of 20 mg / kg of body mass. Evaluation of the state of protein metabolism was conducted according to the spectrum of free plasma amino acids by ion exchange chromatography on ionites. Our studies have shown a decrease in plasma concentrations of free amino acids such as alanine, a-aminobutyric acid, threonine, serine, proline, valine, lysine, and an increase in taurine, aspartic, glutamic acid, glycine, and cysteine. The dynamics of cystine, methionine, leucine, isoleucine, cystianine, tyrosine, phenylalanine, ornithine, histidine, arginine, ammonia were not disrupted as compared with the control observation group. The decrease in the protein synthesizing function and the prevalence of catabolic processes over anabolic processes was also confirmed by the inhibition of the incorporation processes of $^{3}$H-thymidine, $^{3}$H-uridine and $^{14}$C-leucine in hepatocytes and splenocytes, which once again indicates a slowdown of synthetic processes.*

**Key words**: intoxication, blood plasma, spectrum of free plasma amino acids, sodium fluoride, Wistar rats.

### Introduction

The key role of amino acids in the processes of interstitial metabolism is well known. The development of many pathological conditions is inconceivable without structural and metabolic disorders associated with protein or amino acid. If necessary, amino acids can serve as a source of energy, mainly due to the oxidation of their carbon skeleton. In the body, they form a pool, the value of which is constant under physiological conditions. It corresponds to the difference between the intake of amino acids from the outside or sometimes from endogenous sources, and the consumption of amino acids, which serve as substrates in anabolic and catabolic processes. The rate of decomposition and synthesis of individual proteins is different for different tissues of the same organism.

The transformation of the carbon skeleton of amino acids under aerobic conditions leads to the formation of compounds, which are then included in the citric acid cycle and undergo further oxidation with the extraction of energy in the form of macroergic substrates. The process of intake of amino acids for oxidation requires the preliminary removal of the amino group. Most often, this is

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achieved by transamination, during which the amino group of the amino acid is transferred to α-ketoglutaric acid. After that, with specific dehydrogenases, glutamic acid is deaminated to α-ketoglutaric acid and NH4. Transamination of amino acids is an important link between the metabolism of amino acids and sugars. This process involves the interchangeable glycogenic amino acids, which are converted into glycogen by gluconeogenesis through a series of intermediates of the citric acid cycle. Carbon atoms of amino acids can be included in the citric acid cycle by five ways in the form of: actyl-CoA, α-ketoglutaric acid, succinyl-CoA, fumaric acid, oxaloacetate (Musil J. et al., 1984).

Ammonia is formed during the deamination of amino acids. It is toxic and is excreted from the body in the form of various compounds. In humans, white rats and other mammals, the final product is urea, which is formed in the ornithine cycle. Urea biosynthesis requires two ammonia molecules (one for the formation of carbamoyl phosphate and the other for the formation of aspartic acid).

The source of ammonia for the first reaction is the oxidative deamination glutamic acid. For the second reaction, the asparaginic acid ammonia is used, which is formed from glutamic acid when the amino group is transferred to oxaloacetate. Both reactions take place in the matrix of mitochondria of the liver cells. Glutamic acid penetrates into the mitochondria from the cytoplasm using a specific carrier. In the cytoplasm, there is a precursor of glutamic acid, α-ketoglutaric acid, which is the main acceptor of amino groups transferred from amino acids in transamination reactions.

The reaction of ornithine with carbamoyl phosphate forms citrulline, which is then converted to arginine with the participation of aspartic acid. Arginine breaks the urea molecule under the influence of arginase to form ornithine, thus closing the urea cycle, which is an endoergic process and requires 3 ATP molecules.

Studies by many authors convincingly indicate that the quantitative and qualitative spectrum of the pool of free plasma amino acids and the end products of their metabolism are an indicator of the state of the structural and metabolic homeostasis of the organism (Zorkin, A.A. et al., 1985; Grigorova, I.A., et al., 1997; Grigorov, B.I., et al., 1999; Zaitseva, O.V., 1999). The foregoing has determined the relevance of our research.

The aim of the research is to study the state of the pool of free plasma amino acids in the blood of rats under the influence of sodium fluoride in the context of chronic fluoride intoxication.

## Material and methods

The state of the pool of free plasma amino acids was studied on white rats of the Wistar population, which were orally administered a solution of sodium fluoride for 1.5 months, in a dose of 20 mg / kg. At the end of the experiment, the animals were euthanized by decapitation. In the blood of experimental and control groups of animals, the content of free plasma amino acids was studied by ion exchange chromatography on ionites (Zorkin, A.A. et al., 1985). The concentration of the studied amino acids and their spectrum were determined on an automatic amino acid analyzer AAA-339 (Czechoslovakia) by comparison with standard samples. Evaluation of the state of protein metabolism was conducted by the spectrum of such amino acids as: cysteine, taurine, aspartic, threonine, serine, proline, glycine, alanine, valine, cystine, methionine, tyrosine, phenylalanine, leucine, isoleucine, lysine, histidine, arginine, ornithine, glutamine. Metabolites of protein metabolism - urea, creatinine, chlorides and protein in the urine were determined by standard methods (Asatiani V.S., 1969; Predtechensky V.E., 1964).

In order to assess and substantiate the structural and metabolic mechanisms, underlying the formation of modelled fluoride periodontitis, we additionally studied DNA, RNA and protein metabolism in the internal organs and tissues by the radioisotope method. The status of their metabolism was evaluated by the level of incorporation of $^{3}$H-thymidine, $^{3}$H-uridine and $^{14}$C-leucine in the cell, respectively (Rosen, V.B., Smirnov, A.N., 1981).

## Results and discussions

The study of the pool of free plasma amino acids under the condition of chronic fluoride intoxication showed significant differences in the amino acid spectrum in the control and experimental groups of white rats. This primarily indicates that the intake of sodium fluoride at a dose of 20 mg / kg of animal mass leads to profound structural and metabolic shifts in the body during the development of fluoride intoxication. It should be assumed that under the conditions of long-term subtoxic intake of sodium fluoride into the body, there may be a shortage of tissue energy supply and at the same time activation of compensatory-adaptive mechanisms of gluconeogenesis enhancement. These processes, as is known, are stimulated by glucocorticoids, secreted in increased amounts by the action of toxic substances on the body.

Many authors explain the increased number of free amino acids by the fact that the strengthening of catabolic processes in tissues during various kinds of intoxications is a manifestation of adaptive reaction aimed at maintaining homeostasis (Zhukov, V.I. et al., 2000; Zaitseva, O.V., 2001; Krasovsky G.N. and co-authors, 2003).

The increase in the pool of free amino acids as a result of increased protein breakdown contributes to the targeted synthesis of a number of cellular structures and other body needs. Our studies have shown a decrease in plasma concentrations of free amino acids such as alanine, a-aminobutyric acid, threonine, serine, proline, valine, lysine, and an increase in taurine, aspartic, glutamic acid, glycine, and cysteine. The dynamics of cystine, methionine, leucine, isoleucine, cystianine, tyrosine, phenylalanine, ornithine, histidine, arginine, ammonia was not disrupted as compared with the control observation group (Table 1).

The detected shifts in the content of free amino acids may be important in the disorders of proteosynthesis. This assumption is based on a well-known rule: the failure of at least one of the amino acids limits the use of the rest for the synthesis of a protein molecule. In assessing the shifts in the amount of free amino acids, one cannot proceed only from changes in the processes of protein synthesis and decomposition, since metabolism of amino acids is central to the entire metabolic process of the body.

The content of chlorides, protein, urea, creatinine in the urine of experimental and control animals was increased under the modelled conditions of periodontitis, which conforms with the dynamics of the free amino acid pool.

*Table 1*

*The state of pool of free plasma amino acids in the conditions of fluoride modelled periodontitis (nmol / ml)*

| Parameters | Groups of animals, M±t | |
|---|---|---|
| | Control group (n=15) | Study group (n=15) |
| Cysteine | 0.820+0.02 | 2.54±0.15t, P<0.05 |
| Taurine | 18.4+0.65 | 25.7±1.3, P<0.05 |
| Urea | 37.8±2.16 | 21.5±1.03T, P<0.05 |
| Aspartate | 2.40±0.4 | 4.35±0.32T, P<0.05 |
| Threonine | 38.2+1.95 | 20.6±2.204-, P<0.05 |
| Serine | 42.3+2.50 | 27.4+1.804-, P<0.05 |
| Glutamate | 18.3±0.78 | 26.8±1.30T,P<0.05 |
| Glutamine | 280.6+12.3 | 378.4±9.8T, P<0.05 |
| Proline | 50.4+2.20 | 43.8+1.704-, P<0.05 |
| Glycine | 40.6+2.80 | 60.2±3.141, P<0.05 |
| Alanine | 76.3+4.50 | 57.8+3.604-, P<0.05 |
| GABA | 10.40±0.84 | 7.50±0.624-, P<0.05 |
| Valine | 15.8+0.95 | 9.4±0.304-, P<0.05 |
| Cystine + methionine | 8.70±0.42 | 9.10±0.56, P>0.05 |
| Isoleucine + leucine | 5.60+0.37 | 4.80±0.43, P>0.05 |
| Cystianine | 9.85±0.30 | 10.20±0.46, P>0.05 |
| Tyrosine | 8.56+0.28 | 9.16+0.54, P>0.05 |
| Phenylalanine | 12.40±0.53 | 11.90±0.47,P>0.05 |
| Ammonia | 18.60+1.3 | 19.75±0.80, P>0.05 |
| Ornithine | 9.75±0.64 | 10.10±0.75, P>0.05 |
| Lysine | 25.6+1.17 | 14.3±0.824-,P<0.05 |
| Histidine | 10.60+0.73 | 11.25±0.68, P>0.05 |
| Arginine | 21.40±1.85 | 20.70±2.3, P>0.05 |

The decrease in the protein-synthetic function and the prevalence of catabolic processes over anabolic processes was also confirmed by the inhibition of the incorporation of $^{3}$H-thymidine, $^{3}$H-uridine and $^{14}$C-leucine in hepatocytes and splenocytes, which again indicates a slowing down of synthetic processes. (Table 2)

*Table 2*

*Dynamics of inclusion of $^{3}$H-thymidine, $^{3}$H-uridine, $^{14}$C-leucine in the cells of the liver and spleen under the conditions of fluoride intoxication*

| Groups of animals | Organs, M±t | | | | | |
|---|---|---|---|---|---|---|
| | Liver | | | Spleen | | |
| | $^{3}$H-thymidine | $^{3}$H-uridine | $^{14}$C-leucine | $^{3}$H-thymidine | $^{3}$H-uridine | $^{14}$C-leucine |
| Control group (n=15) | 9074.5±20.3 | 8425.3± 30.6 | 15620.8+120.6 | 9680.7± 30.8 | 8920.5±40.4 | 17835.3±140.6 |
| Study group (n=15) | 7025.2± 30.6 P<0.05 | 6210.3±41.2 P<0.05 | 12608.9+90.4 P<0.05 | 7560.3+41.4 P<0.05 | 6807.4± 50.2 P<0.05 | 14206.2±110.7 P<0.05 |

**Conclusions**. The analysis of the state of the free plasma amino acids pool under the condition of chronic fluoride intoxication showed that profound changes in the structural metabolism of proteins and their derivatives occur in the pathogenesis of modelled periodontitis. The dynamic content of amino acids in the blood plasma indicates that in the mechanisms involved in the development of periodontitis, there is a profound structural rearrangement of protein metabolism, which is accompanied by the prevalence of catabolic processes over anabolic and increased gluconeogenesis, aimed at ensuring energy homeostasis, resulting from tissue hypoxia and ischemia of organs and tissues.

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