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Research Article

Full Ligation of both Carotid Arteries within one Hour Causes Significant Changes in the Content of Amino Acids and Their Derivatives in the Brain of White Mongrel Rats

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Abstract

Amino acids and their derivatives are involved in synaptic transmission as neurotransmitters and neuromodulators, and some of them are involved in the formation of neurotransmitters of the nervous system. Therefore, the study of the state of the amino acid pool in simultaneous cerebral ischemia plays a significant role.

Target. Evaluation of the nature of changes in the pool of amino acids and evaluate their participation in oxidative processes in rats with subtotal IHM.

Materials and methods. The experiments were carried out on 16 male outbred white rats weighing 260 ± 20 g in compliance with the requirements of the Directive of the European Parliament and of the Council No. 2010/63/EU of September 22, 2010 on the protection of animals used for scientific purposes.

Results. When modeling partial cerebral ischemia (PCI) by unilateral ligation of a. carotis communis after 1 hour, there were no pronounced morphological changes at the microscopic and ultrastructural levels. Also, there were no pronounced changes in the respiratory parameters of the mitochondrial fraction with a slight decrease in the content of ATP synthase, which reflects the safety of the enzymatic complexes of the electron transport chain in this model of ischemia and changes in the parameters of the prooxidant-antioxidant balance of brain homogenates.

Conclusions. In rats with SIGM, with an ischemic period of 1 hour, there was a tendency to increase the content of the inhibitory neurotransmitter glycine, while changes in the level of amino acids with the properties of excitatory neurotransmitters tended to decrease.

Keywords: amino acid; neurons; ischemia; hippocampus

Relevance

Amino acids (AK) play an important role in the metabolism and functioning of the brain. This is explained not only by the exceptional role of amino acids as sources of synthesis of a large number of biologically important compounds (proteins, mediators, lipids, biologically active amines). Amino acids and their derivatives are involved in synaptic transmission as neurotransmitters and neuromodulators (glutamate, aspartate, glycine, GABA, taurine), and some amino acids are involved in the formation of mediators of the nervous system: methionine – acetylcholine, DOPA, dopamine; tyrosine – catecholamines; serine and cysteine – taurine; tryptophan – serotonin; histidine – histamine; L-arginine – NO; glutamic acid – glutamate [1-3].

Thus, it is of interest to study the state of the amino acid pool in subtotal cerebral ischemia

The purpose of the study is to observe changes in the amino acid pool and biogenic amines in rats with incomplete cerebral ischemia.

Materials and methods

The experiments were performed on 16 male mongrel white rats weighing 260 ± 20 g in compliance with the requirements of the Directive of the European Parliament and of the Council No. 2010/63/EU of 22.09.2010 on the protection of animals used for scientific purposes.

Modeling and GM were performed under intravenous thiopental anesthesia (40-50 mg/kg).

Incomplete cerebral ischemia (SCI) was modeled by simultaneous ligation of both a. carotis communis. The material for the study was collected 1 hour after decapitation.

The control group consisted of falsely operated rats with the same physical characteristics. After the extraction of the brain, a fragment of the hippocampus was taken with its subsequent freezing in liquid nitrogen.

Preparation of the sample for the study included homogenization in a 10-fold volume of 0.2 M perchloric acid, centrifugation for 15 minutes at 13000 g at 4oC with subsequent selection of the supernatant. The analysis was performed by reverse-phase chromatography with pre-column derivatization with o-phthalic aldehyde and 3-mercaptopropionic acid in a Na-borate buffer on a chromatograph Agilent 1100.

To prevent systematic measurement errors, brain samples from the compared control and experimental groups of animals were studied under the same conditions.

Results

No changes in biogenic amines were detected with this method of modeling cerebral ischemia. As a result of the research, quantitative continuous data were obtained. Since the experiment used small samples that had an abnormal distribution, the analysis was carried out using nonparametric statistics using a licensed computer program Statistica 10.0 for Windows (StatSoft, Inc., USA). The data is presented in the form Me (LQ; UQ); Me – median, LQ – the value of the lower quartile; UQ – the value of the upper quartile. The differences between the groups were considered significant when $p<0,05$ (nonparametric Games-Howell test) [4-6].

Previously conducted morphological studies in rats in the dynamics of simultaneous cerebral ischemia (SCI) a decrease in the size of the pericaryons of neurons, an aggravation of their elongation, a decrease in the number of normochromic and hyperchromic neurons and an increase in the proportion of hyperchromic shrunken neurons were revealed [7]. At the ultrastructural level, at SCI, mitochondria swelled with a decrease in the number and length of their crysts, vacuolization of the granular endoplasmic network was noted, and the predominance of free ribosomes over bound ones. These morphological changes were the result of pronounced disturbances of energy metabolism, especially when succinate was used as a substrate in in vitro studies, indicating the most severe damage to the succinate dehydrogenase complex of the electron transfer chain and accompanied by a decrease in the content of ATP synthase, an enzyme that carries out the reaction of ATP formation from ADP [8-10]. Violations of the prooxidant-antioxidant balance in rats with SCI – a decrease in the total SH-groups of proteins and glutathione, the concentration of reduced glutathione and an increase in the content of products reacting with thiobarbituric acid reflected a high activity of oxidative stress [11]. When modeling partial cerebral ischemia (PCI) by unilateral ligation of a. carotis communis after 1 hour, there were no pronounced morphological changes at the microscopic and ultrastructural level. There were also no pronounced changes in the respiration parameters of the mitochondrial fraction with a slight decrease in the content of ATP synthase, which reflects the relative safety of the enzymatic complexes of the electron transfer chain in this model of ischemia and changes in the indicators of the prooxidant-antioxidant balance of brain homogenates. [12-13].

The revealed changes in the content of sulfur-containing amino acids (a decrease in the content of cysteine and methionine) with SCI are the activity of oxidative processes (Table 1) [14-15].

Hyppocampus		
Amino acids	Animal groups	
	Control	SCI 1 hour
Neurotransmitters		
Glycine	174 (150/190)	191 (176/214)
Glutamate	3375 (3146/3574)	3265 (2947/3429)
Aspartate	1603 (1351/1768)	1663 (1398/1952)
Taurine	1032 (983/1125)	1129 (1038/1194)
GABA	523 (485/665)	857 (749/1097)

Endogenous NMDA-receptor antagonist		
α- aminoadipinate	13 (11,5/14,1)	5,08 (4,63/6,51)*
Sulfur – containing		
Cysteate	1,03 (0,278/1,69)	2,19 (1,73/2,72)
Cystathionine	37,7 (34,7/40,8)	46,5 (34,2/52,7)
Taurine	1032 (983/1125)	1129 (1038/1194)
Methionine	19,3 (17,9/23,4)	15,9 (15/16,5)
Cysteinsulfinic acid	2,56 (1,24/4,05)	2,35 (1,41/2,87)
Glycogenic		
Aspartate	1603 (1351/1768)	1663 (1398/1952)
Asparagine	101 (92,5/105)	106 (102/111)
Threonine	425 (345/567)	253 (226/317)
Serine	516 (496/552)	505 (452/528)
Glutamine	1981 (1831/2172)	1740 (1645/1960)
Glutamate	3375 (3146/3574)	3265 (2947/3429)
Glycine	174 (150/190)	191 (176/214)
Alanine	318 (297/334)	456 (446/487)
Valine	74,9 (70,8/79,1)	52,4 (49,3/64,2)
Methionine	19,3 (17,9/23,4)	15,9 (15/16,5)
Histidine	17,7 (16,3/19)	16,7 (16,5/18,2)
Arginine	27,8 (21,2/32,4)	43,4 (32,1/48,6)
Ketogenic		
Lysine	227 (179/259)	134 (117/187)
Leucine	68,2 (64,8/72)	56,3 (51,8/67,9)
Histidine derivative		
3- methylhistidine	4,65 (4,37/5,84)	5,56 (5,23/5,8)
Replaceable		
Glycine	174 (150/190)	191 (176/214)
Alanine	318 (297/334)	456 (446/487)
Glutamine	1981 (1831/2172)	1740 (1645/1960)
Glutamate	3375 (3146/3574)	3265 (2947/3429)
Aspartate	1603 (1351/1768)	1663 (1398/1952)
Asparagine	101 (92,5/105)	106 (102/111)
Serine	516 (496/552)	505 (452/528)

Tyrosine	49,3 (44,6/50,2)	50,3 (46,1/54)
Ornithine	11,2 (9,78/14,2)	15,8 (12,8/20,2)
Essential		
Valine	74,9 (70,8/79,1)	52,4 (49,3/64,2)
Isoleucine	33,2 (31,1/35,1)	26,5 (25,1/32,1)
Leucine	68,2 (64,8/72)	56,3 (51,8/67,9)
Methionine	19,3 (17,9/23,4)	15,9 (15/16,5)
Lysine	227 (179/259)	134 (117/187)
Histidine	17,7 (16,3/19)	16,7 (16,5/18,2)
Threonine	425 (345/567)	253 (226/317)
Tryptophan	29,8 (25,1/31,8)	22,7 (21,5/27,8)
Phenylalanine	31,6 (26/39,2)	31,3 (30,5/32,4)
Aromatic		
Tyrosine	49,3 (44,6/50,2)	50,3 (46,1/54)
Tryptophan	29,8 (25,1/31,8)	22,7 (21,5/27,8)
Phenylalanine	31,6 (26/39,2)	31,3 (30,5/32,4)
BCAA		
Valine	74,9 (70,8/79,1)	52,4 (49,3/64,2)
Isoleucine	33,2 (31,1/35,1)	26,5 (25,1/32,1)
Leucine	68,2 (64,8/72)	56,3 (51,8/67,9)
Amino Acid ratio		
BCAA/AAA	1,56 (1,4/1,7)	1,2 (1,2/1,6)
Replaceable / Essential	8,4 (7,5/9,8)	11,9 (10,2/14,2)
Glycogenic/Ketogenic	28 (27/34,9)	42,2 (32,1/49,1)
AA amount	10835 (9734/11603)	10785 (10198/11542)

Table1: Indicators of the hippocampal amino acid pool of rats with subtotal cerebral ischemia (SCI) lasting 1 hour, Me (LQ/UQ)

Note: * – p<0,05 compared to the control group, AA – amino acid, GABA - Gamma-aminobutyric acid, BCAA - branched-chain amino acid.

Along with this, in rats with SCI, there was an increase the level of NO-synthase substrate L-arginine in the hippocampus by 35% (p>0.05). An increase in the level of L-arginine in SIGM may be associated with a low activity of its utilization reactions due to oxygen deficiency, among which the formation of nitrogen monoxide (NO) plays a significant role. However, the level of the product of this reaction, ornithine, did not change.

After 1 hour, with incomplete cerebral ischemia, there was a tendency to increase the content of the inhibitory neurotransmitter glycine, while changes in the level of amino acids with the properties of excitatory neurotransmitters (aspartate and glutamate), on the contrary, tended to decrease.

Among the group of BCAA, there was a tendency to decrease valine by 30% (p>0.05). The absence of a marked decrease in the amino acids of the BCAA group at SCI is consistent with a significant decrease in energy processes at SIGMA [16-18].

As a result of changes in the levels of BCAA and aromatic amino acids, the ratio of the sum of the levels of BCAA to the sum of the levels of aromatic amino acids in SCI in the hippocampus decreased from 1.6 to 1,2 (p>0,05).

Among the essential amino acids in rats with SCI duration of 1 hour, there was a tendency to decrease valine by 30% (p>0.05), isoleucine by 20% (p>0.05), leucine by 17% (p>0.05), methionine by 18% (p>0.05), lysine by 41% (p>0.05), threonine – by 40% (p>0.05), tryptophan – by 24% (p>0,05).

At the same time, the indicator of the “Replaceable / Essential” amino acids ratio in the SIGM group increased from 8.4 to 11.9 (p> 0.05), which may be

a consequence of impaired utilization of non-essential amino acids in protein synthesis reactions along with increased utilization of essential amino acids.

Conclusions.

So, the following changes in the pool of amino acids are typical for one-hour SCI: a decrease in the content of sulfur-containing amino acids, with a decrease in both methionine and cysteate, as a reflection of the high activity of oxidative stress in SCI. Along with this, with simultaneous cerebral ischemia, an increase in the content of L-arginine, a tendency to an increase in the content of the inhibitory neurotransmitter glycine, and a decrease in aspartate and glutamate as amino acids with the properties of excitatory neurotransmitters, as well as tryptophan, valine and leucine were noted. At the same time, there was no increase in glutamate levels and no decrease in BCAA levels.

Conflict of interest. The authors declares no conflict of interest.

Compliance with ethical principles

The author confirms that they respect the rights of the people participated in the study, including obtaining informed consent when it is necessary, and the rules of treatment of animals when they are used in the study. Author Guidelines contains the detailed information.

References:

1. Razvodovsky Y.E. (2019). The content of amino acids and their derivatives in the cerebral cortex of rats with its partial ischemia Y. E. Razvodovsky, V. Yu. Smirnov, E. M. Doroshenko, N. E. Maksimovich, V.A. Pereverzev Bulletin of the Smolensk State Medical Academy, 5-9.
2. Erecińska M, Nelson D, Wilson DF, Silver IA. (1984). Neurotransmitter amino acids in the CNS. I. Regional changes in amino acid levels in rat brain during ischemia and reperfusion. Brain Res, 304(1):9-22.
3. Clemens J.A. (2000). Cerebral ischemia: gene activation, neuronal injury, and the protective role of antioxidants Free Radic. Biol. Med, 1526-1531.
4. Guo M. F. (2011). Mechanisms related to neuron injury and death in cerebral hypoxic ischaemia M. F. Guo, J. Z. Yu, C. G. Ma Folia Neuropathol, 78-87.
5. Slivka AP, Murphy EJ. (2001). High-dose methylprednisolone treatment in experimental focal cerebral ischemia. Exp Neurol, 167(1):166-72.
6. Rey A, de-Cara A, Calvo L, Puig P, Hechavarría T. (2000). Changes in Plasma Fatty Acids, Free Amino Acids, Antioxidant Defense, and Physiological Stress by Oleuropein Supplementation in Pigs Prior to Slaughter. Antioxidants (Basel), 9(1):45-52.
7. Butin A.A. (2004). Patterns of changes in the vascular-capillary network of the cerebral cortex in response to acute cerebral ischemia // Omsk Scientific Bulletin, 46-57.
8. Bon, E. I. (2021). Disorders of Energy Metabolism in Neurons of the Cerebral Cortex During Cerebral Ischemia E. I. Bon, N.E Maksimovich, S.M Karnyushko, S. M Zimatkin, M.A Lychkovskaya Biomedical Journal of Scientific & Technical Research, 31932-31937.
9. Bon, E.I. (2021). Experimental Cerebral Ischemia Causes Disturbances in Mitochondrial Respiration of Neurons E. I. Bon, N. Ye. Maksimovich, I.K. Dremza, M.A. Lychkovskaya Biomedical Journal of Scientific & Technical Research, 32387-32392.
10. H. Shimizu, S.H. Graham, L.-H. Chang, J. Mintorovitch, T.L. James, A.I. Faden, P.R. (1993). Weinstein,Relationship between extracellular neurotransmitter amino acids and energy metabolism during cerebral ischemia in rats monitored by microdialysis and in vivo magnetic resonance spectroscopy, Brain Research, 33-42.
11. Bon, E. I. (2022). Severity of Oxidative Stress in Stepwise Cerebral Ischemia E. I. Bon, N. Ye. Maksimovich, I.K. Dremza, N.V. Kokhan, I.N. Burak Advance in Medical and Clinical Research, 1-3.
12. Bon, E. I. (2021). Disorders of Energy Metabolism in Neurons of the Cerebral Cortex During Cerebral Ischemia E. I. Bon, N.E Maksimovich, S.M Karnyushko, S. M Zimatkin, M.A Lychkovskaya Biomedical Journal of Scientific & Technical Research, 31932-31937.
13. Stevens J.L, Feelisch M, Martin D.S. (2019). Perioperative Oxidative Stress: The Unseen Enemy Anesth Analg, 1749-1760.
14. Ramon Rodrigo, Rodrigo Fernandez-Gajardo, Rodrigo Gutiérrez, Jose Manuel Matamala, Rodrigo Carrasco, Andres Miranda-Merchak, Walter Feuerhake. (2013). Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities CNS Neurol Disord Drug Targets, 698-714.
15. Bon, E.I. (2022). Characteristics of disorders of the prooxidant-oxidant balance in rats with cerebral ischemia E.I. Bon, N.E. Maksimovich, I.K. Dremza, M.A. Nosovich, K.A. Khrapovitskaya Ulyanovsk Medical Biological Journal, 97-106.
16. Bon, E. I. (2021). Disorders of Energy Metabolism in Neurons of the Cerebral Cortex During Cerebral Ischemia E. I. Bon, N.E Maksimovich, S.M Karnyushko, S. M Zimatkin, M.A Lychkovskaya Biomedical Journal of Scientific & Technical Research, 31932-319373.
17. Taysi S. (2019). Oxidative / Nitrosative Stress and Preeclampsia / Taysi S. [et al.] // Mini Rev Med Chem, 178-193.
18. Robles-Murguia M, Rao D, Finkelstein D, Xu B, Fan Y, Demontis F. (2020). Muscle-derived Dpp regulates feeding initiation via endocrine modulation of brain dopamine biosynthesis. Genes Dev, 34(1-2):37-52.

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