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Incomplete Ischemia Causes Disturbances in the Amino Acid Pool of the Parietal Lobe of Outbred Rats

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Abstract

Amino acids and their derivatives are involved in synaptic transmission as neurotransmitters and neuromodulators, and some amino acids are involved in the formation of nervous system mediators. Therefore, the study of the state of the pool of amino acids in subtotal cerebral ischemia is important.

Target: To evaluate 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 the common carotid artery (CCA), 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. Compared with the indicators in the control group, in rats with SCI with an ischemic period of 1 hour in the parietal lobe, there was a decrease in the content of sulfur-containing amino acids: methionine by 12% ($p<0.05$) and cysteine by 28% ($p<0.05$), apparently because of the activation of oxidative stress. In turn, a decrease in cysteine levels prevents the synthesis of taurine, but the level of the latter did not decrease, possibly due to its long half-life in the brain.

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

Abbreviations: AA: Amino acids; PCI: Partial cerebral ischemia; CCA: Common carotid artery; CI: cerebral ischemia; SCI: Subtotal cerebral ischemia; BCAAs: branched hydrocarbon amino acids.

Introduction

Amino acids (AA) play an important role in the metabolism and functioning of the brain. This is explained not only by the exclusive role of amino acids as sources for the synthesis of many 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 nervous system mediators: 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

To evaluate changes in the amino acid pool in rats with subtotal cerebral ischemia.

Materials and Methods

The experiments were carried out on 16 stray white male rats weighing 260 ± 20 g in accordance with the requirements of Directive 2010/63/EC of the European Parliament and of the Council of 22.09.2010 on the protection of animals for scientific purposes. Modeling of cerebral ischemia (CI) was performed under intravenous anesthesia of thiopental (40-50 mg/kg). Subtotal cerebral ischemia (SCI) was modeled by simultaneous connection of both common carotid arteries (CCA). The material was taken 1 hour after decapitation. The control group consisted of fictitiously operated rats of the same sex and weight. After removal of the brain, a fragment of the parietal cortex was taken, followed by 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. Amino acids were analyzed by reverse-phase chromatography with pre-column derivatization with o-phthalic aldehyde and 3-mercaptopropionic acid in Na-borate buffer on an Agilent 1100 chromatograph.

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

Results

According to the results of the study, quantitative continuous data were obtained. Since the experiment used small samples that had an abnormal distribution, the analysis was carried out by nonparametric statistics using the licensed computer program Statistical 10.0 for Windows (Stat Soft, Inc., USA). The data is presented in the form of Me (LQ; UQ), where Me is the median, LQ is the value of the lower quartile; UQ is the value of the upper quartile. Differences between the groups were considered significant at $p < 0.05$ (nonparametric Games-Howell test) [4-6].

Earlier morphological studies in rats in the dynamics of subtotal cerebral ischemia (SCI) revealed a decrease in the size of perikaryon 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 and cells with pericellular edema IN [7]. At the ultrastructural level, at SCI, mitochondria swelled with a decrease in the number and length of their crystals, 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 in 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 transport 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 Thio barbituric acid reflected a high activity of oxidative stress [11]. When modeling partial cerebral ischemia (PCI) by unilateral ligation of the common carotid artery (CCA) 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 transport chain in this model of ischemia and changes in the prooxidant-antioxidant balance of brain homogenates [12,13].

Changes in the amino acid pool in rats with SCI were of the following nature

Compared with the indicators in the control group, rats with a SCI duration of the ischemic period of 1 hour in the parietal lobe had a decrease in the content of sulfur-containing amino acids: methionine by 12% ($p<0.05$) and cysteine by 28% ($p<0.05$), apparently because of activation of oxidative stress. In turn, a decrease in the level of cysteine interferes with the synthesis of taurine, an amino acid with mediator and antioxidant properties, but the level of the latter did not decrease, possibly due to its long half-life in the brain.

A drop in the level of cysteine in the parietal lobe at SCI did not lead to significant shifts in the levels of cysteine sulfinate, homotaurine and taurine, as noted above, which, along with a decrease in the level of methionine, may reflect a decrease in the flow of sulfur-containing amino acids along the cysteine dioxygenase pathway. The revealed changes in the content of sulfur-containing amino acids (a decrease in the content of cysteine and methionine) in SCI are the activity of oxidative processes [14,15] (Figure 1).

Along with this, in rats with SCI, there was an increase the level of NO-synthase substrate L-arginine in the parietal lobe by 28% ($p<0.05$). An increase in the level of L-arginine in SCI 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 subtotal 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. With SCI in the parietal lobe, there was a tendency to reduce the level of aromatic amino acid - tryptophan (a source of serotonin), while there was no change in the content of other aromatic amino acids (tyrosine, phenylalanine) ($p>0.05$). This may be the result of increased serotonin synthesis or reduced transport to the brain. In this regard, we can assume a violation of the formation of catecholamines in SCI.

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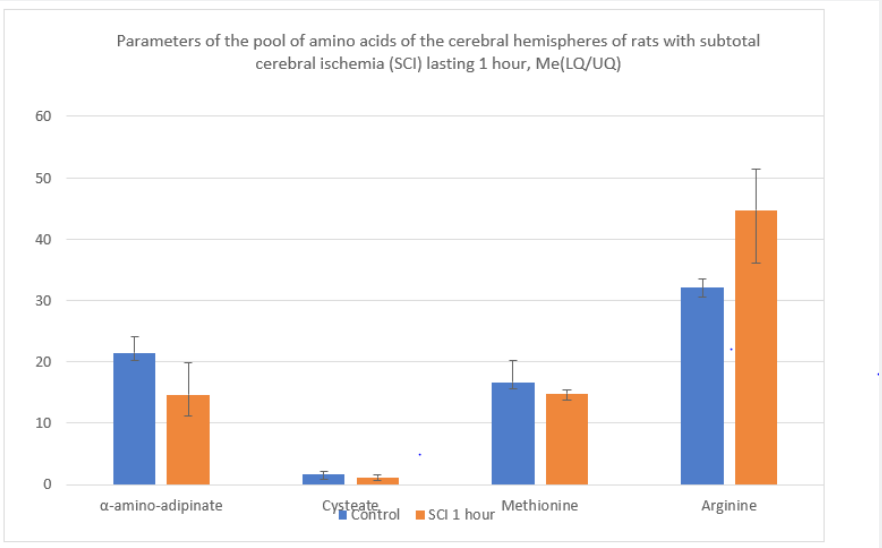

Figure 3. Histopathological examination shows multiply lymphatic channels in loose connective tissue. Flattened endothelial cells on the walls of the channels and infiltration of lymphocytes are also seen. X 100

Among the group of branched hydrocarbon amino acids (BCAAs), there was a trend towards a decrease in valine by 21% in the parietal lobe ($p>0.05$). The absence of a pronounced decrease in amino acids of the BRAAs group in SCI is consistent with a significant decrease in energy processes in SCI [16-18]. As a result of changes in the levels of ARUC and aromatic amino acids, the ratio of the sum of ARUC levels to the sum of aromatic amino acid levels in SCI in the parietal lobe did not change ($p>0.05$). Among the essential amino acids in rats with SCI lasting 1 hour, there was a tendency to decrease in valine - by 21% in the parietal lobe ($p>0.05$), methionine - by 11% in the parietal lobe ($p>0.05$), lysine - by 30 % in the parietal lobe ($p>0.05$), threonine - by 24% in the parietal lobe ($p>0.05$), tryptophan - by 22% in the parietal lobe ($p>0.05$).

At the same time, the "Replaceable/Essential" amino acid ratio in the SCI group increased from 10.0 to 13.1 ($p>0.05$) in the parietal lobe ($p>0.05$), 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. So, the following changes in the pool of amino acids are typical for a one-hour SCI: a decrease in the content of sulfur-containing amino acids, with a decrease in both methionine and cysteine, as a reflection of the high activity of oxidative stress in SCI. Along with this, with subtotal cerebral ischemia, an increase in the content of L-arginine was noted, 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. At the same time, there was no increase in glutamate levels and no decrease in BCAAs levels.

References

1. Razvodovsky Yu E, Smirnov Yu V, Doroshenko EM, Maksimovich NE, Pereverzev VA (2019) The content of amino acids and their derivatives in the cerebral cortex of rats with its partial ischemia. Bulletin of the Smolensk State Medical Academy, Russia 18(1): 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 JA (2000) Cerebral ischemia: gene activation, neuronal injury, and the protective role of antioxidants Free Radic. Biol Me 28(10): 1526-1531.
4. Guo MF, Yu JZ, Ma CG (2011) Mechanisms related to neuron injury and death in cerebral hypoxic ischaemia. Folia Neuropathol 49(2): 78-87.
5. Slivka AP, Murphy EJ (2001) High-dose methylprednisolone treatment in experimental focal cerebral ischemia. Exp Neurol 167(1): 166-172.
6. Rey AI, de-Cara A, Calvo L, Puig P, Hechavarría T et al. (2020) 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):45-56.
7. Butin AA (2004) Patterns of changes in the vascular-capillary network of the cerebral cortex in response to acute cerebral ischemia. Omsk Scientific Bulletin
8. Bon EI, Maksimovich NE, Karnyushko SM, Zimatkin MA, Lychkovskaya MA, et al. (2021) Disorders of Energy Metabolism in Neurons of the Cerebral Cortex During Cerebral Ischemia. Biomedical Journal of Scientific & Technical Research 40(1): 31932-31937pp.
9. Bon EI, Maksimovich N Ye, Dremza IK, Lychkovskaya MA, (2021) Experimental Cerebral Ischemia Causes Disturbances in Mitochondrial Respiration of Neurons. Biomedical Journal of Scientific & Technical Research 40(4): 32387-32392pp.

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10. Shimizu H, Graham SH, LH, Chang L-H, Mintorovitch J, James TL, et al. (1993) 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 605(1): 33-42.

11. Bon EI, Maksimovich N Ye, Dremza IK, Kokhan NV, Burak IN, et al. (2022) Severity of Oxidative Stress in Stepwise Cerebral Ischemia. Advance In Medical and Clinical Research 2: 1-3.

12. Bon EI, Maksimovich NE, Karnyushko SM, Zimatkin SM, Lychkovskaya MA et al. (2021) Disorders of Energy Metabolism in Neurons of the Cerebral Cortex During Cerebral Ischemia. Biomedical Journal of Scientific & Technical Research 40: 31932-319373pp.

13. Stevens JL, Feelisch M, Martin DS (2019) Perioperative Oxidative Stress: The Unseen Enemy. Anesth Analg. 129(6): 1749-1760.

14. Ramon Rodrigo, Rodrigo Fernandez-Gajardo, Rodrigo Gutiérrez, Jose Manuel Matamala, Rodrigo Carrasco et al. (2013) Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities. CNS Neurol Disord Drug Targets 12(5): 698-714.

15. Bon EI, Maksimovich NE, Dremza IK, Nosovich MA, Khrapovitskaya KA et al. (2022) Characteristics of disorders of the prooxidant-oxidant balance in rats with cerebral ischemia. Ulyanovsk biomedical journal 3: 97-106.

16. Bon EI, Maksimovich SM, Karnyushko SM, Zimatkin MA, Lychkovskaya (2021) Disorders of Energy Metabolism in Neurons of the Cerebral Cortex During Cerebral Ischemia. Biomedical Journal of Scientific & Technical Research 40: 31932-31937p.

17. Taysi S (2019) Oxidative Nitrosative Stress and Preeclampsia. Mini Rev Med Chem 19(3): 178-193.

18. Robles-Murguia M, Rao D, Finkelstein D, Xu B, Fan Y et al. (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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