Short Communication

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2023.51.008044

# Changes in Amino Acid Pool in Rats with Brain Ischemia

**EI Bon*, N Ye Maksimovich, EM Doroshenko, VY Smirnov, E Moros and S Holik**

*Grodno State Medical University, Republic of Belarus*

***Corresponding author:** Bon EI, Grodno State Medical University, Grodno, Republic of Belarus

**ARTICLE INFO**

**Received:** June 08, 2023

**Published:** June 14, 2023

**Citation:** EI Bon, N Ye Maksimovich, EM Doroshenko, VY Smirnov, E Moros and S Holik. Changes in Amino Acid Pool in Rats with Brain Ischemia. Biomed J Sci & Tech Res 51(1)-2023. BJSTR. MS.ID.008044.

**ABSTRACT**

In all types of ischemia, changes in the pool of amino acids were observed in both studied structures. An increase in the content of L-arginine was noted in patients with ischemia of varying severity, the most pronounced in ischemia (by 39%, $p<0.05$). Also, with all types of ischemia, there was an increase in glycine, while only with IHM, an increase in the level of aspartate was noted, $p<0.05$. Along with changes in neurotransmitters, in the subtotal ischemia and partial ischemia groups, a decrease in the content of methionine was noted ($p<0.05$), while in total ischemia, on the contrary, an increase in the content of this amino acid was noted ($p<0.05$). In addition, with total ischemia, an increase in the content of threonine and tryptophan was noted. Changes in the pool of amino acids in rats with stepwise subtotal ischemia with intervals between arteria dressings on days 1 and 3 were the closest to changes in the subtotal ischemia group. So, in the 2nd pg. with an interval between dressings of the arteria for 3 days, the decrease in methionine and cysteine was the same as with subtotal ischemia, and there were no significant differences in the content of other amino acids. In the 3rd pg., with an interval between common carotid artery dressings of 1 day, as in subtotal ischemia, there was a decrease in the content of methionine, but, compared with subtotal ischemia, a decrease in cysteine was observed in parietal cortex ($p<0.05$). Changes in the pool of amino acids in the parietal lobe and hippocampus were of a similar nature, however, with stepwise subtotal ischemia, with an interval between dressings of both common carotid artery of 7 days, a decrease in cysteine occurred in the parietal cortex, and with partial ischemia, a more significant decrease in the level of methionine.

**Keywords:** Amino Acid; Neurons; Ischemia

## Introduction

As is known, one of the directions for detailing the mechanisms of development of damage and adaptation of the brain during its ischemia is the study of changes in the pool of amino acids [1,2]. Amino acids and their derivatives play an important role in the functioning of the brain, both in normal and pathological conditions, participating in the biosynthesis of membrane and signal protein and peptide molecules, some lipids, vitamins, hormones, and biogenic amines, and are also directly involved in the implementation of neurotransmitter functions, regulation of the activity of excitation and inhibition processes (glutamate, aspartate, GABA) [3-6]. Aromatic amino acids (phenylalanine, tyrosine, tryptophan, histidine) are of particular importance as precursors of catecholamines and serotonin [7,8]. The study of the pool of amino acids is important for detailing the mechanisms of brain damage in cerebral ischemia [9,10].

## Methods

The study of the pool of amino acids was carried out in prepared homogenates of the studied structures of the brain of experimental animals, isolated 1 hour after ischemia modeling. To do this, after removing the brain, a fragment of the parietal cortex and hippocampus was taken, followed by freezing in liquid nitrogen. Preparation of samples for the study included homogenization in a 10-fold volume of 0.2 M perchloric acid, centrifugation for 15 min. at 13000 g at 4°C, followed by collection of the supernatant. Amino acids were analyzed by reversed-phase chromatography with pre-column derivatization with o-thioaldehyde and 3-mercaptopropionic acid in Na-borate buffer on an Agilent 1100 chromatograph [11]. To prevent a systematic measurement error, brain samples from the compared control and experimental groups of animals were studied under the same conditions. Statistical processing. As a result of the research,

quantitative continuous data were obtained. Since the experiment used small samples that had a non-normal distribution, the analysis was performed by nonparametric statistics using the licensed computer program Statistical 10.0 for Windows (Stat Soft, Inc., USA). The data are presented as Me (LQ; UQ), where Me is the median, LQ is the value of the lower quartile; UQ is the value of the upper quartile. When the applicability conditions were met (normality of the samples and homogeneity of the variances), parametric analysis of variance was used with a posteriori comparison of the selected contrasts.

## Results

Changes in the pool of amino acids in rats with ischemic brain injury of varying severity were studied with partial (one-sided ligation of the common carotid artery, CCA), subtotal (simultaneous bilateral ligation of both CCAs), stepwise subtotal (alternate ligation of both CCAs at different time intervals) and total (complete cessation of cerebral blood flow) cerebral ischemia. In partial cerebral ischemia (PCI), the following changes in the pool of amino acids (AA) were noted: an increase in the level of glutamate and GABA without changing the ratio of the content of excitatory and inhibitory amino acid transmitters, an increase in the content of L-arginine, a decrease in the level of essential AAs with an increase in the "Nonessential/ Essential" AA ratio, as a reflection of the increased utilization of essential AAs. Changes in sulfur-containing AAs (cysteine, cystathionine, taurine, cysteine sulfonic acid) were absent, except for a decrease in the content of methionine in the parietal lobe, which indicates minor violations of the prooxidant-oxidant balance in this model of CI [11-14]. There was a decrease in the content of branched hydrocarbon amino acids (BHAAs) and a trend towards a decrease in the level of aromatic AAs (tyrosine, tryptophan, phenylalanine), with a decrease in their ratio, as a reflection of a more pronounced utilization of BHAAs, compared with aromatic AAs.

As shown earlier, PCI was accompanied by minimal changes in energy and prooxidant-antioxidant balance and a slight neurological deficit in terms of motor activity [15-18]. In subtotal cerebral ischemia (SCI), there was a decrease in the content of sulfur-containing AAs, with a decrease, in contrast to SCI, not only in methionine, but also in cysteate, as a reflection of a higher activity of oxidative stress [19,20]. Along with this, in SCI, as in PCI, 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 AA with the properties of excitatory neurotransmitters, as well as tryptophan, valine and leucine, were noted. At the same time, in contrast to PCI, there was no increase in the level of glutamate and a decrease in the levels of AA in the branched chain amino acids group. The revealed changes in the AA pool in the brain structures of rats with subtotal ischemia, modeled by simultaneous ligation of both CCAs, were accompanied by pronounced morphological changes in PL and Hp [18], high activity of oxidative stress [17], and the development of energy deficiency [21].

The introduction of Omega-3 fats at a dose of 5 g/kg of body weight for a week to rats with SCI did not have a corrective effect on the level of taurine, methionine, L-arginine and lysine, the change of which occurred in SCI, however, mythological studies revealed the presence of corrective properties on structural level [18], which is caused by the presence of Vaso protective and anti-inflammatory effects [22-24]. In rats with stepped cerebral ischemia (SSCI) with different intervals between ligation of both common carotid arteries (CCA), changes in the AA content were as follows. In the SSCI subgroup with the longest interval between CCA dressings of 7 days (subgroup 1), there was a decrease in the content of BHAAs - valine and leucine, an increase in the content of tryptophan, methionine, L-arginine, and glycine. As in the case of PCI, in rats of the 1st subgroup of SSCI, there was an increase in the content of L-arginine, a decrease in the content of amino acids with a branched hydrocarbon chain, however, in contrast to PCI, there was an increase in the content of valine, leucine and tryptophan, but there was no change in the content of sulfur-containing compounds. In PCI and SCI there was a decrease in the level of methionine. When compared with SCI, in rats of the 1st subgroup of SSCI, as in SCI, there was an increase in glycine ($p<0.05$), however, in contrast to SCI, there was a decrease in the content of cysteine and methionine ($p<0.05$). In the subgroup SSCI with an interval between CCA dressings of 3 days (subgroup 2), as in SSCI with an interval between CCA dressings of 7 days, PCI and SCI, there was an increase in the content of L-arginine, a decrease in the content of methionine [25].

However, in contrast to the 1st subgroup of SSCI and from other groups of CIS (PCI and SCI), in the 2nd subgroup of SSCI there was an increase in the content of taurine and nonessential AA - asparagine and alanine. As with PCI, there was an increase in the content of glutamate, and, as with SCI, a decrease in the level of cysteine, but, compared with PCI, there was a decrease in the content of asparagine and an increase in the level of alanine [26-28]. Changes in the 3rd subgroup of SSCI with the shortest interval between CCA dressings - 1 day (subgroup 3) manifested themselves as an increase in the content of tyrosine, L-arginine and citrulline, taurine, asparagine, and alanine, as well as a decrease in the content of ornithine and methionine [29]. Compared with the 1st subgroup of SSCI, in this subgroup there was a decrease in the content of methionine and lysine, as well as an increase in the content of asparagine, alanine, L-arginine, ornithine and citrulline. Compared to the 2nd subgroup of SSCI, there was a significant increase in the level of asparagine, lysine, tyrosine, arginine and citrulline. The nature of the shifts in the AA pool in the 2nd and 3rd subgroups of SSCI was of the same type, except for an increase in the level of citrulline in the 3rd subgroup, which indicates a more significant decrease in the synthesis of nitric oxide in this subgroup of SSCI.

In the 3rd subgroup of SSCI, similarly to the group "PCI", there was a decrease in the content of methionine, but an increase in the content of several AAs - asparagine, alanine, lysine, and tyrosine

was observed [30]. In addition, similarly to the SCI group, there was a decrease in the content of methionine, but, compared with SCI, a decrease in the content of cysteate was observed in the PL [31]. When comparing the changes in SSCI in 3 subgroups with different intervals between CCA ligations, a similar pattern of changes in the AA pool was revealed, which manifested itself in an increase in the content of L-arginine, which was also noted in rats with PCI and SCI [32]. However, changes in other AAs in in the 3rd subgroup, SSCI with a minimum interval between ligation of both common carotid arteries, 1 day was the most pronounced and included an increase in the content of tyrosine, citrulline, taurine, asparagine, and alanine, as well as a decrease in the content of ornithine and methionine, as a reflection of the ineffectiveness of compensatory mechanisms in this model of CI. Changes in the pool of amino acids in the parietal lobe and hippocampus were framed along the same lines, however, with SSCI with an interval between ligation of both common carotid arteries of 7 days, a decrease in cysteate occurred in the PL, and a more significant decrease in the level of methionine than in HP [33]. In previous studies, it was found that SSCI with an interval between CCA dressings of 7 days is manifested by a slight difference in the size of neuronal perikaryons and the ratio of neurons in terms of the degree of cytoplasmic achromatophilia, energy exchange indicators and prooxidant-antioxidant balance from those in the group with PCI, which can be explained by the inclusion of compensatory mechanisms that prevented the development of significant disorders [34].

SSCI with an interval of 1 and 3 days between dressings of both CCAs leads to damage to neurons, which manifests itself in a decrease in their size, deformation of perikaryons, an increase in the number of shriveled neurons and shadow cells, a significant disorganization of neuronal organelles, inhibition of energy exchange and activation of oxidative stress [15-17]. In total cerebral ischemia (TCI), the studied brain structures showed an increase in the content of aromatic AAs of tyrosine and tryptophan. The content of methionine also increased, in contrast to SCI and PCI, in which the content of methionine decreased, which may be due to the lack of activation of oxidative processes in this form of cerebral ischemia. Along with this, with TCI, as with SCI, PCI, and all types of SSCI, an increase in the content of L-arginine was noted, a tendency to an increase in the content of the inhibitory neurotransmitter glycine [35-37]. Also, when modeling TCI, the most pronounced morpho functional disorders were revealed: shriveled and disturbances in the ultrastructure of neurons, depression of mitochondrial respiration, and suppression of antioxidant protection [16-39]. The amount of research carried out fully reveals the tasks set for studying changes in the pool of amino acids in rats with cerebral ischemia of varying severity.

## References

1. Gusev EI, Skvortsova VI (2001) Cerebral ischemia. M Medicine 327.
2. H Shimizu, SH Graham, LH Chang, J Mintorovitch, TL James, 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.
3. Li P, Wu G (2020) Composition of amino acids and related nitrogenous nutrients in feedstuffs for animal diets. Amino Acids 52(4): 523-542.
4. Lili Liang, Jun Ye, Lianshu Han, Wenjuan Qiu, Huiwen Zhang, et al. (2020) Examining the blood amino acid status in pretherapeutic patients with hyperphenylalaninemia. J Clin Lab Anal 34( 3): 23-29.
5. Swider O, Roszko M, Szymczyk K, Michał Wójcicki (2020) Biogenic Amines and Free Amino Acids in Traditional Fermented Vegetables-Dietary Risk Evaluation. J Agric Food Chem 68(3): 856-868.
6. M Erecińska, D Nelson, D F Wilson, I A Silver (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.
7. Choi S, Disilvio B, Fernstrom MH, Fernstrom JD (2009) Meal ingestion, amino acids and brain neurotransmitters: effects of dietary protein source on serotonin and catecholamine synthesis rates. Physiol Behav 98(1-2): 156-162.
8. Sujean Choi, Briana Disilvio, Madelyn H Ferns Trom, John D Ferns Trom (2013) Oral branched-chain amino acid supplements that reduce brain serotonin during exercise in rats also lower brain catecholamines. Amino Acids 45(5): 1133-1142.
9. Imai K, S Uzu, T Toyo'oka (1990) Fluorogenic reagents, having benzofurazan structure, in liquid chromatography. J Pharm Biomed Anal 7(12): 1395-1403.
10. Fernstrom JD (2013) Large neutral amino acids: dietary effects on brain neurochemistry and function. Amino Acids 45(3): 419-430.
11. Yu E Razvodovsky (2020) Influence of combined administration of L-arginine and N-nitro-L-arginine methyl ether on the fund of amino acids and biogenic amines of the hippocampus of rats with subtotal cerebral ischemia. Bulletin of the Smolensk State Medical Academy 3: 5-10.
12. Stephanie L Gage, Samantha Calle, Natalia Jacobson, Mark Carroll, Gloria DeGrandis-Hoffman, et al. (2020) Pollen Alters Amino Acid Levels in the Honeybee Brain and This Relationship Changes with Age and Parasitic Stress. Front Neurosci 14: 231-239.
13. Polis B, Samson AO (2020) Role of the metabolism of branched-chain amino acids in the development of Alzheimer's disease and other metabolic disorders. Neural Regen Res 15( 8): 1460-1470.
14. Ana I Rey, Almudena de-Cara, Luis Calvo, Patricia Puig, Teresa Hechavarría, 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): 45-52.
15. Bon EI, Maksimovich NE, Karnyushko SM, Zimatkin SM, Lychkovskaya, et al. (2021) Disorders of Energy Metabolism in Neurons of the Cerebral Cortex During Cerebral Ischemia. Biomedical Journal of Scientific & Technical Research 40(1): 31932-31937.
16. EI Bon, Maksimovich N Ye, Dremza IK, Lychkovskaya (2021) Experimental Cerebral Ischemia Causes Disturbances in Mitochondrial Respiration of Neurons. Biomedical Journal of Scientific Technical Research 40(4): 32387-32392.
17. EI Bon (2022) Characteristics of disorders of the prooxidant-oxidant balance in rats with cerebral ischemia. Ulyanovsk biomedical journal 3: 97-106.

18. Maksimovich NE , Bon EI , Zimatkin SM (2020) The rat brain and its response to ischemia: monograph. Grodno: GrGMU 240.
19. Gilfix BM, DW Blank, DS Rosenblatt (1997) Novel reductant for determination of Total Plasma Homocysteine. Clinical Chemistry 43(4): 687-698.
20. Chowdhury VS (2019) Heat Stress Biomarker Amino Acids and Neuropeptide Afford Thermotolerance in Chicks. J Poult Sci 56(1): 1-11.
21. EI Bon (2022) Respiration of mitochondria of brain neurons in rats with cerebral ischemia of varying severity. Ulyanovsk biomedical journal 2: 128-138.
22. Niemoller TD, Stark DT, Bazan NG (2009) Omega-3 fatty acid docosahexaenoic acid is the precursor of neuroprotectin D1 in the nervous system. World Review of Nutrition and Diet 99: 46-54.
23. Hung-Chuan Pan, Tsung-Kuei Kao, Yen-Chuan Ou, Dar-Yu Yang, Yu-Ju Yen, et al. (2009) Protective effect of docosahexaenoic acid against brain injury in ischemic rats. J Nutrition and Biochemistry 20(9): 715-725.
24. EI Bon (2022) Correction by ω-3 polyunsaturated acids of disturbances in energy processes in the brain tissues of rats with subtotal cerebral ischemia. Science and innovation 5(231): 78-83.
25. Ludmila Belayev, Larissa Khoutorova, Kristal D Atkins, Nicolas G Bazan (2009) Robust Docosahexaenoic Acid–Mediated Neuroprotection in a Rat Model of Transient. Focal Cerebral Ischemia. Stroke 40(9): 3121-3126.
26. Maricela Robles-Murguia, Deepti Rao, David Finkelstein, Beisi Xu, Yiping Fan, et al. (2020) Muscle-derived Dpp regulates feeding initiation via endocrine modulation of brain dopamine biosynthesis. Genes Dev 34(1-2): 37-52.
27. V Nechiporuk, A Melnyk, M Korda, N Pentiuk, S Kachula, et al. (2019) Influence of chronic hyperhomocysteinemia on metabolism of sulfur containing amino acids in the rats' heart and brain on the background of hyperthyreosis and hypothyreosis. Georgian Med News 29(5): 127-132.
28. Zablocka B, Domanska-Janik K (1996) Enhancemen of 3[H]D-aspartate release during ischemia like conditions in rat hippocampal slices: source of excitatory amino acids. Acta Neurobiologiae Experimentalis 56(1): 63-70.
29. SuJean Choi, Briana DiSilvio, Madelyn H Ferns Trom, John D Ferns Trom (2011) Effect of chronic protein ingestion on tyrosine and tryptophan levels and catecholamine and serotonin synthesis in rat brain. Nutr Neurosci 14(6): 260-267.
30. Yu E Razvodovsky (2019) Influence of blockade of nitrogen monoxide synthesis by N-nitro-L-arginine methyl ester (L-NAME) on the content of free amino acids and biogenic amines in the cerebral cortex of rats with subtotal cerebral ischemia. National Academy of Sciences of Belarus News Medical science series 16(3): 291-297.
31. Bowsher R, Henry DP (2020) Purification, characterization and identification of rat brain cytosolic tyrosine transaminase as glutamine Transaminase-K Neurochem. Int 133: 104-110.
32. Yu E Razvodovsky (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 18(1): 5-9.
33. Ludmila Belayev, Larissa Khoutorova, Kristal D Atkins, Tiffany N Eady, Song Hong, et al. (2011) Docosahexaenoic Acid Therapy of Experimental Ischemic Stroke. Translational Stroke Research 2(1): 33-41.
34. EI Bon, N Ye Maksimovich, IK Dremza, NV Kokhan, IN Burak, et al. (2022) Severity of Oxidative Stress in Stepwise Cerebral Ischemia. Advance In Medical and Clinical Research 2(1): 1-3.
35. Minghui Wang, Ao Niu, Zhibo Gong, Zhengwen Xu, Lili Li, et al. (2020) PEG-amino acid-przewaquinone a conjugation: Synthesis, physicochemical properties, and protective effect in a rat model of brain ischemia-reperfusion. Bioorg Med Chem Lett 30(1): 110-121.
36. Slivka AP, Murphy EJ (2001) High-dose methylprednisolone treatment in experimental focal cerebral ischemia. Exp Neurol 167(1): 166-172.
37. EI Bon (2021) Changes in the content of ATP synthase in neurons of the parietal cortex and hippocampus of rats with cerebral ischemia. News of biomedical sciences 21(4): 121-125.
38. EI Bon (2022) Violations of the Ultrastructure of Neurons in The Cerebral Cortex of Rats with Total Cerebral Ischemia. International Journal of Stem cells and Medicine1(2): 1-9.
39. EI Bon, Maksimovich N Ye, Zimatkin SM, Karnyushko OA, Bakush UA, et al. (2023) Effects of Neuroglobin in Cerebral Ischemia and Introduction of Omega-3 Polyunsaturated Fatty Acids. J of Psychiatry and Psychological Disorders 1(2): 1-8.


**ISSN: 2574-1241**

DOI: 10.26717/BJSTR.2023.51.008044

**Bon EI.** Biomed J Sci & Tech Res