

CHANGES IN THE AMINO ACID POOL OF CEREBRAL HEMISPHERES  
IN RATS WITH TOTAL CEREBRAL ISCHEMIAE. I. Bon<sup>1</sup>, N. E. Maksimovich<sup>1</sup>, V. Yu. Smirnov<sup>1</sup>, E. M. Doroshenko<sup>1</sup>, Yu. E. Razvodovsky<sup>2</sup>, E. V. Moroz<sup>1</sup>, E. I. Vishnevskaya<sup>1</sup>, N. V. Kokhan<sup>1</sup><sup>1</sup>Grodno State Medical University, Grodno, Belarus<sup>2</sup>Institute of Biochemistry of Biologically Active Compounds of the Belarus National Academy of Sciences, Grodno, Belarus**For citation:** Bon EI, Maksimovich NE, Smirnov VYu, Doroshenko EM, Razvodovsky YuE, Moroz EV, Vishnevskaya EI, Kokhan NV. Changes in the amino acid pool of cerebral hemispheres in rats with total cerebral ischemia. *Saratov Journal of Medical Scientific Research*. 2023; 19 (4): 395–398. EDN: BMNILM. DOI: <https://doi.org/10.15275/ssmj1904395> (In English)**Abstract:** *Objective:* assessment of changes in the pool of amino acids (AA) in rats with total cerebral ischemia (TCI). *Material and Methods.* Experiments were performed on 16 male outbred white rats weighing 260±20 g. TCI was modeled by decapitation of animals. Brain tissue was sampled 1 hour after decapitation. *Results.* In the parietal lobe (PL) and hippocampus (HC) of TCI group animals 1 hour after their decapitation, we detected an increase in the content of tyrosine (by 43%,  $p=0.044$ , and 40%,  $p=0.044$ , respectively) and tryptophan (by 24%,  $p=0.036$ , and 23%,  $p=0.046$ , respectively). Similar trend was observed for methionine that increased by 32% in PL ( $p=0.046$ ) and by 27% in HC ( $p=0.046$ ). Analogous increase in the content of L-arginine was noted in PL and HC (by 20%,  $p=0.037$ , and 33%,  $p=0.037$ , correspondingly). Isoleucine content increased by 12% in PL ( $p=0.054$ ), while valine content decreased by 15% in HC ( $p=0.053$ ). The ratio of the combined total content of branched-chain amino acids (BCAA) to the combined content of aromatic AA in TCI significantly declined from 1.4 to 1.0 in PL ( $p=0.053$ ) and from 1.6 to 1.0 in GC ( $p=0.053$ ). We observed an increase in methionine content by 33% ( $p=0.046$ ) in PL and an increase in tryptophan content by 24% ( $p=0.046$ ) in HC. *Conclusion.* One-hour TCI caused the following changes in the AA pool: an increase in the content of aromatic AA (tyrosine and tryptophan) and methionine; an increase in the content of L-arginine; and also, an increase in the concentration of the inhibitory neurotransmitter glycine.**Keywords:** total cerebral ischemia, incomplete cerebral ischemia, amino acid pool, brain homogenates**Introduction.** Amino acids (AA) are important in metabolism and brain function. This is due not only to their crucial role as the sources of synthesis of various biologically important compounds (proteins, mediators, lipids, biologically active amines), but also to their involvement in synaptic transmission as neurotransmitters and neuromodulators (glutamate, aspartate, glycine, GABA, taurine). Some AA are in charge of the formation of nervous system mediators: e. g., methionine (acetylcholine, DOPA, dopamine), tyrosine (catecholamines), serine and cysteine (taurine), tryptophan (serotonin), histidine (histamine), L-arginine (NO), glutamic acid (glutamate) [1–3].

Major cerebral circulatory disorders, which are modeled by total cerebral ischemia (TCI), lead to severe brain dysfunction. TCI affects the normal ratio of AA playing an important role in the functioning of the nervous system. AA and their compositions can act as therapeutic agents for cerebral ischemia. To develop new AA preparations, it is necessary to study in detail how their ratios and

quantities change during cerebral ischemia of varying severity, including TCI [4–7].

The *objective* of our study was to assess changes in the pool of AA in rats with TCI.**Material and methods.** The experiments were performed on 16 outbred white male rats weighing 260±20 g in compliance with the requirements of the Directive of the European Parliament and Council #2010/63/EC of 22 September 2010 on the protection of animals used for scientific purposes (Protocol #1 of 5 January 2022).

The simulation was carried out under intravenous thiopental anesthesia (40–50 mg/kg). TCI was modeled by decapitation of animals. Brain samples were collected 1 hour after decapitation. After this time, according to the results of our previous studies, significant histological disorders of neurons occur (wrinkling and hyperchromia) [4–7]. At the same time, it was of interest to determine changes in the AA pool corresponding to these disorders. This model was developed by the authors.

The control group consisted of decapitated rats; their heads were immediately immersed in liquid nitrogen, and the AA composition of the brain was examined after short-term cooling.

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**Method for studying the amino acid pool of the brain.** After the brain was removed, fragments of the parietal lobe cortex and hippocampus were sampled and frozen 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 minutes at 13,000 g at 4°C, followed by collection of the supernatant. AA were analyzed by reverse-phase chromatography with pre-column derivatization using o-phthalaldehyde and 3-mercaptopropionic acid in sodium borate buffer on an Agilent 1100 chromatograph.

To avoid systematic measurement errors, we examined brain samples from the compared control and experimental groups of animals under the same conditions.

Continuous data were obtained. Since our experiment involved small samples following a non-normal distribution, the analysis was performed by nonparametric statistics using the licensed software STATISTICA 10.0 for Windows (StatSoft, Inc., USA). All data are presented as *Me* (LQ; UQ), where *Me* is the median, LQ is the lower quartile value, and UQ is the upper quartile value. Differences between groups were considered significant at  $p=0.046$  (non-parametric Games-Howell post-hoc test).

**Results.** The main pool of AA in the cerebral cortex was characterized by the parameters identified for the control group (Table).

Compared with the values characteristic for the control group, 1 hour after decapitation, the TCI group animals exhibited in their parietal lobe (PL) and

**Amino acid pool in cerebral hemispheres of rats with total cerebral ischemia (TCI), nmol/g; Me (LQ/UQ)**

amino acids	Parietal lobe		Hippocampus	
	group of animals		group of animals	
	control	TCI	control	TCI
<b>Neurotransmitters</b>				
Glycine	176 (161/201)	206 (187/223)	174 (150/190)	199 (193/251)
Glutamate	3.137 (3.040/3.277)	3.427 (3.349/3.619)	3.375 (3.146/3.574)	3.224 (3.018/3.527)
Aspartate	1.653 (1.501/1.820)	1.696 (1.524/1.862)	1.603 (1.351/1.768)	1.407 (1.357/1.608)
Taurine	1.035 (909/1.120)	1.165 (1.044/1.283)	1.032 (983/1.125)	1.147 (673/1.226)
GABA	481 (446/534)	930 (851/1.016) *	523 (485/665)	1.235 (860/1.313) *
<b>Endogenous antagonist of NMDA receptors</b>				
A-aminoadipic acid	21.5 (20.2/24)	26.6 (21.7/29.5)	13 (11.5/14.1)	9.19 (6.36/10.9)
<b>Sulfur-containing</b>				
Cysteine	1.66 (0.767/2.16)	1.67 (1.57/2.25)	1.03 (0.278/1.69)	1.55 (1.24/1.8)
Cystathionine	36.7 (30.3/40.2)	35.7 (28.3/38.7)	37.7 (34.7/40.8)	56.7 (31.5/82.7)
Taurine	1.035 (909/1.120)	1.165 (1.044/1.283)	1.032 (983/1.125)	1.147 (673/1.226)
Methionine	16.7 (15.6/20.3)	25 (23.6/26.6) *	19.3 (17.9/23.4)	25.7 (23.3/26.6)
Cysteine sulfinic acid	1.27 (0.757/3.07)	3.03 (1.9/4.69)	2.56 (1.24/4.05)	1.98 (1.81/4.4)
<b>Glycogen amino acids</b>				
Aspartate	1.653 (1.501/1.820)	1.696 (1.524/1.862)	1.603 (1.351/1.768)	1.407 (1.357/1.608)
Asparagine	92.3 (87.8/98.1)	110 (104/119)	101 (92.5/105)	120 (109/126)
Threonine	330 (282/443)	367 (334/402)	425 (345/567)	371 (350/391)
Serine	566 (535/580)	598 (554/649)	516 (496/552)	518 (360/535)
Glutamine	1.937 (1.600/2.084)	2.221 (1.966/2.293)	1.981 (1.831/2.172)	2.069 (1.993/2.153)
Glutamate	3.137 (3.040/3.277)	3.427 (3.349/3.619)	3.375 (3.146/3.574)	3.224 (3.018/3.527)
Glycine	176 (161/201)	206 (187/223)	174 (150/190)	199 (193/251)
Alanine	264 (251/276)	443 (431/461) *	318 (297/334)	480 (423/498) *
Valine	61.1 (54.8/78.3)	65.4 (60.8/67)	74.9 (70.8/79.1)	63.7 (60.8/67.5)
Methionine	16.7 (15.6/20.3)	25 (23.6/26.6) *	19.3 (17.9/23.4)	25.7 (23.3/26.6)
Histidine	19.9 (16.2/21.4)	19.7 (17.8/23.9)	17.7 (16.3/19)	16.9 (14.8/17.5)
Arginine	32.1 (30.5/33.5)	39.7 (38/45.3) *	27.8 (21.2/32.4)	41.9 (38.2/47.6) *
<b>Ketogenic amino acids</b>				
Lysine	160 (129/191)	134 (122/147)	227 (179/259)	195 (168/277)
Leucine	56.7 (49.8/67.2)	60 (57.4/62.4)	68.2 (64.8/72)	62.9 (61.2/64.8)
<b>Histidine derivatives</b>				
3-methylhistidine	5.57 (4.96/5.9)	6.54 (5.41/6.71)	4.65 (4.37/5.84)	5.43 (4.68/5.99)

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amino acids	Parietal lobe		Hippocampus	
	group of animals		group of animals	
	control	TCI	control	TCI
<b>Nonessential amino acids</b>				
Glycine	176 (161/201)	206 (187/223)	174 (150/190)	199 (193/251)
Alanine	264 (251/276)	443 (431/461) *	318 (297/334)	480 (423/498) *
Glutamine	1.937 (1.600/2.084)	2.221 (1.966/2.293)	1.981 (1.831/2.172)	2.069 (1.993/2.153)
Glutamate	3.137 (3.040/3.277)	3.427 (3.349/3.619)	3.375 (3.146/3.574)	3.224 (3.018/3.527)
Aspartate	1.653 (1.501/1.820)	1.696 (1.524/1.862)	1.603 (1.351/1.768)	1.407 (1.357/1.608)
Asparagine	92.3 (87.8/98.1)	110 (104/119)	101 (92.5/105)	120 (109/126)
Serine	566 (535/580)	598 (554/649)	516 (496/552)	518 (360/535)
Tyrosine	46.2 (40.1/50.1)	81.2 (71.6/87) *	49.3 (44.6/50.2)	82 (78/90.6) *
Ornithine	10.6 (8.89/13.4)	11.5 (9.06/13.7)	11.2 (9.78/14.2)	13.9 (11.7/17.2)
<b>Essential amino acids</b>				
Valine	61.1 (54.8/78.3)	65.4 (60.8/67)	74.9 (70.8/79.1)	63.7 (60.8/67.5)
Isoleucine	29.7 (26.2/34.4)	34.3 (32.5/35.1)	33.2 (31.1/35.1)	32.3 (30.7/33.5)
Leucine	56.7 (49.8/67.2)	60 (57.4/62.4)	68.2 (64.8/72)	62.9 (61.2/64.8)
Methionine	16.7 (15.6/20.3)	25 (23.6/26.6) *	19.3 (17.9/23.4)	25.7 (23.3/26.6)
Lysine	160 (129/191)	134 (122/147)	227 (179/259)	195 (168/277)
Histidine	19.9 (16.2/21.4)	19.7 (17.8/23.9)	17.7 (16.3/19)	16.9 (14.8/17.5)
Threonine	330 (282/443)	367 (334/402)	425 (345/567)	371 (350/391)
Tryptophan	27.7 (24.6/32)	37.6 (32/40.4) *	29.8 (25.1/31.8)	39 (36.4/40.9) *
Phenylalanine	29.4 (24.6/33.7)	36.2 (34.4/38.2)	31.6 (26/39.2)	37.1 (36.2/42.4)
<b>Aromatic amino acids</b>				
Tyrosine	46.2 (40.1/50.1)	81.2 (71.6/87) *	49.3 (44.6/50.2)	82 (78/90.6) *
Tryptophan	27.7 (24.6/32)	37.6 (32/40.4) *	29.8 (25.1/31.8)	39 (36.4/40.9) *
Phenylalanine	29.4 (24.6/33.7)	36.2 (34.4/38.2)	31.6 (26/39.2)	37.1 (36.2/42.4)
<b>Branched-chain amino acids</b>				
Valine	61.1 (54.8/78.3)	65.4 (60.8/67)	74.9 (70.8/79.1)	63.7 (60.8/67.5)
Isoleucine	29.7 (26.2/34.4)	34.3 (32.5/35.1)	33.2 (31.1/35.1)	32.3 (30.7/33.5)
Leucine	56.7 (49.8/67.2)	60 (57.4/62.4)	68.2 (64.8/72)	62.9 (61.2/64.8)
<b>Ratios of amino acids</b>				
Branched-chain amino acids/aromatic amino acids	1.4 (1.4/1.6)	1.04 (0.979/1.08) *	1.56 (1.4/1.7)	1 (0.95/1.03) *
Nonessential/Essential	10.0 (9.5/10.9)	11.1 (10.8/11.6)	8.4 (7.5/9.8)	9.59 (8.71/10.8)
Glycogen/Ketogenic	40.1 (33.4/46.5)	46.4 (42.2/55.1)	28 (27/34.9)	31.9 (24.4/42.1)
Amino acid content	10.033 (8.904/10.741)	11.716 (11.056/12.182)	10.835 (9.734/11.603)	11.639 (10.720/12.050)

Note: \* —  $p=0.046$ , compared with the control group.

hippocampus (HC) samples the following trends: an increase in the content of aromatic AA (precursors of biogenic monoamines): tyrosine (by 43%,  $p=0.047$ , and 40%,  $p=0.047$ , respectively) and tryptophan (by 24%,  $p=0.049$ , and 23%,  $p=0.049$ , respectively).

Also, in TCI group vs. the control animals, the level of methionine increased by 32% in PL ( $p=0.046$ ) and by 27% in HC ( $p=0.056$ ). Methionine is a precursor of other sulfur-containing AA (serine, cystathionine, cysteine). In addition, we observed elevated levels of other sulfur-containing AA (cysteine, taurine), with the exception

of cystathionine in PL and cysteine sulfinic acid in HC. In the TCI group, we noted an increase in the content of L-arginine in both PL and HC (by 20%,  $p=0.038$  and 33%,  $p=0.038$ , correspondingly).

In contrast to the HC, in the PL of the TCI animals, the content of the citrulline, which is a precursor of L-arginine, increased by 22%,  $p=0.046$ .

As for the group of branched-chain amino acids (BCAA) (valine, isoleucine, leucine), the changes in TCI were multidirectional: specifically, we observed an increase in the isoleucine level by 12% in PL ( $p=0.046$ ) and

a decrease in valine content by 15% in HC ( $p=0.054$ ). Levels of other BCAA did not change ( $p=0.057$ ).

The ratio of the combined total content of BCAA to the combined content of aromatic AA in TCI significantly declined from 1.4 to 1.0 in PL ( $p=0.046$ ) and from 1.6 to 1.0 in GC ( $p=0.039$ ). We observed an increase in methionine content by 33% ( $p=0.046$ ) in PL and an increase in tryptophan content by 24% ( $p=0.046$ ) in HC.

The content of the inhibitory neurotransmitter glycine in TCI tended to increase in PL and HC (by 15% and 13%, respectively,  $p=0.049$ ), while the content of excitatory neurotransmitters (aspartate and glutamate) did not change ( $p=0.058$ ).

Changes in the content of essential amino acids (valine, isoleucine, leucine, methionine, lysine, histidine, threonine, tryptophan, phenylalanine) in TCI animals were multidirectional both in PL and HC. In PL, the methionine content increased by 33% ( $p=0.046$ ), and in HC, tryptophan level increased by 24% ( $p=0.047$ ).

However, the ratio of nonessential to essential AA in the TCI group did not change ( $p=0.053$ ).

**Discussion.** The increase in methionine content in TCI reflects the identified lack of activation of oxidative processes in this grade of cerebral ischemia [2, 3, 8–11].

An increase in the content of sulfur-containing AA echoes actively occurring transsulfuration and dioxygenase reactions in the context of the pathway of cysteine transformations, including the synthesis of taurine [2, 10].

An increase in the level of L-arginine during cerebral ischemia may be associated with the low activity of reactions of its utilization due to oxygen deficiency, among which the formation of nitrogen monoxide plays a significant role. In turn, NO performs mediator functions, participates in the regulation of cerebral blood flow as a vasodilator, and is capable of exhibiting antioxidant, antiplatelet and anti-inflammatory properties [7–9, 12, 13].

An increase in the level of L-arginine and citrulline reflects the active course of reactions associated with the cytoplasmic part of the ornithine cycle, with the exception of those involving arginine [2, 3, 7].

**Conclusion.** One-hour TCI caused the following changes in the AA pool: an increase in the content of aromatic AA (tyrosine and tryptophan) and methionine; an increase in the content of L-arginine; and also, an increase in the concentration of the inhibitory neurotransmitter glycine.

Changes in the AA pool in the PL and HC were similar, which made it possible to exclude a significant increase in methionine levels in the PL as a reflection of active oxidative effects in this part of the brain in combination with more pronounced morphological changes in the PL of rats with PCI.

The article was submitted on 06.05.2023; approved after review on 01.06.2023; accepted for publication on 24.11.2023.

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**Authors' contributions:** all authors gave equivalent contributions to the publication.

**Conflict of interest.** The authors declare no conflicts of interest.

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