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Research Article

The Effect of L-NAME on the Spectrum of Amino Acids and Biogenic Amines in Hippocampus and Brain Cortex During Subtotal Cerebral Ischemia

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Abstract

The Aim of this Study: The aim of this study was to estimate the changes in the pool of free amino acids and biogenic amines in hippocampus and brain cortex of rats undergoing subtotal cerebral ischemia (SCI) and treated with L-NAME.

Methods: The experiments were carried out on 18 white outbred female rats (6 animals in each group), weighing 180 - 220g. Subtotal cerebral ischemia (SCI) was simulated in the rats of the experimental groups by ligation of both carotid arteries for one hour. L-NAME was injected intravenously at a dose of 5 mg/kg immediately before ligation of the common carotid artery. The spectrum of compounds determined included proteinogenic amino acids, ornithine, citrulline, a number of related compounds (taurine, α-aminobutyrate, etc.), and biogenic amines. The analysis was carried out on an Agilent 1100 chromatograph by reverse phase chromatography with precolumn derivatization with o-phthalic aldehyde and 3-mercaptopropionic acid in Na-borate buffer.

Results: Subtotal cerebral ischemia induced imbalance in the pool of amino acids and their derivates in hippocampus and brain cortex. The effect of SCI on the pool of free AA and neuroactive compounds in the studied sections of the rats brain is basically the same, however, there is a group of compounds, including aspartate, ornithine, and GABA, for which the nature of the effect is specific for the hippocampus and the brain cortex. Preventive administration of L-NAME partially prevents this imbalance; however induce the changes in the levels of bran chain amino acids (BCAA), arginine, gistidine, treonine and taurine.

Keywords: Amino Acids; Biogenic Amines; Hippocampus; Brain Cortex; Subtotal Cerebral Ischemia; L-NAME

Introduction

Stroke is one of the leading causes of disability and death in many countries of the world [6]. The high mortality rate from ischemic stroke is largely due to insufficient knowledge of its pathogenesis [5,7]. Therefore, one of the tasks of experimental medicine is to elucidate and detail the mechanisms of ischemic and reperfusion injuries of the brain.

Studies carried out with the use of inhibitors of various NO synthase isoforms indicate the important role of nitrogen monoxide (NO) in the pathogenesis of ischemic brain damage [5,8]. One of the directions of detailing the pathogenetic mechanisms of ischemic stroke is the study of changes in the pool of amino acids and their derivatives in various parts of the brain [2-4,8].

The aim of this study was to characterize the changes in the pool of amino acids and biogenic amines in the hippocampus and cerebral hemispheres of rats in subtotal cerebral ischemia with L-NAME management.

Methods

The experiments were carried out on 18 white outbred female rats (6 animals in each group), weighing 180-220g. Subtotal cerebral ischemia (SCI) was simulated in the rats of the experimental groups by ligation of both carotid arteries for one hour. L-NAME was injected intravenously at a dose of 5 mg/kg immediately before ligation of the common carotid artery. The control group consisted of sham-operated animals that received an equal volume of isotonic NaCl solution. All surgical manipulations were performed under conditions of intravenous thiopental anesthesia (60 mg/kg). After extraction of the brain, a fragment of the hippocampus and a fragment of the frontal lobe of the cerebral hemispheres (cortex with the underlying white matter) on the side of the ligation were removed, followed by freezing in liquid nitrogen. Sample preparation for the study included homogenization in a 10-fold volume of 0.2M perchloric acid, centrifugation for 15 min at 13000 g at 4 ° C, followed by collection of the supernatant.

The spectrum of compounds determined included proteinogenic amino acids, ornithine, citrulline, a number of related compounds (taurine, α-aminobutyrate, etc.), and biogenic amines. The analysis was carried out on an Agilent 1100 chromatograph by reverse phase chromatography with precolumn derivatization with o-phthalic aldehyde and 3-mercaptopropionic acid in Na-borate buffer. Photometric detection at a wavelength of 338 nm (for the determination of AA) and fluorometric (276/345 nm) (for biogenic amines). A Zorbax Eclipse Plus C18 column, 3.5 μm, 2.1 x 150 mm, was used. Identification and quantitative analysis were performed using the Agilent Chem Station B.04.01 software; the method was calibrated using a standard mixture of amino acids from Sigma-Aldridge. Used mobile phases: 0.1 M Na-acetate buffer (pH 6.25 and 5.75); aqueous solutions of acetonitrile and methanol (60% v/v). Separation was carried out with gradient elution in 78 min; column temperature 34 ° C. For the separation of biogenic amines, a mobile phase was used: 0.05 M NaH2PO4, 0.024 M CH3COOH, 480 mg/L sodium octyl sulfonate, 1.5 ml/L dibutyl amine, 7% acetonitrile (vol.). We used reagents of at least reagent grade. Tridistilled water for mobile phases was passed through a Norganic cartridge (Millipore, USA), mobile phases were filtered through a 0.22 μm membrane filter [1].

Statistical processing of the data was carried out using the R program. To assess the influence of factors, a parametric analysis of variance (ANOVA) was used with the use of Tukey's correction for a posteriori comparison of indicators. If the conditions for the applicability of the parametric DA were not met, the nonparametric permutation test ezPerm from the ez package was used. Comparison of the influence of factors in the two studied GM departments was performed using DA using a contrast matrix for a posteriori comparison of groups (glht procedure from the multcomp package). The methods of correlation and discriminant analysis were also used.

Results and Discussion

Subtotal ischemia caused a change in the pool of amino acids and their derivatives in the brain cortex: there was an increase in the levels of aspartate, β -alanine, valine and leucine, a decrease in glutamate, asparagine, threonine, GABA, and tyrosine (Figure 1). The changes mainly concerned essential and neurotransmitter amino acids (Figure 2) and contributed to the depletion of the total pool of free amino acids in the GM cortex. Analysis of the integral indicators of the amino acid pool also revealed an increase in the ratio of the total concentration of branched chain amino acids (ARUC) and AAA (Figure 3), due to both an increase in ARUC concentrations and a decrease in AAA. A decrease in 5-HIAA in the cortex (Figure 4), the main metabolite of tryptophan, indicates inhibition of serotonin degradation pathways. The reason for this may be the development of a deficiency in the pool of aromatic amino acids (this is indirectly evidenced by a decrease in the level of tyrosine). The latter, in turn, may be caused by a disruption in the processes of AAC transport across the blood-brain barrier in SCI, which is confirmed by the growth of BCAA in the brain cortex, which is the main competitor of AAA for the general transport system.

The introduction of L-NAME, despite the normalization of the majority levels of compounds (aspartate, glutamate, asparagine, methionine, GABA, β -alanine, 5-HIAA) (Figure 1 to 4), induced an amino acid imbalance in the brain cortex, manifested in a decrease in levels of glutamine, histidine, taurine, tryptophan, phenylala-

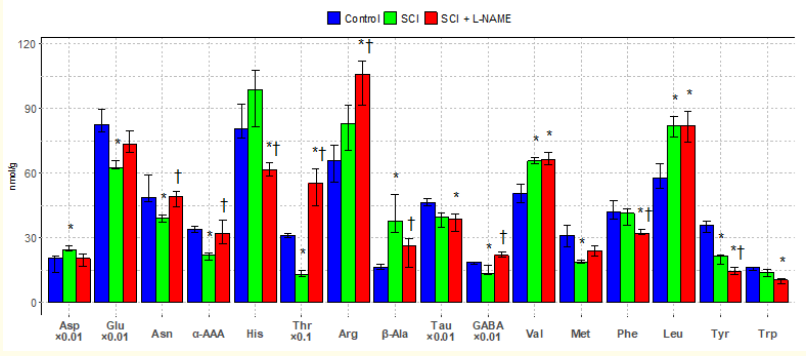

Figure 1: Concentration of amino acids and their derivatives in the brain cortex with subtotal ischemia during administration of L-NAME, nmol / g.

Note, here and in fig. 2-4: Median values with interquartile range are presented;

* - p <0.05 when compared with control; † - p <0.05 when compared with SIGM.

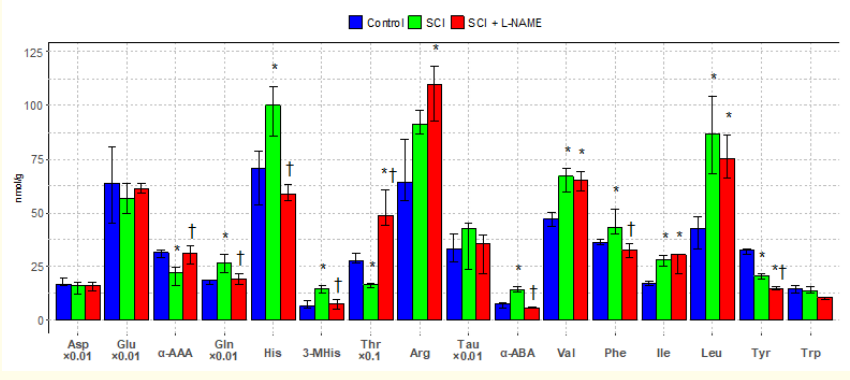

Figure 2: Concentration of amino acids and their derivatives in the hippocampus of rats during subtotal ischemia of brain and administration of L-NAME against its background.

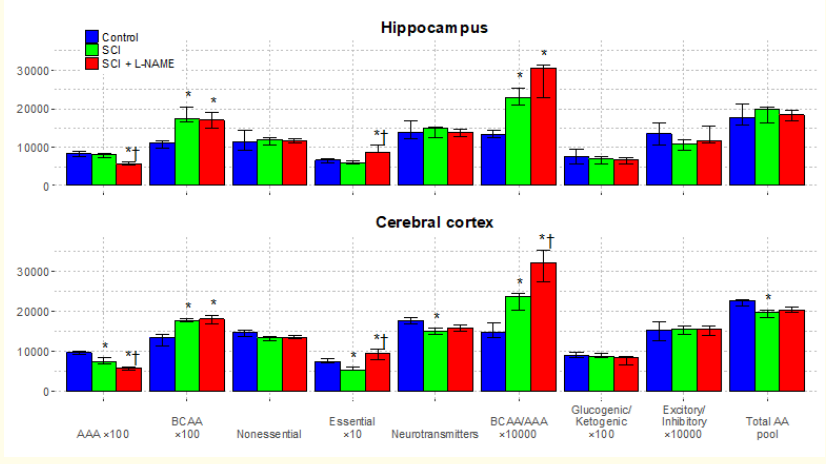

Figure 3: Integral indices of the amino acid fund of the hippocampus and cortex of GM rats (nmol / g) and their ratio during subtotal ischemia of brain and administration of L-NAME against its background.

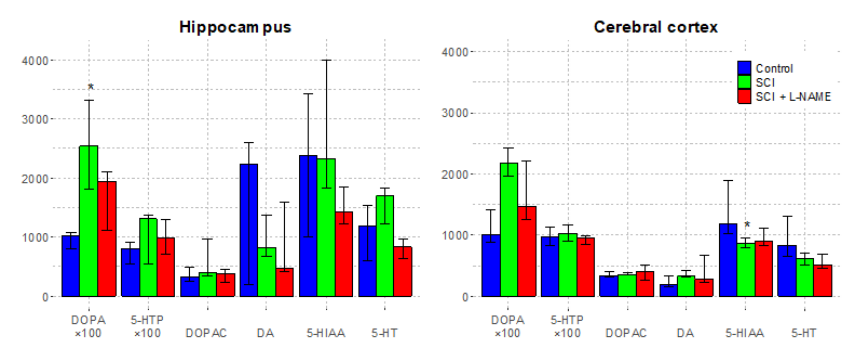

Figure 4: Concentration of biogenic amines in the cortex and hippocampus of brain rats during subtotal ischemia of brain and administration of L-NAME on its background, pmol / g.

nine, tyrosine, as well as increased concentrations of threonine and arginine. Thus, the deficiency of amino acids increased upon administration of L-NAME, as did an increase in the BCAA/AAA ratio (Figure 3). However, the content of irreplaceable components of the AA pool became higher than the control values (Figure 3). The increase in arginine is explained by the action of L-NAME as an antagonist of NO synthase and, thus, indicates the adequacy of the model.

In the rat hippocampus, SCI caused an increase in the levels of phenylalanine, histidine, 3-methylhistidine, glutamine, β-aminobutyrate, and BCAA (Figure 2). Simultaneously, there was a decrease in the levels of threonine, tyrosine and α-aminoadipino acid (α-AAA). There were no disturbances in the pool of biogenic amines in the rat hippocampus during SIGM (except for an increase in the level of dioxyphenyl acetate (DOPA)) (Figure 4). However, correlation analysis indicates the effect of ischemia on the activity of their transformations. Thus, in normal conditions, serotonin and its metabolite, 5-hydroxyindole acetate (5-HIAA), correlate positively ($r = 0.98$, $p < 0.05$), with SCI their connection is disrupted, the 5-HIAA level begins to negatively correlate with level 5-oxytryptophan (5-HTP) ($r = –0.83$, $p < 0.05$). Also, with SCI, there is a violation of the bonds between tyrosine and its metabolites (DOPA and DOPAC). All this may indicate functional disorders of the serotonin and dopamine systems in the hippocampus during ischemia, which, however, is not accompanied by changes in the levels of their components.

The effect of ischemia on the integral indices of the amino acid pool of the hippocampus was almost identical to that in the cortex of the GM (Figure 3). Only a decrease in the levels of AAA, the total pool of AA and its irreplaceable components were not recorded. The administration of L-NAME prevented SCI-induced disruption of the levels of phenylalanine, histidine, 3-methylhistidine, glutamine, α-aminobutyrate, and α-aminoadipic acid, which may indicate a normalizing effect of L-NAME on the pool of these amino acids in the hippocampus. At the same time, the administration of L-NAME did not affect the levels of BCAA and increased the concentrations of threonine and arginine. The decrease in the level of tyrosine during ischemia was enhanced by the administration of L-NAME.

The introduction of L-NAME against the background of SCI had no effect on the levels of biogenic amines, except for the normalization of the DOPA level (Figure 4). At the same time, the correlation analysis indicates the normalizing effect of L-NAME on the serotonin system of the hippocampus against the background of SCI.

A decrease in AAA levels upon administration of L-NAME intensified, against the background of an increased ARUC content, an increase in the BCAA/AAA ratio (Figure 3). Along with this, there was an increase in the total concentration of essential components of the amino acids pool, and the resulting decrease in the proportion of nonessential amino acids in the total pool.

Comparison of the effects of SCI and the introduction of L-NAME in the cortex and hippocampus makes it possible to identify a group of compounds that have specific reactions to these factors depending on the department of the brain. Thus, the level of aspartate against the background of SCI grew only in the cortex, remaining unchanged in the hippocampus; after the introduction of L-NAME, its level returned to normal (Figure 5). The level of ornithine and GABA in the hippocampus became higher than its values in the brain cortex, despite the absence of statistically significant changes in relation to the control. On the other hand, the concentrations of a number of compounds (including arginine, threonine, BCAA, tyrosine, αABA, α-AAA, lysine and tryptophan) changed synchronously in both departments. A decrease in the concentration of taurine in the brain cortex upon administration of L-NAME was accompanied by the appearance of a negative correlation between the levels of taurine in the hippocampus and brain cortex (r = - 0.82 versus - 0.14 and - 0.26 in the control and with SCI). This may indicate a redistribution of taurine pools between these departments.

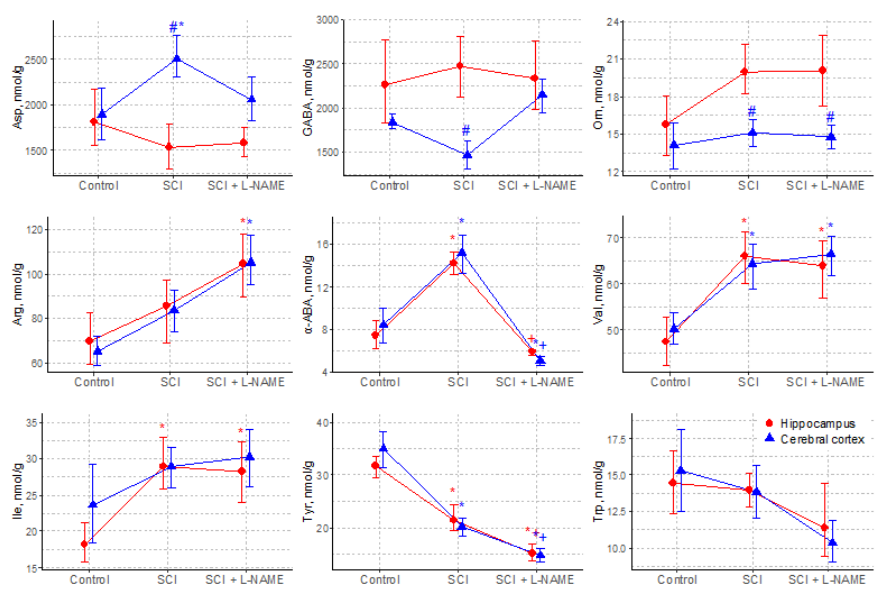

Figure 4: Comparison of changes in some indicators of the amino acids fund of the hippocampus and cortex during subtotal ischemia of brain and the introduction of L-NAME on its background, nmol / g

Note:Mean values are presented with 95% confidence intervals;

* - p <0.05 when compared with control; † - p <0.05 when compared with SIGM;

+ - p <0.05 when between GM departments.

The most significant indicators in the discrimination of groups were: in the cortex - tyrosine, threonine, glutamate, histidine, tryptophan and arginine (F-excl > 10), in the hippocampus - threonine, tyrosine and leucine (F-ex = 30.3; 11.9 and 3.79, respectively). With this set of predictors, highly significant discrimination between groups was achieved (Wilks' Lambda = 0.00047 (cortex) and 0.012 (hippocampus), F = 75.2 and 35.3, respectively, p < 10 10).

Conclusions

1. Subtotal ischemia of the rat brain induces an increase in the brain cortex levels of aspartate, β-alanine, BCAA and a decrease in glutamate, asparagine, threonine, GABA, tyrosine and 5-hydroxyindole acetate; in the hippocampus, it disrupts the levels of phenylalanine, histidine, glutamine, BCAA, as well as the activity of the serotonin and dopamine systems.

2. The effect of SCI on the pool of free amino acids and neuroactive compounds in the studied sections of the rats brain is basically the same, however, there is a group of compounds, including aspartate, ornithine, and GABA, for which the nature of the effect is specific for the hippocampus and the brain cortex.
3. Preliminary administration of L-NAME partially normalizes disorders caused by SCI in the cortex and hippocampus, simultaneously induces an amino acid imbalance affecting the levels of AAA, arginane, histidine, threonine and taurine.

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