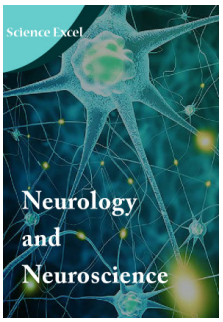
Neurology & Neuroscience

Original Article



The effect of combaine administration of L-arginine and omega-3 polyunsaturated fatty acids on the spectrum of free amino acids and biogen amines in hyppocampus of rats undergoing subtotal cerebral ischemia

Razvodovsky YE[1*], Smirnov VY[2], Doroshenko EM[2], Bon EI[2], Korotkevich TV[3], Maksimovich NYe[2] and Semenenia IN[1]

Institute biochemistry of biologically active substances Academy of science of Belarus 230009, Republic of Belarus, Grodno, str. Boulevard of Lenin's komsomol,50, Belarus, Russia

*Correspondence

Yury Evgeny Razvodovsky

Institute biochemistry of biologically active substances Academy of science of Belarus 230009, Republic of Belarus, Grodno, str. Boulevard of Lenin's komsomol,50, Belarus, Russia

Tel: +966552958971

E-mail: yury_razvodovsky@mail.ru

- Received Date: 25 Feb 2021
- Accepted Date: 30 Mar 2021
- Publication Date: 06 Apr 2021

Keywords: amino acids, biogenic amines, hippocampus, subtotal cerebral ischemia, L-arginine, omega-3 PUFA.

Abstract

The aim of this study was to estimate the changes in the pool of free amino acids and their derivatives in hippocampus of rats undergoing subtotal cerebral ischemia (SCI) and treated with L-arginine and omega-3 polyunsaturated fatty acids (PUFA). Experiment was held on 18 rats: 12 animals were undergoing bilateral filament occlusion of arteries carotid, 6 of them L-arginine and omega-3 PUFA was administrated. The drug omega-3 PUFA "Omegamed" (at a dose of 5 g/kg of body weight) was injected intragastrically during the week preceding the simulation of SIMG. L-arginine (at a dose of 100 mg/kg body weight) was injected intravenously just before ligation of the common carotid arteries. The analyses of free amino acids and their derivates levels in the blood plasma extracts were carried out by reversed phase HPLC. In the hippocampus of rats with SIGM, there was an increase in the levels of histidine, 3-methylhistidine, glutamine, α--aminobutyrate, isoleucine, leucine, valine, as well as a decrease in the levels of threonine, tyrosine, and α--aminoadipic acid. Administration of L-arginine and omega-3 PUFAs prevented ischemia-induced disruption of threonine, histidine, glutamine, α--aminobutyrate, α--aminoadipic acid levels, and also had a corrective effect on the serotonin system of the hippocampus.

Introduction

Stroke is one of the leading causes of death and the leading cause of disability in the developed countries of the world [1,2]. Despite the advances in modern medicine, the effectiveness of stroke treatment remains rather low. An urgent direction of experimental research is the search for neuroprotective agents that improve the recovery of nerve cells damaged by ischemia-reperfusion among biologically active compounds and natural metabolites [3].

The amino acid L-arginine plays an important role in metabolic processes, including being a precursor of nitrogen monoxide, which is involved in the regulation of many biochemical reactions and physiological processes [4]. There is experimental and clinical evidence of the biological activity of L-arginine in various pathological conditions, including ischemic stroke [5,6].

Polyunsaturated fatty acids (PUFA) perform many functions in the nervous tissue; therefore they are necessary for the normal functioning of the brain [7,8]. Currently, a lot of experimental data have been accumulated regarding the neuroprotective effects of PUFA in ischemic brain damage [9,10]. Moreover, PUFA have both prophylactic and therapeutic effects [9]. It has been shown that additional intake of PUFA in the diet in the period preceding ischemia potentiates postischemic oligodendrogenesis in rats [10].

Administration of PUFA two hours after ischemic reperfusion reduces neuronal damage, improves cognitive processes, and accelerates the recovery of sensorimotor functions in mice by increasing neurogenesis in the hippocampus [11]. The administration of PUFA within five hours after ischemia reduces the volume of the affected area of the brain, improves sensory functions, and increases the survival rate of rats [9]. The neuroprotective effects of PUFA are realized through a number of mechanisms, including activation of the survival signaling cascade in neurons, anti-inflammatory effect of the neuroprotectin D1 metabolite, inhibition of NF-kB activation, stabilization of the cell membrane, decrease in thromboxane B2 production, and decrease in oxidative stress [7-11].

The aim of the study was to characterize the changes in the pool of amino acids and biogenic amines in the hippocampus of rats during subtotal cerebral ischemia against the background of the combined use of L-arginine and omega-3 PUFA

Citation: Razvodovsky YE, Smirnov VY, Doroshenko EM, et al. The effect of combaine administration of L-arginine and omega-3 polyunsaturated fatty acids on the spectrum of free amino acids and biogen amines in hyppocampus of rats undergoing subtotal cerebral ischemia. Neurol Neurosci. 2021; 2(1):1-3.

Materials and methods

The experiments were performed on 18 white outbred female rats (6 animals in each group), weighing 180-220 g. Subtotal cerebral ischemia (SIGM) was simulated in the rats of the experimental groups by ligation of both common carotid arteries for one hour. SIGM was modeled by ligation of both carotid arteries for one hour. The drug omega-3 PUFA "Omegamed" (at a dose of 5 g/kg of body weight) was administered intragastrically during the week preceding the modeling of SIGM. L-arginine (at a dose of 100 mg/kg body weight) was injected intravenously just before ligation of the common carotid arteries. The control group consisted of sham-operated animals that received an equivalent volume of isotonic NaCl solution. All surgical manipulations were performed under conditions of intravenous thiopental anesthesia (60 mg/kg). After the extraction of the brain, a fragment of the hippocampus was removed on the side of the ligation, followed by freezing in liquid nitrogen. Sample preparation for research included homogenization in a 10-fold volume of 0.2 M perchloric acid, centrifugation for 15 min at 13000 g and 4 ° C, followed by collection of the supernatant.

The spectrum of the 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). The column used was Zorbax Eclipse Plus C18, 3.5 μm, 2.1 x 150 mm. Identification and quantitative analysis were performed using the Agilent ChemStation B.04.01 software [12]. The method was calibrated using a standard mixture of amino acids from the Sigma-Aldridge company. Mobile phases used: 0.1 M Na-acetate buffer (pH 6.25 and 5.75), aqueous solutions of acetonitrile and methanol (60% vol). Separation was performed 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 dibutylamine, 7% acetonitrile (vol.). In the work, reagents of at least reagent grade were used Tridistilled water for the mobile phases was passed through a Norganic cartridge (Millipore, USA). The mobile phases were filtered through a 0.22 μm membrane filter [13].

Statistical data processing was performed using the R program. To assess the influence of factors, parametric analysis of variance (ANOVA) with Tukey's a posteriori comparison was used. In the case of violation of dispersion homogeneity, DA was performed in Welch's modification with a posteriori comparison according to Games-Howell. In the absence of normality of the distribution of indicators, the nonparametric Kruskal-Wallis AN was used with the Benjamini-Hochberg correction for the multiplicity of comparisons. 95% confidence intervals were obtained by the nonparametric bootstrap method (R = 1000). The methods of correlation and linear discriminant analysis were also used.

Results and discussion

In the hippocampus of rats with SIGM, an increase in the levels of histidine, 3-methylhistidine, glutamine, β-aminobutyric acid, and branched-chain amino acids (BCAA) - isoleucine, leucine, and valine was observed (Figure 1). At the same time, there was a decrease in the levels of threonine, tyrosine and α-aminoadipic acid. An increase in the total content of ARUC was responsible for an increase in the ratio of BCAA/AAC in the hippocampus during SIGM (Figure 2). SIGM did not cause significant changes in other integral indicators of the amino acid pool of the hippocampus (Figure 2).

A study of the levels of biogenic amines in the rat hippocampus showed that the effect of SIGM on the pool of these compounds resulted in an increase in the level of dioxyphenylalanine (Figure 1). Correlation analysis indicates the effect of ischemia on the rate of conversion of biogenic amines. So, if in normal levels of serotonin and its metabolite 5-hydroxyindole acetate correlate positively, then with SIGM this relationship was disrupted and the level of 5-hydroxyindoleacetic acid began to negatively correlate with the level of 5-hydroxyindoleacetic acid. Also, with SIGM there is a weakening of the relationship between the concentration of tyrosine and its metabolites - dioxyphenylalanine and dioxyphenyl acetate (Table 1). All this may indicate functional disorders of the serotonin and dopamine systems in the hippocampus during ischemia, which, however, are not accompanied by changes in the levels of their components. The reason for these changes (including the accumulation of dioxyphenylalanine) may be the inhibition of the decarboxylase of aromatic amino acids and, accordingly, the synthesis of dopamine. Since this enzyme is present in both dopaminergic and serotonergic neurons, it may also contribute to the inhibition of serotonin synthesis [2]. An additional evidence of the latter may be the observed constancy of the level of tryptophan in the hippocampus, which is unlikely under conditions of ischemia at a normal rate of serotonin synthesis.

Administration of L-arginine and omega-3 PUFA prevented ischemia-induced changes in the levels of threonine, histidine, glutamine-aminobutyrate, and α--aminoadipic acid, which indicates a normalizing effect of these compounds on the hippocampal amino acid pool (Figure 1). At the same time, the introduction of these substances did not affect the levels of BCAA, the values of which remained above the control, and also contributed to an increase in the concentration of lysine. Against the background of the increased content of BCAA, the ratio of BCAA/AAA continued to grow (Figure 2). Along with this, there was an increase in the total content of essential amino acids in the total amino acids pool and the resulting decrease in the proportion of nonessential amino acids (Table 2).

The introduction of L-arginine and omega-3 PUFA against the background of SIGM prevented an increase in the level of dioxyphenylalanine in the hippocampus (Figure 1), which, given the increased positive correlations tyrosine - dioxyphenylalanine and tyrosine - normetanephrine, can be explained by the activation of the hydroxylase pathway of tyrosine degradation (Table 1). At the same time, the disappearance of the negative correlation of 5-hydroxyindolylacetic acid-5-hydroxytryptophan and the prevention of the violation of the positive correlation of serotonin-5-hydroxyindoleacetic acid indicates the normalizing effect of the introduction of L-arginine and omega-3 PUFA on the functioning of the serotonin system and is most likely associated with with the restoration of the normal activity of the decarboxylase of aromatic amino acids (which can be indirectly indicated by the normalization of the level of dioxyphenylalanine).

Threonine, leucine, tyrosine, and valine were the most significant indicators in the hippocampus, making the greatest contribution to the total variance (Table 2). With this set of predictors, highly significant discrimination between groups was achieved (Wilks' Lambda = 0.0248, F = 16, p <10 7). Noteworthy is the absence in this list of L-arginine, one of the two injected compounds. This, as well as the constancy of its level in this experimental situation, is evidence of the impossibility of increasing the level of arginine in the central nervous system by intravenous administration. Consequently, its possible effects in the brain, including the hippocampus, are mediated through its effect on the amino acid pool in the periphery.

The location of the realizations on the plane of the two main components (Figure 3) suggests that the introduction of L-arginine and omega-3 PUFA against the background of SIGM eliminates the shift along the first main component, which determines more than 88% of the total variance, but promotes a slight shift along the second main component. components (determining less than 11.5% of the

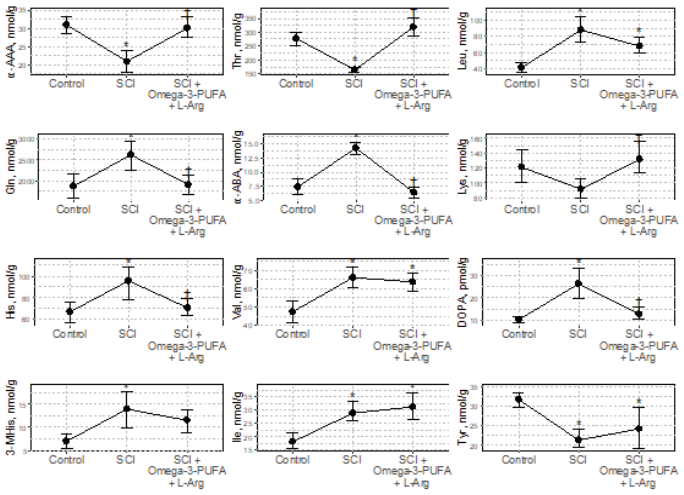


Figure 1. *Concentration of amino acids and their derivatives in the hippocampus of rats with subtotal ischemia of GM and against the background of joint administration of L-arginine and omega-3 PUFA. Note: here and in Fig. 2, the indicators are presented as mean and 95% confidence intervals.*

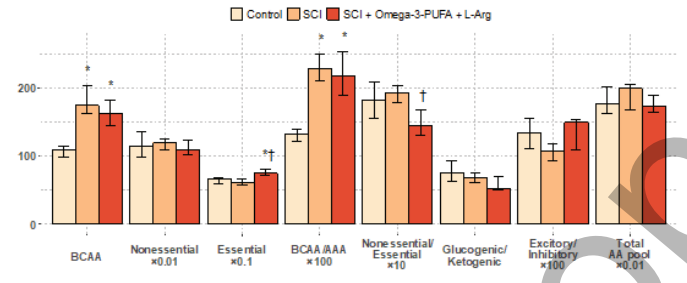


Figure 2. *Integral indicators of the amino acid fund of the rat hippocampus (nmol / g) and their ratio in subtotal ischemia of GM and against the background of the combined administration of L-arginine and omega-3 PUFA and L-Arg.*

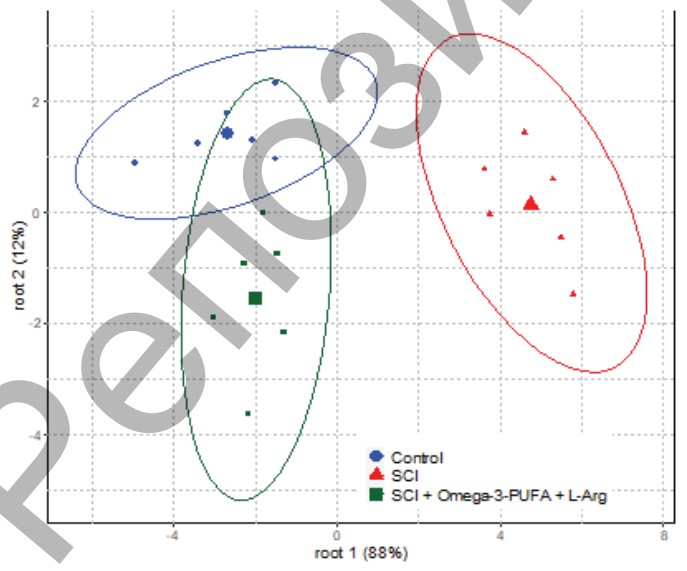


Figure 3. *The projection of the experimental groups on the plane of the two main components.*

total variance). Analysis of the coefficients of discriminant functions (Table 2) makes it possible to associate the change in the level of leucine with the effects of SIGM in the hippocampus, and the change in the concentrations of threonine, valine and tyrosine - with the introduction of L-arginine and omega-3 PUFA.

Conclusions

SIGM induces an imbalance in the pool of free amino acids and their derivatives in the hippocampus in rats, including the levels of threonine, tyrosine histidine, 3-methylhistidine, glutamine, ARUC, dioxyphenylalanine, as well as functional disorders of the serotonin and dopamine systems. The combined administration of L-arginine and omega-3 PUFA prevented the disruption of the levels of threonine, histidine, glutamine and dioxyphenylalanine, and also had a corrective effect on the serotonin system of the hippocampus..

References

1. Feigin VL, Norrving B, Mensah GA. Global burden of stroke. Circ Res. 2017; 120: 439-448.
2. Razvodovsky YE. Alcohol attributable fraction of stroke mortality in Russia. J Neurol Sci. 2013; 33: 231.
3. Deutch AY, Roth RH. Pharmacology and Biochemistry of Synaptic Transmission. (3rd edn), Molecules to Networks. 2014; pp: 212-223.
4. Maksimovich NYe. Tolerance of hypoxic hypoxia in rats with cerebral ischemia treated by NO-synthase modulators. Hypoxia Medical. 2004; 1: 20-23.
5. Almakaeva LG. Litvinova EV. Arginine and its application in medicine and pharmacy. Liki Ukraini. 2011; 1: 23-26.
6. Khalimova HM, Rashidova NS. Experience of using L-arginine in the treatment of patients with ischemic stroke. Ukrainian Chemotherapy J. 2012; 3: 247-248.
7. Eady TN, Belayev L, Khoutorova L, et al. Docosahexaenoic Acid Signaling Modulates Cell Survival in Experimental Ischemic Stroke Penumbra and Initiates Long-Term Repair in Young and Aged Rats. PLoS ONE. 2012; 7: e46151.
8. Pan HC, Kao TK, Ou YC, et al. Protective effect of docosahexaenoic acid against brain injury in ischemic rats. J Nutr Biochem. 2009; Vol. 20: 715-725.
9. Niemoller TD, Stark DT, Bazan NG. Omega-3 fatty acid docosahexaenoic acid is the precursor of neuroprotectin D1 in the nervous system. World Rev Nutr Diet. 2009; 99: 46-54.
10. Belayev L, Khoutorova L, Atkins KD, et al. Robust Docosahexaenoic Acid–Mediated Neuroprotection in a Rat Model of Transient, Focal Cerebral Ischemia. Stroke. 2009; 40: 3121-3126.
11. Belayev L, Khoutorova L, Atkins K, et al. Docosahexaenoic Acid Therapy of Experimental Ischemic Stroke. Transl Stroke Res. 2011; 2: 33-41.
12. Barkovsky EV, Bokut SB, Borodinsky AN. Modern problems of biochemistry. Research methods. Minsk: Higher School. 2013; pp: 491.
13. Smirnov VYu, Razvodovsky YuE, Doroshenko EM, et al. Influence of the composition of amino acids with a branched hydrocarbon chain, tryptophan and taurine on the exchange of amino acids in experimental models of alcoholism. Ukrainian Biochemical J. 2003; 75: 101-107.