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Disorders Of Mitochondrial Respiration in Rats with Subtotal Cerebral Ischemia Under Conditions of The Use of Modulators of the L-Arginine-No Pathway and Against the Background of The Administration of Omega-3 Polyunsaturated Fatty Acids

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Abstract

The urgency of the problem of cerebrovascular diseases can rightfully be defined as an emergency, requiring the concentration of efforts of specialists. This is due to the fact that cerebrovascular diseases of ischemic origin tend to grow, rejuvenate, are associated with a severe clinical course, high rates of disability and mortality.

Omega-3 polyunsaturated fatty acids are involved in the implementation of the basic functions of neurons, such as the transmission of impulses and the functioning of receptors. In this regard, it is of interest to study the morphofunctional features of brain neurons in rats with subtotal cerebral ischemia against the background of the introduction of Omega- 3 polyunsaturated fatty acids and L-NAME.

There was a significant improvement in the parameters of mitochondrial respiration when using succinate as a substrate in the group of animals treated with Omega-3 PUFAs and even greater activation in the groups of animals SCI

+ L-NAME + Omega-3 and SCI + L-NAME + L-arginine + Omega-3. When malate/glutamate was used as a substrate in these groups, the same trend in respiration was observed as when succinate was used to activate mitochondrial respiration. However, changes in succinate-dependent respiration were more pronounced.

Keywords: *Omega-3 polyunsaturated fatty acids, subtotal cerebral ischemia, neurons*

Introduction

Cerebrovascular diseases of ischemic origin tend to grow, rejuvenate, are associated with a severe clinical course, high rates of disability and mortality. The urgency of the problem of cerebrovascular diseases can rightfully be defined as extraordinary, requiring the concentration of efforts of specialists of different profiles to solve it [2, 6, 7].

The search for new approaches to the treatment of acute ischemic stroke is one of the urgent problems of experimental and clinical neurology [2, 7].

One of the promising neuroprotective amino acids is L-arginine. Most of the effects caused by this amino acid are associated with its ability to increase the formation of NO, acting as a source for its formation. It has been shown that the use of L-arginine reduces the size of the infarct, reduces vascular tone and causes a hypotensive effect, prevents and corrects ischemic and reperfusion damage to the brain and other organs [3, 4, 5, 8].

An important role of ω-3 polyunsaturated fatty acids (Omega-3 PUFAs) is to ensure the functioning of cell membranes, transmembrane ion channels and the regulation of physiological processes through the synthesis of lipid mediators, which, lining up in the

phospholipid layer of cell membranes, affect their fluidity. Omega-3 PUFAs control the functioning of the immune and reproductive systems, being precursors of the biosynthesis of prostaglandins, leukotrienes and thromboxanes and other cytokines [9, 10].

Brain neurons, being electrically active cells rich in ion channels, are the most sensitive to deficiency of polyunsaturated fatty acids.

Omega-3 PUFAs are involved in the implementation of the main functions of neurons, such as the transmission of impulses and the functioning of receptors [2, 9].

In this regard, it is of interest to study the morphological and functional features of brain neurons in rats with subtotal cerebral ischemia against the background of the administration of Omega-3 PUFAs and L-NAME [2, 9, 10].

The aim of the work is to study the parameters of mitochondrial respiration in rats with subtotal cerebral ischemia under conditions of the use of modulators of the L-arginine-NO pathway and against the background of the administration of Omega-3 polyunsaturated fatty acids.

Materials and methods of research

The studies were carried out on animals represented by 8 groups of 6 rats each. The control group (group 1) consisted of sham-operated rats receiving 0.5 ml of isotonic NaCl solution. Subtotal cerebral ischemia (SCI) was modeled by ligation of both common carotid arteries (CCA) under conditions of intravenous thiopental anesthesia (40-50 mg/kg) - group 2. Rats of the 3rd group immediately before CCA ligation were injected intramuscularly with L-NAME at a dose of 5 mg/kg, animals of the 4th group were additionally injected with L-arginine at a dose of 200 mg/kg of body weight (SCI + L-NAME + L-arginine), and rats of the 5th group received only L-arginine at a similar dose before surgery (SCI + L-arginine), animals of the 6th group were additionally intragastrically given Omega-3 PUFA at a dose of 5 mg/kg of body weight (SCI + L-NAME + Omega-3 PUFA) intragastrically for a week, rats of group 7 were injected before surgery only Omega-3 PUFAs in the same dose (SCI + Omega-3 PUFAs), while the rats of the 8th group were given L-NAME, L-arginine and Omega-3 PUFAs in the above doses (SCI + L-NAME + L-arginine + Omega-3 PUFA). The duration of SCI was 60 minutes, after which the rats were decapitated.

The tissue respiration of mitochondria in brain homogenates was studied in rats.

To determine mitochondrial respiration, the brain was removed in the cold (0–4°C), dried with filter paper, weighed, and homogenized in an isolation medium containing 0.25 M sucrose, 0.02 M Tris-HCl, and 0.001 M EDTA, pH 7.2. Mitochondria were isolated by differential centrifugation. The nuclear fraction was separated by centrifugation at 600 g for 10 min at 4°C. The resulting supernatant was centrifuged at 8500 g for 10 min at 4°C, the mitochondrial precipitate was washed twice in the isolation medium and resuspended to a protein concentration of 35–40 mg/ml. The protein concentration was determined by the Lowry method [1].

The rate of mitochondrial respiration was recorded polarographically by changing the oxygen content in the mitochondrial suspension using a laboratory-made Clark electrode built into a thermostatically sealed polarographic cell at a temperature of 25°C. A concentrated suspension of mitochondria was introduced into a polarographic cell with an incubation medium (0.05 M sucrose, 0.01 M Tris-HCl, 0.125 M KCl, 0.0025 M KH2PO4, 0.005 M MgSO4, 0.001 M EDTA, pH 7.4) in an amount that ensured the final protein concentration in cell 1 mg/ml. After recording the rate of basal (endogenous) respiration (V1), respiration substrates (succinate – 5 mM or malate – 2 mM/glutamate – 5 mM) and ADP 200 nmol/mL were alternately introduced into the mitochondrial suspension. The rate of mitochondrial respiration was recorded in various metabolic states: V2 is the rate of substrate-dependent respiration, V3 is the rate of respiration associated with phosphorylation (after the addition of ADP), and V4 is the rate of respiration after completion of phosphorylation of the added ADP. The indicators characterizing the conjugation of the processes of oxidation and phosphorylation in mitochondria were determined: the acceptor control coefficient (AC = V3/V2), the respiratory control coefficient (RC = V3/V4), and the phosphorylation coefficient – ADP/O.

Research results

In the study of respiration in the mitochondria of the brain, the rate of basal respiration in the absence of a substrate (V1), the rate of respiration in the presence of a substrate (succinate or malate/glutamate) (V2), the rate of respiration associated with ADP phosphorylation (V3), and the rate of respiration after completion of phosphorylation were determined ATP – (V4), acceptor control coefficient (V3/V2), respiratory control coefficient (V3/V4), phosphorylation coefficient (ADF/O). The results obtained are presented in table 1.

Table 1: Respiratory parameters of mitochondria in the brain of rats of the control group, with SCI, SCI + Omega-3 PUFA, SCI + L-NAME + Omega-3 PUFA and SCI + L-NAME + L-arginine + Omega-3, Me (LQ; UQ)

Groups	V1 (ng at O/min×mg protein)	V2 (ng at O/min×mg protein)	V3 (ng at O/min×mgprotein)	V4 (ng at O/min×mgprotein)	(V3/V2)	(V3/V4)	(АДФ/О)
Substrate succinate							
Control	*17*	*34*	*66*	*38*	*1,2*	*1,8*	***1,9***
	(15;17)	*(28;36)*	*(65;68)*	*(36;40)*	*(1,2;2,0)*	*(1,7;1,9)*	***(1,8;1,9)***
SCI	*27**	*39**	*50**	*40*	*1,3**	*1,4**	***1,2****
	(19;27)	*(36;42)*	*(48;54)*	*(37;41)*	*(1,2;1,3)*	*(1,2;1,4)*	***(1,1;1,2)***
SCI + Omega 3	*15#*	*35*	*65#*	*37*	*1,8#*	*1,8#*	***1,4***
	(14; 15)	*(34;36)*	*(64;68)*	*(36;38)*	*(1,8; 1,8)*	*(1,8;1,8)*	***(1,4;1,5)***
SCI + L-NAME+ Omega 3	*15#*	*36*	*71#*	*38*	*1,9#*	*1,9#*	***1,9#***
	(14;17)	*(35;37)*	*(68;74)*	*(35;41)*	*(1,9;2,0)*	*(1,8;2,0)*	***(1,8;1,9)***
SCI + L-NAME + L-arginine + Omega-3	*16#*	*35*	*67#*	*39*	*1,7#*	*1,6#*	***1,8***
	(14;17)	*(32;37)*	*(63;72)*	*(32;42)*	*(1,6;1,9)*	*(1,5;2,1)*	***(1,7;2,0)***
Substrate malate/glutamate							
Control	*18*	*27*	*51*	*31*	*2,0*	*1,6*	***2,0***
	(14;19)	*(26;27)*	*(48;56)*	*(27;34)*	*(1,8,3,0)*	*(1,6;1,7)*	***(1,9;2,1)***
SCI	*18*	*36*	*50*	*32*	*1,5**	*1,4*	***1,5****
	(18;24)	*(35;38)*	*(48;51)*	*(30;37)*	*(1,3;1,5)*	*(1,3;1,6)*	***(1,4;1,5)***
SCI + Omega 3	*17*	*34*	*56*	*37*	*1,6*	*1,5*	***1,8***
	(16;18)	*(33;36)*	*(51;61)*	*(35;39)*	*(1,6;1,7)*	*(1,4;1,6)*	***(1,7;1,9)***
SCI + L-NAME + Omega 3	*16*	*24*	*55*	*31*	*2,3#*	*1,7*	***2,1***
	(14;19)	*(21;26)*	*(52;58)*	*(28;35)*	*(2,2; 2,5)*	*(1,6;1,9)*	***(1,9;2,4)***
SCI + L-NAME + L-arginine + Omega-3	*18*	*25*	*53*	*34*	*2,1#*	*1,8*	***2,2***
	(15;20)	*(22;31)*	*(49;60)*	*(31;38)*	*(1,9; 2,7)*	*(1,5;1,9)*	***(1,7;2,6)***

Notes

* – p<0>

– p<0>

SCI – subtotal cerebral ischemia

L-NAME – Nw-nitro-L-arginine

Omega-3 – Omega-3 PUFA

Subtotal cerebral ischemia caused by ligation of both common carotid arteries in rats was accompanied by significant disturbances in mitochondrial respiration of brain cells.

When succinate was used as a substrate, the rate of endogenous (basal) and substrate-dependent respiration increased, but the rate of respiration associated with ADP phosphorylation decreased, which led to a decrease in the coefficients of acceptor and respiratory control. In addition, the phosphorylation coefficient (ADP/O) sharply decreased.

The use of a mixture of malate with glutamate as a substrate showed unidirectional shifts in the parameters of mitochondrial respiration with the substrate succinate, but significant changes were observed only for the acceptor control coefficient and the phosphorylation coefficient.

There was a significant improvement in the parameters of mitochondrial respiration when using succinate as a substrate in the group of animals treated with Omega-3 PUFAs and even greater activation in the groups of animals SCI + L-NAME + Omega-3 and SCI + L-NAME + L-arginine + Omega-3. When malate/glutamate was used as a substrate in these groups, the same trend in respiration was observed as when succinate was used to activate mitochondrial respiration. However, changes in succinate-dependent respiration were more pronounced.

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