

# **The Effect of the Heat Shock Protein HSP<sub>70</sub> Modulators on the Energy Metabolism of the Rats Brain in Acute Cerebral Ischemia**

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## **Abstract**

The study is devoted to the actual problem of medicine - optimization of the treatment of cerebral strokes. Ischemic brain damage, as a result of a decrease in cerebral blood flow, leads to a disturbance of the function of the respiratory chain of mitochondria and energy metabolism, as well as changes in the activity of the antioxidant system.

We studied the ability of drugs HSP<sub>70</sub> modulators to normalize energy metabolism in ischemic brain. The research study was performed on 148 Wistar rats. An acute cerebrovascular disturbance was modeled by bilateral ligation of the common carotid arteries under thiopental anesthesia (40 mg/kg). Within 4 days, each group received treatment with one of the selected drugs (tamoxifen 1 mg/kg, melatonin 5 mg/kg, HSF-1 200 µl/kg, glutamine 25 mg/kg, piracetam 500 mg/kg).

The ability of HSP<sub>70</sub> modulators to normalize energy metabolism in ischemic brain was established, which is confirmed by an increase in the level of ATP, ADP, pyruvate, malate and the activity of NAD-MDGmt against the level lowering of lactate in all experimental groups. The HSP<sub>70</sub> modulators provide for the normalization of energy metabolism and enhancement of ATP production in conditions of acute cerebral ischemia due to HSP<sub>70</sub>-dependent mechanisms of activation and regulation of compensatory malate-aspartate shunt.

**Keywords:** tamoxifen, melatonin, glutamine, HSF-1, HSP<sub>70</sub>, cerebral ischemia, energy metabolism

## **Introduction**

In recent years, there has been an increase in the prevalence of vascular diseases, including acute cerebrovascular accident. Ischemic brain damage is accompanied by severe neurological disorders, such as cognitive impairment, motor, verbal and other CNS functions [1, 3]. The stroke ranks first among the causes of persistent disability [1, 2]. Among all types of stroke, ischemic brain damage prevails. With ischemic brain damage as a result of reduction of cerebral blood flow there is a violation of the respiratory chain of mitochondria and energy metabolism, glutamate "exitotoxicity", violation of ionic cell homeostasis with an increase in intracellular content of calcium ions, increased NO synthesis, membranes and cell death [3, 4].

One of the complex clinical problems is intensive therapy in the acute period of cerebral stroke. There is a large group of intensive care units is being conducted during this period, among which an important place belongs to the prevention and treatment of cerebral ischemia through the normalization of energy metabolism and mitoprotection [5, 6].

For this purpose, drugs which improve neurometabolism is used. At the same time, based on the ideas about the pathobiochemical mechanisms of the development of acute cerebrovascular accident, it is believed that one of the promising directions of increasing the effectiveness of therapy of cerebral ischemia is the use of drugs that activate their own energy processes, directing them along the natural physiological channel, and created on the basis of natural metabolites [4-6].

Based on this, in the 80's of the XX century, intermediates of succinate oxidase and many compensatory mechanisms which directed on the anaerobic succinate synthesis (Kondrasheva transaminase cycle, Roberts cycle, etc.) were widely used [5].

Many years of clinical application of amber acid (rehabilitin, yantvate, polisar, cytoflavin, etc.) have demonstrated their moderate therapeutic efficacy in the treatment of cerebral stroke. In the early 2000s, a group of researchers led by Prof. I.F. Belenichev were found that in response to the formation of cerebral ischemia, HIF-1 $\alpha$  is expressed, which initiates the launch of compensatory mechanisms for energy production.

Subsequently, the regulation of these processes is switched to HSP<sub>70</sub>, which "prolongs" the action of HIF-1 $\alpha$ , as well as independently supports the expression of the activity of NAD-MDGMt, thus sustaining the long-term activity of the malate-aspartate shuttle mechanism [7-9].

Therefore, attempts to use tamoxifen, melatonin, glutamine and the factor of thermal shock (HSF-1) as potential neuroprotectors modulators HSP<sub>70</sub> have been made. Initial studies showed promising results [7, 10].

## **The object and methods of research**

The experimental part was performed on 148 Wistar rats that were kept in vivarium with natural light and had a standard nutrition. Experimental studies were conducted in accordance with the main provisions of the Council of Europe Convention on the Protection of Vertebrate Animals, which are used in experiments and other scientific purposes (Strasbourg, 1986), and others [11, 12].

An acute cerebrovascular accident (ACVA) by type of ischemic stroke was modeled by bilateral irreversible occlusion of the common carotid arteries under thiopental with sodium anesthesia (40 mg / kg) [13].

Animals were divided into VII groups by randomization method: I - pseudo-operated rats (PO, n = 10); II - animals with ACVA (n = 10); III - ACVA + tamoxifen (Pharmaceutical Company "Zdorovie", Ukraine) (1 mg/kg) [11], (n = 10); IV - ACVA + melatonin (JSC "Kiev Vitamin Plant", Ukraine) (5 mg/kg) [12], (n = 10); V - ACVA + heat shock factor (HSF-1) (Sigma, USA) (200 µl/kg), (n = 10); VI - ACVA + glutamine (Sigma, USA) (25 mg/kg) [13], (n = 10); VII - ACVA + piracetam (PC "Borschahovsky Chemical and Pharmaceutical Plant", Ukraine) (500 mg/kg) [14], (n=10). Drugs were administered daily starting immediately after the animals were given anesthesia.

On the 4th day, the animals were taken out of the experiment under thiopental anesthesia (40 mg/kg).

Blood was rapidly removed from the brain, separated from the cerebellum, and the bits were placed in liquid nitrogen. Then crushed in liquid nitrogen to a powdered state and homogenized using a homogenizer SilentCrusher S (Heidolph) at (2°C) in a 10-fold volume of solution containing (in mmol): sucrose - 250, tris-HCl buffer - 20, EDTA-1 (pH 7.4) [13]. A mitochondrial fraction was isolated by differential centrifugation at a refrigerated centrifuge Sigma 3-30k (Germany) (at +4°C). For mitochondrial fraction purification for 7 minutes at 1000g centrifugation was performed, and then the supernatant was re-centrifuged for 20 minutes at 17,000g. The supernatant was poured out and stored at -80°C [13].

To assess the energy metabolism, the level of macroergic molecules (ATP, ADP, AMP) was measured by thin layer chromatography (TLC). The obtained data were used to calculate the energy charge (EC), energy potential (EP), index of phosphorylation (IP), thermodynamic control of respiration (TCR). The levels of malate and lactate were also determined by the Hohorst method, the level of pyruvate by the Tsoh-Lamprehta method [13].

The level of mitochondrial and cytoplasmic HSP<sub>70</sub> was determined by enzyme linked immunosorbent Enzo kit (Sweden). The concentration of total protein was measured by the Lowry method [14].

The results were processed using the statistical package of the licensed program STATISTICA® for Windows 6.0 (StatSoftInc.), Microsoft Excel 365.

Data are presented as arithmetic mean and standard error ( $M \pm m$ ). The significance of the differences ( $p$ ) in the experimental data was calculated using Shapiro-Wilk criterion. The reliability of the differences ( $p$ ) of the experimental data was calculated using Student's  $t$ -criterion, with a parametric distribution, or by the Mann-Whitney  $U$ -criterion, with nonparametric values. Differences were considered statistically significant at  $p \leq 0,05$ .

## Results and Discussion

The ACVA modeling leads to typical ischemic violations of all parts of the brain energy metabolism - discoordination in the Krebs cycle, the activation of low-productive anaerobic glycolysis and, as a consequence, the expressed energy deficit [3,4,9]. Thus, at the 4th day of cerebral ischemia in the control group, a significant decrease was observed for the pseudo-operated group of the macrophage phosphates ATP and ADP by 60.8% ( $p < 0,05$ ) and 45.2% ( $p < 0,05$ ) respectively. At the same time, the level of AMP increased by 104.4% ( $p < 0,05$ ) (Table 1), which indicates a progressive energy deficit and intensification of damage, since AMP acts as a prooxidant [9].

It is known that the violation of the oxygen regime of tissues, the discoordination in the Krebs cycle, the activation of anaerobic glycolysis, metabolic acidosis, transmittent autocooidosis, accumulation of  $Ca^{2+}$  in mitochondria, damage of the mitochondria membrane with ROS and NO, increases the pores opening and the apoptotic proteins release from damaged mitochondria. [3, 4]

Under the action of ROS occurs mitochondrial pore opening and mitochondrial charge dropping. The opening of the pore occurs due to oxidation or nitrozing of the the protein thiol groups cysteine-dependent region of the internal membrane of mitochondria (ATP/ADP-antporter), which transforms it into a permeable non-specific channel – pore.

The pores opening transforms mitochondria from "power stations" into "furnaces" of oxidation substrates without the formation of ATP [3,4]. Against of the modulators of HSP<sub>70</sub>, an increase in macroergy levels was observed. The most effective was HSF-1, which increased the levels of ATP and ADP by 101.8% ( $p < 0,05$ ) and 58.1% ( $p < 0,05$ ), with the AMP level decreased by 53.6% ( $p < 0,05$ ) according to the control group.

Other test samples also had a positive effect on energy metabolism in the brain and significantly increased the level of ATP-ADP: tamoxifen by 50.4-26.5%, melatonin by 66.4-36.5%, glutamin by 52.2-29%. Piracetam significantly ( $p < 0,05$ ) increased only the level of ATP by 30%.

Also, all tested samples reliably ( $p < 0,05$ ) decreased the level of proantioxidant AMP: tamoxifen by 46.4%, melatonin by 53.6%, glutamin and piracetam by 42.9% (Table 1).

Table 1. Evaluation of the adenine nucleotides level in the rats brain on the 4th day of cerebral ischemia and with pharmacological correction by HSP<sub>70</sub> modulators (M ± m)

Group of animals (n=10)	ATP μmol/g of tissue	ADP μmol/g of tissue	AMP μmol/g of tissue
Pseudo-operated animals (PO)	2,88±0,08	0,56±0,03	0,14±0,01
Animals with ACVA	1,13±0,06*	0,31±0,03*	0,28±0,02*
Animals with ACVA + tamoxifen	1,7±0,06**	0,39±0,03**	0,15±0,01**
Animals with ACVA + melatonin	1,88±0,07**	0,42±0,02**	0,13±0,013**
Animals with ACVA + HSF-1	2,28±0,10**	0,49±0,02**	0,13±0,012**
Animals with ACVA + glutamine	1,72±0,12**	0,4±0,03**	0,16±0,01**
Animals with ACVA + piracetam	1,47±0,11**	0,36±0,04	0,16±0,019**

\* -  $p \leq 0,05$  in relation to PO

\*\* -  $p \leq 0,05$  in relation to control

For a more in-depth assessment of the violation of energy metabolism, its basic parameters were determined: energy charge (EC), energy potential (EP), phosphorylation index (PI), thermodynamic control of respiration (TCR).

In the control group, there was a 1.2-fold decrease in EC relative to the group of pseudo-operated animals, indicating that the system of ATP/ADP/AMP was degraded by macroergic bonds. Indicators of EP, PI and TCR also had a reliable ( $p < 0.05$ ) tendency to decrease: in 1.3, 2.1, 3.7 times according to the pseudo-operated group (Table 2).

Reduction of PI indicates a decrease in the level of ATP in relation to other fractions of adenyl nucleotides, and the fall of TCR on the depletion of the respiratory chain of mitochondria as a result of inhibition of phosphorylation [3,9].

Reducing the rate of oxidative phosphorylation leads to a decrease in the expression of genes and the synthesis of proteins, a decrease in the ATP/ADP+AMP and the activation of phosphofructokinase, which intensifies anaerobic glycolysis [3].

When the test samples were administered, the main parameters of the energy exchange were restored. The leading position in the restored macroergic balance was occupied by the heat shock factor HSF-1, which significantly ( $p < 0.05$ ) increased EC, PI and TCR relative to the control group in 1,2, 1,9 and 3,4 times, respectively.

In other groups, there was an increase of EC relative to the control (tamoxifen, melatonin, glutamine, piracetam in 1.1 times ( $p < 0.05$ )), PI (tamoxifen and melatonin 1.7 times ( $p < 0.05$ ), glutamine 1.6 times ( $p < 0.05$ ), piracetam 1.5 times ( $p < 0.05$ )) and TCR (melatonin 3.3 times ( $p < 0.05$ ), tamoxifen 2.4 times ( $p < 0.05$ ), glutamin in 1,9 times ( $p < 0,05$ ), piracetam in 2,2 times ( $p < 0,05$ )).

All test samples tended to increase the mitochondrial energy potential, which, however, was not reliably confirmed (Table 2).

Table 2. Estimation of energy metabolism parameters in the rats brain on the 4th day of cerebral ischemia and with pharmacological correction by HSP<sub>70</sub> modulators (M ± m)

Group of animals (n=10)	Energy charge	Energy potential	Phosphorylation index	Thermodynamic breath control
Pseudo-operated animals (PO)	0,88±0,005	5,31±0,31	4,25±0,23	4,40±0,5
Animals with ACVA	0,75±0,013*	4,00±0,43*	1,97±0,12*	1,16±0,12*
Animals with ACVA + tamoxifen	0,85±0,007**	4,7±0,54	3,27±0,26**	2,78±0,28**
Animals with ACVA + melatonin	0,86±0,004**	4,45±0,26	3,41±0,18**	3,78±0,59**
Animals with ACVA + HSF-1	0,87±0,006**	4,76±0,30	3,70±0,19**	3,96±0,44**
Animals with ACVA + glutamine	0,85±0,006**	4,74±0,31	3,21±0,25**	2,26±0,24**
Animals with ACVA + piracetam	0,83±0,011**	4,29±0,36	2,96±0,27**	2,6±0,39**

\* -  $p \leq 0,05$  in relation to PO

\*\* -  $p \leq 0,05$  in relation to control

At the 4th day of the experimental ACVA, a significant level of lactate in the brain was recorded at 130.1% ( $p < 0.05$ ) with a decrease of pyruvate and malate level by 56.6% ( $p < 0.05$ ) and 55.6% ( $p < 0.05$ ) respectively (Table 3). The data indicate the final transfer of cells to anaerobic respiration and, as a result, the accumulation of undioxified lactate, decrease in the level of pyruvate and the formation of lactate acidosis. At the same time, a sharp drop in the activity of NAD-MDGmt was detected in the control group by 63.2% ( $p < 0.05$ ), which suggests a suppression of transport and energy-producing functions of mitochondria and suppresses the activity of the compensatory malate-aspartate shuttle mechanism [3,9].

The designation of the HSP<sub>70</sub> modulators led to the normalization of the main parts of the energy exchange - the inhibition of unproductive anaerobic glycolysis and the production of ATP due to the activation of energetically more productive systems, in particular, due to the malate-aspartate shunt. Thus, HSP<sub>70</sub> modulators reduced the level of lactate: HSF-1 by 50.2% ( $p < 0.05$ ), melatonin by 33.7% ( $p < 0.05$ ), tamoxifen by 27.1% ( $p < 0,05$ ) relative to the control group. Also, there was a significant increase of the pyruvate level (HSF-1 by 108.7%, melatonin by 65.2%, tamoxifen by 47.8%) and malate (HSF-1 by 133.3%, melatonin and tamoxifen by 75% each). Glutamin significantly ( $p < 0.05$ ) increased

the content of malate only by 50%. The reference drug piracetam, on the contrary, provoked lactate acidosis, which is evidenced by an increase in the content of lactate by 28.7% ( $p < 0.05$ ) relative to the control group. All the test samples reliably ( $p < 0.05$ ) restored the activity of NAD-MDGMt, the most active was HSF-1, which increased the activity of NAD-MDGM in 2.7 ( $p < 0.05$ ) times relative to control (Tab 3).

The increase in the activity of NAD-MDGMt in the mitochondria of the animals brain with the ACVA with HSP<sub>70</sub> modulators occurred to an increase in their malate level, which indicates the activation of the compensatory malate-aspartate shuttle mechanism [9].

Table 3. Estimation of parameters of carbohydrate-energy metabolism in the rats brain on the 4th day of cerebral ischemia and with pharmacological correction by HSP<sub>70</sub> modulators ( $M \pm m$ )

Group of animals (n=10)	Pyruvate $\mu\text{mol} / \text{g}$ of tissue	Lactate, $\mu\text{mol} / \text{g}$ of tissue	Malate, $\mu\text{mol} / \text{g}$ of tissue	NAD-MDGM, $\mu\text{mol} / \text{g}$ of tissue / min
Pseudo-operated animals (PO)	0,53±0,022	2,76±0,185	0,27±0,022	1,562±0,118
Animals with ACVA	0,23±0,013*	6,35±0,436*	0,12±0,009*	0,575±0,079*
Animals with ACVA + tamoxifen	0,34±0,015**	4,63±0,203**	0,21±0,018**	1,208±0,103**
Animals with ACVA + melatonin	0,38±0,023**	4,21±0,203**	0,21±0,014**	1,276±0,082**
Animals with ACVA + HSF-1	0,48±0,027**	3,16±0,206**	0,28±0,016**	1,533±0,123**
Animals with ACVA + glutamine	0,29±0,027	5,3±0,275	0,18±0,023**	0,972±0,09*
Animals with ACVA + piracetam	0,26±0,03	8,17±0,61**	0,16±0,019	0,69±0,097

\* -  $p \leq 0,05$  in relation to PO

\*\* -  $p \leq 0,05$  in relation to control

The established changes in energy metabolism in the ischemic brain of animals occurred with changes in the concentration of HSP<sub>70</sub> in cytosol and mitochondria of brain neurons. In the control group at day 4 of ischemia, there was a decrease in the HSP<sub>70</sub> concentration in the cytosolic and in the mitochondrial fraction: by 89.7% ( $p < 0.05$ ) and by 68.4% ( $p < 0.05$ ), respectively, by fractions relative to pseudo-operated animals (table 4). This fact confirms our previous work, which showed parallelism in changes in the level of malate and activity of NAD-MDGMt mitochondria, cytoplasmic AsT and the content of HSP<sub>70</sub>

and HIF-1a. A mathematical analysis established a direct relationship between the concentration of HSP<sub>70</sub> protein and the level of activity of NAD-MDGmt [3, 7, 9].

Appointment of investigated drugs had a positive effect on the recovery of HSP<sub>70</sub> levels with varying degrees of severity. Thus, the factor of thermal shock HSF-1 took the leading position: there was an increase ( $p < 0,05$ ) of the level of HSP<sub>70</sub> in 11,3 times (cytosol) and in 2,6 times (mitochondria) in relation to control.

The appointment of tamoxifen increased the HSP<sub>70</sub> level by 5.1 times (cytosol) and 1.5-fold (mitochondria). The appointment of melatonin increased the cytosolic HSP<sub>70</sub> level in 2.6 ( $p < 0,05$ ) times, the mitochondrial HSP<sub>70</sub> was increased in 1.2 ( $p < 0,05$ ) times in relation to control.

The course appointment of glutamin significantly ( $p < 0,05$ ) increased the protein chaperone 70 in the cytosolic / mitochondrial fraction in 1,5 / 1,1 ( $p < 0,05$ ) times relative to control (Table 4).

Table 4. Concentration of HSP<sub>70</sub> in cytosolic and mitochondrial fractures of animals brains with ACVA ( $M \pm m$ ).

Group of animals (n=10)	HSP <sub>70</sub> , cytosolic fraction, ng/ml	HSP <sub>70</sub> , mitochondrial fraction, ng/ml
Pseudo-operated animals (PO)	16,83±0,64	8,60±0,58
Animals with ACVA	1,73±0,11*	2,72±0,19*
Animals with ACVA + tamoxifen	8,81±0,51**	4,15±0,20**
Animals with ACVA + melatonin	4,55±0,27**	3,3±0,19**
Animals with ACVA + HSF-1	19,50±1,05**	7,08±0,23**
Animals with ACVA + glutamine	2,61±0,16**	3,01±0,17**
Animals with ACVA + piracetam	2,24±0,17**	2,80±0,19

\* -  $p \leq 0,05$  in relation to PO

\*\* -  $p \leq 0,05$  in relation to control

Thus, the modulators of HSP<sub>70</sub> are capable of restoring the energy-producing function of mitochondria under the conditions of the ACVA.

The effect of the heat shock factor HSF 1 is due to its physiological transcription activity in relation to the HSPs family. The HSF-1 leads to the expression of the genes responsible for the subsequent translation of the HSP<sub>70</sub> protein in the cytoplasm and mitochondria. The high level of HSP<sub>70</sub> supports the functioning of the alternative malate-aspartate pathway of ATP production, because of the high level of NAD-MDGmt. HSF-1 increases the expression of HSP<sub>70</sub> prolonging HIF-1a [3,9] in an ischemic condition, as well as independently supports the expression of NAD-MDGmt, thereby sustaining the activity of the malate-aspartate shutter mechanism [9].

Melatonin in ischemia conditions increases the HSP<sub>70</sub> level by activating melatonin receptors MT1 and MT2. In addition, the unique structure of the melatonin molecule makes it an effective ROC / RNC scavenger and prevents total



damage of polypeptide bonds, inactivation of enzyme systems and antioxidant endogenous proteins, including HSP<sub>70</sub>. Also, melatonin is able to exhibit cytoprotective effects by supporting the activity of glutathione peroxidase, Cu, Zn- and Mn-superoxidedismutase, and  $\gamma$ -glutamylcysteine ligases [15]. At the same time, the ability of the hormone to inhibit a number of prooxidant enzymes such as lipoxygenase and NO synthase, which, in ischemia/reperfusion conditions, reduces ROC production. The positive flow of melatonin to energy metabolism is due to its ability to prevent damage to aconitase and thus maintain Krebs' cycle at the citrate-isocitrate stage [16].

The synthetic estrogen receptor modulator, tamoxifen, activates ER- $\beta$  receptors that are inactivated by the HSP<sub>70</sub> proteins under resting conditions. Under the action of the drug is the separation of chaperone molecules and increase its mitoprotective activity. In addition, our previous studies have established direct genomic protection tamoxifen against the HSP<sub>70</sub> mRNA [10]. Tamoxifen is also capable of demonstrating mitoprotective action, protecting the membrane structures of the mitochondria from the effects of ROC and thereby maintaining the functional activity of these organelles [3,17].

Glutamine acts as a substrate for the recovery of depleted resources of the thiol-disulphide system, namely glutathione. Increasing the level of recovered forms of glutathione restores intracellular redox potential and signaling/activity of transcription factors. Support for the optimal ratio of reduced and oxidized forms of glutathione is an important link in the survival of cells. Also, glutathione prevents damage to the structure of HSP<sub>70</sub> in conditions of oxidative stress, maintaining its cytoprotective functions.

## **Conclusions**

1. Bilateral ligation of common carotid arteries leads to typical signs of ischemic brain damage in experimental animals: suppression of the Krebs cycle, activation of low productive anaerobic glycolysis, energy deficit against the reduction of the 70 kDa heat shock proteins concentration. All this lead to reduction of ATP by 60.8% ( $p < 0,05$ ) and ADP by 45,2% ( $p < 0,05$ ), reduction of pyruvate and malate levels by 56,6% ( $p < 0,05$ ) and 55,6% ( $p < 0,05$ ), increase the lactate level by 130,1% ( $p < 0,05$ ), the decrease in the concentration of HSP<sub>70</sub> in cytosol by 89,7% ( $p < 0,05$ ) and mitochondria by 68,4% ( $p < 0,05$ ) respectively to pseudo-operated group.
2. HSP70 modulators: HSF-1 (200  $\mu$ l / kg), tamoxifen (1 mg/kg), melatonin (5 mg/kg), and glutamine (25 mg/kg) significantly increased ( $p < 0.05$ ) the heat shock protein HSP70 in 11.3 times, in 5.1 times, in 2.6 times and 1.5 times in the cytosolic fraction, as well as the concentration of chaperone protein increased in the mitochondrial fraction of the brain in 2.6 times, 1.5 times, 1.2 times and 1.1 times, respectively, in relation to the control group.
3. HSP70 modulators resulted in the normalization of the main parameters of energy metabolism of the brain. ATP/ADP levels were significantly increased ( $p < 0.05$ ) against of HSF-1 by 101.8%/58.1%, melatonin - by 66.4%/

36.5%, tamoxifen - by 50.4%/26.5%, glutamine - by 52.2%/29%. Lactate level was decreased: HSF-1 by 50.2% ( $p<0.05$ ), melatonin by 33.7% ( $p<0.05$ ), tamoxifen by 27.1% ( $p<0.05$ ), glutamin by 16.5%. Increased pyruvate level: HSF-1 by 108.7 % ( $p<0.05$ ), melatonin by 65.2% ( $p<0.05$ ), tamoxifen by 47.8% ( $p<0.05$ ), glutamin by 26.1%. Increased malate level: HSF- 1 on 133.3% ( $p<0,05$ ), melatonin and tamoxifen by 75% ( $p<0.05$ ), glutamine by 50% ( $p<0.05$ ). NAD-MDGmt activity was significantly increased: HSF-1 in 2.7 times, melatonin in 2.2 times, tamoxifen in 2.1 times, glutamine in 1.7 times relative to the control group.

4. The HSP70 modulators provide for the normalization of energy metabolism and enhancement of ATP production in conditions of acute cerebral ischemia due to HSP70-dependent mechanisms of activation and regulation of compensatory malate-aspartate shunt.
5. The leading position in restoring the capacity of mitochondria was revealed by the factor of thermal shock, which increased the content of ATP by 101,8% ( $p<0,05$ ), ADP by 58,1% ( $p<0,05$ ), malate by 133,3% ( $p<0,05$ ), pyruvate by 108,7% ( $p<0,05$ ) and NAD-MDGmt in 2,7 ( $p<0,05$ ) relative to control.

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