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Research Article

Changes In the Course of Energy and Oxidative Processes of The Brain During Its Ischemia

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Abstract

Energy deficiency and oxidative stress are stages of the biochemical cascade in brain damage of ischemic origin. There is information in the literature about their changes in certain types of ischemia, but there are no data on the features and comparative characteristics in cerebral ischemia of varying severity. It was found that the most pronounced disturbances in the prooxidant-oxidant balance and energy metabolism were observed in total cerebral ischemia. Similar, however, less pronounced disorders were found in daily subtotal ischemia and in the subgroup of stepped subtotal ischemia with an interval between ligation of the common carotid arteries of 1 day. The least pronounced disorders were in the subgroup with an interval between ligation of the common carotid arteries 7 days

The aim of the work is to assess the state of the energy and oxidative processes of the brain during its ischemia of varying severity.

Key words: energy metabolism, mitochondria, oxidative stress.

Introduction

Energy deficiency and oxidative stress are stages of the biochemical cascade in brain damage of ischemic origin [3, 4]. There is information in the literature about their changes in certain types of ischemia, but there are no data on the features and comparative characteristics in cerebral ischemia of varying severity.

The aim of the work is to assess the state of the energy and oxidative processes of the brain during its ischemia of varying severity.

Materials and research methods

The experiments were carried out on 60 male outbred white rats weighing 260 ± 20 g in compliance with the requirements of the Directive of the European Parliament and of the Council No. 2010/63/EU of September 22, 2010 on the protection of animals used for scientific purposes.

The studies used models of partial, total, subtotal and stepped subtotal cerebral ischemia [2,6,10]. Partial cerebral ischemia (PCI) was modeled by ligation of one CCA on the right. Total cerebral ischemia (TCI) was modeled by decapitation of animals. Subtotal cerebral ischemia (SCI) was modeled by simultaneous ligation of both common carotid arteries (CCA). Stepwise subtotal cerebral ischemia (SSCI) was performed by successive ligation of both CCAs with an interval of 7 days (subgroup 1), 3 days (subgroup 2), or 1 day (subgroup 3). The control group consisted of sham operated rats of similar sex and weight [1, 2,10].

Modeling of cerebral ischemia (CI) was performed under conditions of intravenous thiopental anesthesia (40-50 mg/kg). The material was taken 1 hour after decapitation. Energy and oxidative processes have been studied, and the severity of morphological changes in brain neurons during its ischemia of varying severity has been assessed [6,8].

The state of energy was assessed by the activity of mitochondrial respiration using succinate and the malate/glutamate complex as substrates, which makes it possible to assess the degree of functional activity of the electron transport chain (ETC) in mitochondria as a whole, in particular, I and II of the ETC complex [1,6].

The following indicators of mitochondrial respiration were recorded: V_1 – basal respiration rate; V_2 – substrate-dependent respiration rate; V_3 –respiration rate associated with phosphorylation (after ADP addition); V_4 –the respiratory rate 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 coefficient of acceptor control (AC= V_3/V_2), the coefficient of respiratory control (RC= V_3/V_4) and the coefficient of phosphorylation - ADP/O. The study of mitochondrial respiration of the brain was carried out after its homogenization in the isolation medium according to the modified method [1,2,6,10].

To assess the severity of oxidative processes in brain homogenates, the level of antioxidant protection (indicators of total SH-groups, GSH concentrations, glutathione peroxidase activity), and the state of lipid peroxidation (the content of products that react with thiobarbituric acid) were determined on a spectrophotometer PV 1251C (Solar, Belarus) [5].

In order to study the consequences of energy disorders and changes in oxidative stress activity, a morphological study of neurons in the parietal cortex of the rat brain was carried out using an Axioscop 2 plus microscope (Zeiss, Germany), a digital video camera (LeicaDFC 320, Germany) and ImageWarp image analysis program (Bitflow, USA). In histological studies, the size and shape of the perikaryons of rat brain neurons were determined, and changes in cytoplasmic chromatophilia were studied using conventional methods [1,2,4].

Statistical research.

Since the experiment used small samples that had a non-normal distribution, the analysis was performed by nonparametric statistics using the licensed computer program Statistica 10.0 for Windows (StatSoft, Inc., USA). The data are presented as Me (LQ; UQ), where Me is the median, LQ is the value of the lower quartile; UQ is the value of the upper quartile. Differences between groups were considered significant at $p<0.05$ (Kruskell-Walli's test with Bonferoni correction).

Research results

The study of the respiration of the mitochondrial fraction of brain homogenates in rats with PCI for 1 hour did not reveal any changes in the parameters of mitochondrial respiration and prooxidant-antioxidant balance compared with the control (p>0.05). There were no morphological changes either [3,6].

In the "TCI" group, with a complete cessation of blood circulation, the most pronounced changes in the studied parameters were noted. In the study of respiration of the mitochondrial fraction of brain homogenates, in comparison with the indicators in the control group, in the presence of malate/glutamate, V_1 decreased by 65(58;67) %, p<0.05; V_2 – by 41(38;48) %, p<0.05; V_3 – by 25(22;38) %, p<0.05; phosphorylation coefficient – by 78(71;84) %, p<0.05. Acceptor control and respiratory control coefficients did not change (p>0.05). In the presence of succinate, V_1 decreased by 44(38;52) %, p<0.05, V_2 – by 60(48;64) %, p<0.05, V_3 – by 59(38;65) %, p<0.05, V_4 – by 32(28;46) %, p<0.05. The respiratory control coefficient (V_3/V_4) decreased by 45(42;48) % (p<0.05), the phosphorylation coefficient (ADP/O) in TCI was equal to zero. The decrease in the rate of basal respiration V_1 was more pronounced when using the succinate substrate (by 21%, p<0.05), which indicates a greater damage to the II complex (succinate dehydrogenase). Changes in energy metabolism were accompanied by the most pronounced changes in the prooxidant-antioxidant balance [4,7]. Thus, compared with the level in the control group, there was a decrease in the indicators of antioxidant defense mechanisms - total SH-groups of proteins and glutathione by 82 (79; 87) %, p<0.05, GSH concentration – by 75 (71; 81) %, p<0.05, as well as zero activity of glutathione peroxidase, which may be associated with both impaired expression of the enzyme and damage to it by radicals [3,4,6]. The content of TBARS didn't change (p>0.05), due to the complete absence of oxygen necessary for their production. Violations of energy and oxidative processes were reflected at the morphological level: hyperchromic wrinkled neurons made up a large proportion of the cell population, both normochromic and hyperchromic neurons were absent, a decrease in the size of neuronal perikaryons was observed (by 74 (68; 78) %, p <0.05), aggravation of their elongation (by 35(28;39) %, p<0.05) [3,4,6].

In the " SCI " group, which provides for the shutdown of 90% of the blood flow, compared with the "control" group, in the presence of malate / glutamate, an increase in V_2 by 24 (18; 27) %, p < 0.05, was noted, the coefficients of acceptor control and phosphorylation decreased by 25(17;29) %, p<0.05. In the presence of the succinate substrate, an increase in V_1 was also noted – by 38 (34;42) %, p<0.05, V_2 – by 13(9;18) %, p<0.05, V_3 – by 26(21;32) %, p<0.05. At the same time, the coefficient of acceptor control, the coefficients of respiratory control and phosphorylation decreased by 35(31;39) %, p<0.05, 20(18;28) %, p<0.05 and by 36(30;41) %, p<0.05, respectively, which indicates a decrease in energy production. An increase in V_1 and и V_2 and a decrease in the phosphorylation coefficient (ADP/O) indicates proton transfer bypassing the ATP synthase complex. The enzymes of the mitochondrial matrix and cytochromes in this model of CI do not yet have pronounced damage, as evidenced by the high rates of V_1 and V_2, however, a decrease in the coefficients of acceptor control, respiratory control, and phosphorylation indicates uncoupling of the processes of oxidation and phosphorylation and a decrease in ATP production. More pronounced disturbances with the use of succinate than with the use of malate/glutamate, as in TCI, indicate greater damage to the succinate dehydrogenase complex of the ETC [3,4,6,7].

However, changes in a number of indicators of mitochondrial respiration (V_1, V_2 and V_3) with 1-hour SCI and 1-hour TCI were multidirectional. Their increase in SCI is associated with uncoupling of oxidation and phosphorylation, while their decrease in TCI is associated with a lack of substrates for mitochondrial respiration, in both cases leading to a decrease in energy production [3,7,10]. Energy disorders in the "SCI " group were accompanied by a decrease, compared with the "control" group, in the indicators of non-

enzymatic mechanisms of antioxidant defense - total SH-groups of proteins and glutathione by 56 (49; 61) %, p<0.05, GSH concentration - by 57(51;63) %, p<0.05, as well as high intensity of enzymatic mechanisms (glutathione peroxidase activity increased by 12(9;18) %, p<0.05). In addition, there was an increase in the content of TBARS – by 32(27;38) % p<0.05, which is a marker of oxidative stress. Compared with TCI, the decrease in the content of total SH-groups of proteins, glutathione and GSH concentration was less significant (the difference was 26%, p<0.05 and 18%, p<0.05, respectively). At the histological level, with SCI, there was an increase in the proportion of hyperchromic wrinkled neurons, a decrease in the number of normochromic and hyperchromic neurons, however, in contrast to TCI, they persisted [6,7,9]. The sizes of neuronal perikaryons decreased by 52(47;58) %, p<0.05, while their elongation increased by 20(18;29) %, p<0.05. The decrease in the size of neurons was less significant than with TBI (by 22%, p<0.05) [9].

Compared with the "control" group, in the 1st subgroup of SSCI with an interval between ligation of both common carotid arteries of 7 days, in the presence of malate/glutamate in the 1st subgroup of SSCI, an increase in V_2 by 46 (39;56) % was observed, p<0.05 and the coefficient of respiratory control by 56 (51;63) %, p<0.05, indicating the activation of the transport of hydrogen protons through the ATP-synthase complex. In the presence of succinate, there was an increase in V_1, V_4 by 45 (39;52) %, p<0.05 and by 47 (37;55) %, p<0.05, respectively, the respiratory control coefficient - by 48 (41; 56) %, p<0.05. In this subgroup, there was a decrease in the content of total SH-groups of proteins and glutathione by 8(4;12) %, p<0.05, GSH concentration by 20(17;28) %, p<0.05, as well as an increase in the activity of glutathione peroxidase – by 5(3;9) %, p<0.05. The content of TBARS increased by 23 (18;29) % p<0.05 [5,7].

In the 2nd subgroup of SSCI with an interval between dressings of 3 days, compared with the "control" group in the presence of the "malate/glutamate" substrate, there was only a decrease in V_4 by 49(32;54) %, p<0.05. V_2 and V_4 values were less by 58(42;69) %, p<0.05, and the phosphorylation coefficient was by 50(41;65) %, p<0.05. In the presence of succinate, the rates of V_2, V_3 and V_4 decreased by 61 (51;69) %, p<0.05, 48 (40;52) %, p<0.05 and 30 (28;39) %, p <0.05, respectively. In addition, when studying the prooxidant-oxidant balance, there was a decrease in the content of total SH-groups of proteins and glutathione by 30(25;37) %, p<0.05, GSH concentration by 31(27;38) %, p<0.05, as well as an increase in the activity of glutathione peroxidase - by 8(5;13) %, p<0.05. The content of TBARS increased by 29(23;37) % p <0.05 [1,5,7,10].

In the 3rd subgroup of SSCI with a minimum interval between dressings of both CCAs of 1 day, compared with the control group in the presence of succinate, there was a decrease in the rates of V_2, V_3 and V_4 by 52(43;65) %, p<0.05, 61(52;69) %, p<0.05 and 65(58;71) %, p<0.05, respectively. The coefficient of respiratory control also decreased by 42(37;49) %, p<0.05 and the coefficient of phosphorylation by 37(28;45) %, p<0.05. A decrease in V_2, V_3, V_4 and respiratory control coefficient in the 3rd subgroup of SSCI indicates damage to electron carriers in mitochondria and Krebs cycle enzymes, which, in turn, led to a decrease in energy production, as evidenced by a low phosphorylation coefficient. When studying oxidative processes, it was found that in this subgroup there was a decrease in the content of total SH-groups of proteins and glutathione by 46(35;52) %, p<0.05, GSH concentration by 57(49;65) %, p<0 .05, as well as an increase in glutathione peroxidase activity by 9(4;15) %, p<0.05. The content of TBARS increased by 31(26;39) %, p <0.05 [3,4,6].

In general, the greatest disturbances in the energy and oxidative processes of the brain were observed in the 2nd and 3rd subgroups of SSCI, which indicates the highest activity of oxidative stress. At the same time, the indicators of mitochondrial respiration and prooxidant-antioxidant balance in these subgroups were the closest to those in the SCI group, while in the 1st SSCI subgroup with an interval between ligation of both common carotid

arteries of 7 days, they were the same as in the PCI group. At the morphological level, step-by-step SCI with an interval of 1 and 3 days between dressings of both CCAs led to neuronal damage, which manifests itself in a decrease in their size, deformation of perikaryons, and an increase in the number of shrunken neurons and shadow cells. The most pronounced changes were observed in the subgroup with an interval between dressings of 1 day. These changes were similar to those in SCI ($p>0.05$). SSCI with an interval between CCA ligations of 7 days, on the contrary, is manifested by a lesser severity of histological changes: the size of the neuronal perikaryons and the ratio of neurons in terms of the degree of cytoplasmic chromatophilia differed slightly from those in the "PCI" group [5,7,9].

Thus, the most pronounced morphological and functional disorders (depression of mitochondrial respiration, suppression of antioxidant protection, shrinkage of neurons) occur during the simulation of TCI [10]. Subtotal ischemia, modeled by simultaneous ligation of both CCAs, and stepwise ligation of both CCAs with an interval of 1 day also led to severe irreversible neuronal damage: an increase in the number of hyperchromic shriveled neurons corresponded to inhibition of respiration of the mitochondrial fraction of brain homogenates and activation of peroxide processes [10]. When modeling SCI, the blood circulation in the circle of Willis is compensated, which explains a slight, compared with TCI and SCI, decrease in respiratory rates of the mitochondrial fraction of brain homogenates, as well as maintaining the prooxidant-antioxidant balance [1,2,8,10]. When the CCA was ligated with an interval of 7 days, the ratio of neurons according to the degree of chromatophilia of the cytoplasm and the size of the pericaryons of neurons did not differ from the values of the indicator in the control group. In the same animals, the respiratory parameters of the mitochondrial fraction of brain homogenates and the prooxidant-antioxidant balance were insignificant [1,2,8]. It was noted that the antioxidant protection of the brain was preserved as a manifestation of the activation of compensatory mechanisms: an increase in the efficiency of the processes of utilization of oxygen and oxidation substrates and their delivery to the mitochondria of neurons due to the effects of neuroglobin, an increase in the synthesis of nucleic acids and proteins, the transport of O_2 and metabolic substrates, the dominance of the activity of anabolic processes over catabolic [4], which reduces the severity of oxidative stress.

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