



Amino Acid Pool Disorders in Rats in the Hippocampus in Modeling Partial Cerebral Ischemia

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RESEARCH ARTICLE

ABSTRACT

Cerebrovascular diseases are widespread throughout the world, with a significant change in the brain pool. As shown earlier, when modeling partial cerebral ischemia (PCI) by unilateral ligation of the common carotid artery (CCA), after 1 hour, there were no pronounced morphological changes at the microscopic and ultrastructural levels. At the same time, no pronounced changes in the parameters of respiration of the mitochondrial fraction and changes in the parameters of the prooxidant-antioxidant balance of brain homogenates, compared with the control, were observed, which reflects the relative safety of the enzymatic complexes of the electron transport chain in this model of ischemia. However, the results of behavioral tests indicated the development of a minor neurological deficit, and the existing decrease in the content of ATP synthase is the rationale for a possible cause of its development. Thus, changes in the pool of amino acids in the hippocampus were of a similar nature, with the exception of a more significant decrease in the level of methionine in the parietal lobe as a reflection of a higher activity of oxidative processes in this area of the brain.

KEYWORDS

Ischemia, Amino acid pool, Rat, Experimental, Hippocampus

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INTRODUCTION

Cerebrovascular diseases are widespread throughout the world, with a significant change in the brain pool [1-10]. As shown earlier, when modeling partial cerebral ischemia (PCI) by unilateral ligation of the common carotid artery (CCA), after 1 hour, there were no pronounced morphological changes at the microscopic and ultrastructural levels [7]. At the same time, no pronounced changes in the parameters of respiration of the mitochondrial fraction and changes in the parameters of the prooxidant-antioxidant balance of brain homogenates, compared with the control, were observed, which reflects the relative safety of the enzymatic complexes of the electron transport chain in this model of ischemia [1-10]. However, the results of behavioral tests indicated the development of a minor neurological deficit [4], and the existing decrease in the content of ATP synthase is the rationale for a possible cause of its development [1,2,3].

MATERIALS AND RESEARCH METHODS

The experiments were carried out on 16 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. IHM modeling was carried out under conditions of intravenous thiopental anesthesia (40-50 mg/kg). Partial cerebral ischemia (PCI) was modeled by ligation of one common carotid artery on the right. The control group consisted of sham-operated rats of similar sex and weight.

The material was taken 1 hour after the operation. After removing the brain, a fragment of the hippocampus was taken, followed by freezing in liquid nitrogen. Sample preparation for the study included homogenization in a 10-fold volume of 0.2 M perchloric acid, centrifugation for 15 min. at 13000 g at 4°C, followed by collection of the supernatant. Amino acids were analyzed by reversed-phase chromatography with pre-column derivatization with

o-phthalaldehyde and 3-mercaptopropionic acid in Na-borate buffer on an Agilent 1100 chromatograph. When studying the content of amino acids (AA) in the homogenates of the hippocampus in rats with one-hour PCI, an increase in the median values of the content of AA with neurotransmitter properties, an increase in glutamate by 19%, $p < 0.05$, was revealed.

An increase in glutamate levels while maintaining glutamine and GABA levels may be associated with an increase in glutaminase activity and/or transamination/reductive amination in neurons. At the same time, changes in the levels of AA with the properties of excitatory neurotransmitters (aspartate and glutamate) had a multidirectional character: a tendency to an increase in the level of glutamate and to a decrease in aspartate. The decrease in aspartate, in contrast to glutamate, can be explained by its increased utilization as a glycolytic AA in oxidation reactions with the formation of energy.

As for inhibitory mediators (GABA, glycine, taurine), one can note a trend towards an increase in their content in the hippocampus: taurine by 29% ($p > 0.05$), GABA - by 41% ($p > 0.05$). At the same time, the median GABA level differed from its value in the control group. Changes in the level of glycolytic AA (aspartate, asparagine, threonine, serine, glutamine, glutamate, glycine, alanine, valine, methionine, histidine, arginine) and ketogenic AA (leucine, lysine) for the brain, from the point of view of biochemical research, are mostly non-specific, since the processes of gluconeogenesis and ketogenesis do not occur in the brain. However, it should be noted that aspartate, glutamate and glycine play the role of neurotransmitters; methionine is a precursor to a number of compounds with antioxidant properties; arginine is a substrate for NO synthase, and lysine is a precursor of the endogenous NMDA receptor antagonist α -amino adipate.

With PCI, there was an increase in the median value of L-arginine by 46% ($p < 0.05$). An increase in the content of L-arginine and citrulline indicates a decrease in the utilization of the substrate for NO synthase, which may be the cause of the existing slight neurological deficit due to the lack of NO, one of the mediators of the central nervous system that mediates the regulation of many processes, including higher nervous activity. At the same time, in the hippocampus, there was a tendency to increase the median values of the levels of other sulfur-containing AAs, most of all taurine by 29% ($p > 0.05$) and cysteine sulfinic acid by 29% ($p > 0.05$), without changing the levels of cysteate and cystathionine, as a reflection of the low activity of oxidative processes in this type of cerebral ischemia.

In rats with PCI, there was a decrease in the median values of the content of branched hydrocarbon amino acids (BCAAs), as AA, included in the energy processes in the hippocampus: valine - by 37% ($p < 0.05$), isoleucine - by 36% ($p < 0.05$) and leucine by 32% ($p < 0.05$), which may be due to their utilization in transamination reactions. Along with changes in amino acids with a branched hydrocarbon chain, there was a trend towards a decrease in the levels of aromatic AAs (tyrosine, tryptophan, phenylalanine), which may reflect their utilization in catecholamine and serotonin synthesis reactions or a decrease in transport to the brain. At the same time, in the hippocampus there was a tendency to reduce the ratio of ARUC to aromatic AA from 1.56 to 1.13 ($p > 0.05$).

There was a decrease in the median values of all essential AA in the hippocampus of valine by 37% ($p < 0.05$), isoleucine - by 36% ($p < 0.05$) and leucine by 32% ($p < 0.05$), and there was also a tendency to a decrease in lysine by - 55% ($p > 0.05$) and threonine - by 32% ($p > 0.05$) in the hippocampus, which indicates its possible inclusion in metabolic processes, primarily as alternative energy substrates, as the result of inhibition of glucose utilization processes. At the same time, the indicator of the "Replaceable/Essential" AA ratio in the PHI group increased from 8.4 to 16.4 in the hippocampus ($p > 0.05$), which may be a consequence of impaired utilization of nonessential AAs in protein synthesis reactions along with increased utilization of irreplaceable AAs.

DISCUSSION & RESULT

One-hour PCI is characterized by the following changes in the AA pool: an increase in glutamate and GABA without changes in the ratios of excitatory and inhibitory amino acid transmitters, a decrease in the level of essential AAs with an increase in the "Essential/Essential" AA ratio, as a reflection of increased utilization of essential AAs. There was a decrease in the content of amino acids with a branched hydrocarbon chain and a trend towards a decrease in the level of aromatic AAs (tyrosine, tryptophan, phenylalanine) with a decrease in their ratio as a reflection of a more pronounced utilization of BCAAs, compared with aromatic AAs.

Thus, changes in the pool of amino acids in the hippocampus were of a similar nature, with the exception of a more significant decrease in the level of methionine in the parietal lobe as a reflection of a higher activity of oxidative processes in this area of the brain.

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