Kinetic Characteristics of the Energy Production System in Rat Brain under Conditions of Posthypoxic Encephalopathy and Its Correction with *Bergeniae crassifolia* Extract

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Posthypoxic encephalopathy is characterized by inhibition of the succinate oxidase stage in cerebral energy production. When administered to rats exposed to hypoxia, *Bergeniae crassifolia* extract reduced mortality and restricted the inhibition of rapid metabolic cluster reactions.

Key Words: *Bergenia crassifolia extract; posthypoxic encephalopathy; brain mitochondria; NADH*

This study analyzes the role of rapid and slow metabolic clusters [2,3] in the maintenance of brain energy homeostasis by measuring the level of NADH fluorescence in rat brain mitochondria during posthypoxic encephalopathy (PHE) and its correction with extract from *Bergenia crassifolia* leaves. Previous screening revealed high cerebroprotective activity of this preparation in hypoxia [4,5]. The present study used an original approach to the assessment of energy production based on the analysis of kinetic characteristics of pyridine nucleotide reduction (PNR) during phosphorylation of exogenous ADP [4].

MATERIALS AND METHODS

The study was carried out on 2-month-old male Wistar rats weighing 180-200 g (Laboratory of Experimental Biomedical Modeling, Tomsk Research Center). Piracetam (400 mg/kg) and extract of *Bergenia crassifolia* leaves (300 mg/kg) were suspended in water and administered intragastrically for 5 days starting from day 14 after hypoxia. Hypoxia was modeled in a hermetically sealed 5-liter jar until the start of agony (3.5-4.0 h), after which the jar was open, and the animal was returned to its home cage. After the 19th hypoxia session the rats were decapitated under ether anesthesia.

Isolated brains were homogenized in an ice-cold medium containing: 1.2×10^{-1} M KCl, 2×10^{-3} M K_2CO_3, 10^{-2} M HEPES, 2×10^{-4} M EDTA (pH=7.2), 2×10^{-3} M KH_2PO_4 (pH=7.2 at 26°C) was added during incubation. The functional state of the energy production system was evaluated by the level of reduced pyridine nucleotides [9].

The data were analyzed statistically using paired Wilcoxon—Mann—Whitney test.

RESULTS

In the experimental animals, the time of NAD reduction after the addition of ADP to cerebral mitochondria oxidizing an endogenous substrate was 2.65-fold prolonged and the rate of transition from *2AP* to *2R* metabolic state was 2.8 times lower compared to the control (Table 1). During oxidation of exogenous succinate, the time of NAD reduction was 2.3-fold prolonged and the rate of transition from metabolic state *3* to *4R* was 2.5 times lower than the corresponding control values. During oxidation of NAD-dependent substrates, Tr_3 increased 1.95-fold and V_3 decreased 2.9-fold. These changes indicate that hypoxic injury causes pronounced negative shifts in the brain energy metabolism [1].

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Taking into account the contribution of rapid metabolic cluster to maintenance of brain energy homeostasis [2,3], we assumed that inhibition of *3* to *4R* transition and PNR during utilization of NAD-dependent endogenous substrates is caused by succinate. In the control animals, the SDH inhibitor malonate significantly accelerated PNR (by 120%), while the aminotransferase inhibitor aminooxyacetate 3-fold increased the rate of RPN and 10-fold decreased the rate of transition from metabolic state *3* to *4R*. In rats subjected to hypoxic injury malonate 2-fold decreased V_3 during NAD-dependent substrate oxidation. The aminotransferase inhibitor 3-fold accelerated RPN and 3.5-fold decreased the rate of transition from state *3* to *4R* (Fig. 1). Therefore, the inhibition of endogenous succinate oxidation and restriction of substrate production by transamination considerably affected mitochondrial energetics. Our data confirm the contribution of succinate-dependent energy production to maintenance of the reduced pyridine nucleotide pool [10] and emphasize the role of rapid metabolic cluster in maintenance of the energy homeostasis in brain mitochondria.

Taking into account reactions of the energy production system to ischemia [6,8] and the inhibition of succinate-dependent energy production in the brain under conditions of extreme energy demands [7], we can assume that inhibition of the NAD-reductase stage of the mitochondrial respiratory chain is associated with inhibition of rapid metabolic cluster reactions.

The extract of *Bergenia crassifolia* prevented the death of experimental animals which occurred in 33-45% cases on day 21 after hypoxic exposure [5]. Administration of the extract normalized the time of NAD reduction during oxidation of endogenous substrate (Tr_2 decreased 2-fold), reduced the level of oxidized products at the initial stage of mitochondrial respiration during the oxidation of exogenous substrates in all metabolic states (normalization of F_{4S}, F_3, and F_{4R} values), and normalized the time of NAD reduction during the oxidation of both endogenous and exogenous substrates (Tr_3 decreased 2-fold). The extract of *Bergenia crassifolia* normalized kinetic characteristics of the energy production system accelerating reduction of NAD coupled to the phosphorylation of exogenous ADP during oxidation of both endogenous (2-fold increase in V_2) and exogenous (1.5-fold increase in V_3) substrates.

Experiments with SDH and transaminase inhibitors showed that the cerebroprotective effect of *Bergenia crassifolia* extract is associated with normalization of succinate-dependent energy production and rapid metabolic cluster reactions (Fig. 1). In animals exposed to hypoxia and treated with the *Bergenia crassifolia* extract, all studied indices approached the control level.

TABLE 1. Effect of PHE and Test Preparations on Characteristics of Rat Brain Mitochondria (n=5)

Parameters	Intact control		PHE		PHE+BCE		PHE+piracetam		Control+BCE		Control+piracetam	
	Succinate	Malate+ glutamate	Succinate	Malate+ glutamate	Succinate	Malate+ glutamate	Succinate	Malate+ glutamate	Succinate	Malate+ glutamate	Succinate	Malate+ glutamate
F_{2S}	0.489	0.502	0.499	0.499	0.509	0.502	0.519	0.527	0.509	0.512	0.500	0.497
F_{2AP}	0.400	0.402	0.399	0.386	0.402	0.400	0.410	0.414	0.403	0.400	0.399	0.387
F_{2R}	0.445	0.442	0.44	0.429	0.454	0.448	0.482	0.470	0.463	0.460	0.456	0.441
Tr_2	38.6	39	109*	112*	53*,+	53.4*,+	41.4*,+	49.8*,*,+,+	34*	33*	42*	41
F_{4S}	0.529	0.534	0.495*	0.508*	0.531	0.520	0.539	0.556	0.528	0.524	0.532	0.511
F_3	0.424	0.447	0.393*	0.418*	0.424	0.441	0.421	0.468	0.413	0.445	0.410	0.429
F_{4R}	0.507	0.514	0.468*	0.479*	0.500	0.510	0.515	0.536	0.513	0.509	0.517	0.489
Tr_3	38.2	26	86.5*	50.8*	42.6+	26+	42*	24.6*,+	29*	19*	34*	21.2*
V_2	3.16	3.26	1.34*	1.32*	2.64*,+	3.52*,+	3.44*,+,+	3.37*,+,+	4.96*	4.29	4.20*	4.47
V_3	6.58	8.12	2.59*	2.8*	3.74*,*,+	4.96*,+	4.87*,+,+	5.9*,*,+,+	6.28	8.11	5.37*	8.97

Note. BCE: *Bergenia crassifolia* extract. p<0.05 in comparison with: *intact control; **PHE; +control+BCE; ++control+piracetam.

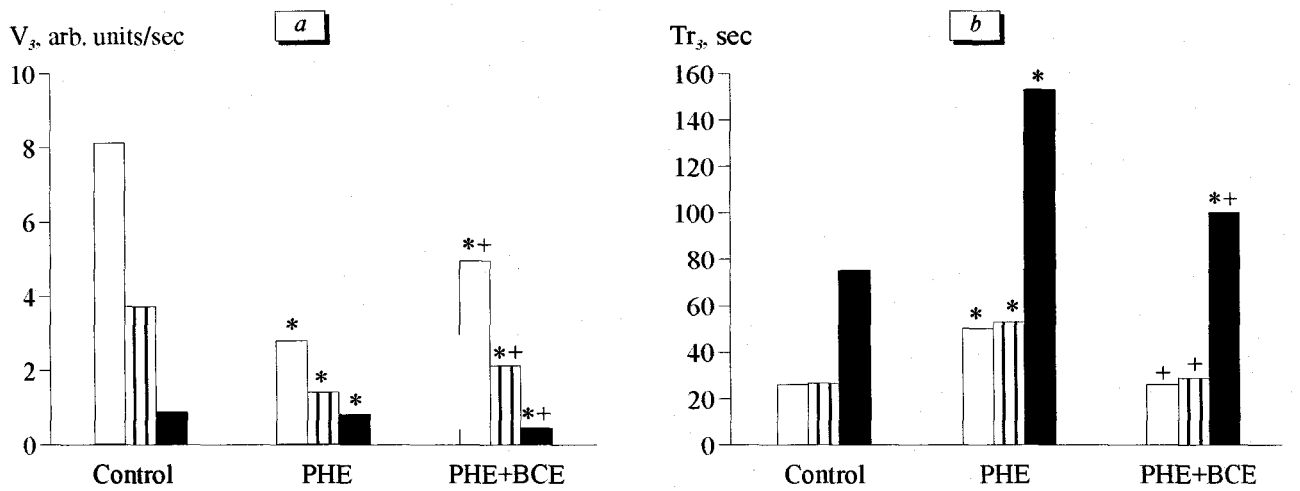

Fig. 1. Effect of malonate and aminoacetate on the NAD fluorescence of rat brain mitochondria. Open bars: malate with glutamate; shaded bars: malate with glutamate+malonate; filled bars: malate with glutamate +aminooxyacetate. $p<0.05$ in comparison with the control (*) or posthypoxic encephalopathy, PHE (*). BCE: *Bergenia crassifolia* extract.

Thus, evaluation of cerebral energy production by the rate of transition from *3* to *4R* mitochondrial metabolic state and the time of RPN in combination with inhibitory analysis made it possible to reveal a considerable contribution of rapid metabolic cluster reactions to metabolic disturbances in PHE. *Bergeniae crassifolia* extract exerted a cerebroprotective effect by preventing inhibition of the succinate oxidaze system, more resistant to oxygen deficiency than NAD-dependent energy production. The observed antiradical properties of this preparation can also contribute to the mechanisms of its cerebroprotective effect.

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