PHARMACOLOGY AND TOXICOLOGY

Effect of Meadowsweet (*Filipendula vulgaris*) Extract on Bioenergetics of the Brain during Experimental Posthypoxic Encephalopathy

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We studied the effect of *Filipendula vulgaris* aqueous extract on mitochondrial energy production system in the brain of rats with posthypoxic encephalopathy developing 19 days after hypoxic injury. *Filipendula* extract more effectively than valerian extract improved kinetic characteristics of respiratory activity of mitochondria, increased substrate oxidation–phosphorylation coupling, and inhibited LPO.

Key Words: *experimental posthypoxic encephalopathy; brain mitochondria; Filipendula vulgaris extract*

The disintegration of brain systems providing search, perception, processing of information, and emotional reaction play an important role in the pathogenesis of anxious disorders [2]. Insufficient energy supply for psychophysiological processes can contribute to the development of psychopathology [10,11]. Herbal anxiolytics are now widely used in the therapy of anxiety disorders [1,6]. Meadowsweet improving GABAergic inhibition in the brain is a promising plant for developing effective anxiolytics [6].

Here we studied the effect of meadowsweet aqueous extract on brain bioenergetics in rats with experimental encephalopathy caused by hypoxic injury.

MATERIALS AND METHODS

Experiments were carried out on 60 outbred male rats weighing 200-220 g (Institute of Pharmacology, Siberian branch of the Russian Academy of Medical Sciences) in winter and spring. The animals were kept under standard vivarium conditions at natural illumination with free access to food and water. Studies were carried out in accordance with Manual on Experimental (Preclinical) Study of New Pharmacological Agents [5].

Posthypoxic encephalopathy was modeled by placing the rats in a sealed chamber (volume 3 liters) until the first agonal breath. Thereafter, the animals were removed and left free breathing room air. Starting from day 14 after hypoxic injury, aqueous meadowsweet extract in an effective therapeutic dose of 50 mg/kg was administered into the stomach for 5 days. The dose was determined by antianxious activity in an experimental conflict situation. Aqueous valerian root and rhizome extract (Dal'khimfarm) in the same dose was used as the reference preparation. Aqueous meadowsweet extract includes simple phenols (saligenin, salicin), flavonoids (quercetin, kaempferol, apigenin, luteolin, dihydroquercetin, isoquercitrin, avicularin, spiraeoside, rutin), coumarins (coumarin, umbelliferone, esculetin, fraxetin). The plant material was standardized by the total flavonoid content, which amounts

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1.7±0.1% in the above-ground parts of *Filipendula vulgaris*.

The control animals received the same volume of distilled water. On day 6, the rats were decapitated under light ether anesthesia.

Functional state of mitochondria of brain homogenate was studied by polarography (Expert-001 polarograph) by the rate of oxygen consumption in different metabolic states after Chance. Oxygen consumption rate was calculated before (V_4), during (V_3) and after (V_{3-4}) cycle of phosphorylation of ADP (50 μM) added during oxidation of endogenous substrates, flavin adenine dinucleotide-dependent substrate succinate (1 mM) and NAD-dependent substrates malate and glutamate (3 mM each). To determine the degree of inhibition of succinate dehydrogenase, its activator, isocitrate, was added (1.5 mM). The contribution of flavin adenine dinucleotide-dependent respiration to mitochondrial oxidation of NAD-dependent substrates was assessed by changes in the rate of phosphorylation after addition of succinate dehydrogenase (SDH) inhibitor malonate (2 mM). To estimate the energy status of mitochondria, indices of respiration stimulation ($RS=V_3/V_4$), respiratory control ($RC=V_3/V_{3-4}$), and oxidation-phosphorylation coupling (ADP/O) were calculated. LPO intensity was estimated by the rate of malonic dialdehyde (MDA) formation in the presence of ascorbate as the initiator of oxidation and contents of conjugated dienes and Schiff bases [9].

Statistical analysis was performed by pairwise comparisons using nonparametric Mann–Whitney test with probability of an erroneous conclusion less than 5% ($p<0.05$).

RESULTS

A decrease in oxygen supply below the physiological level triggers a cascade of biochemical events underlying tissue degradation. The mechanisms of neuronal damage include depletion of energy resources, disturbance of ion homeostasis, accumulation of excitatory amino acids, and hyperproduction of reactive oxygen species [3,7,12]. At the functional level, energy-dependent functions specific for nervous tissue are inhibited due to suppression of the aerobic energy production and ATP depletion [4].

In our experiments, encephalopathy caused by hypoxic injury was accompanied by significant changes in brain energy production (Table 1). During oxidation of endogenous substrates, the rates of oxidative phosphorylation V_4 and V_{3-4} 2-fold increased, while V_3 rate increased by 14% compared to that in intact animals. ADP/O decreased by 18%. Under conditions of substrate load with succinate, active phosphorylation (V_3) was accelerated by 22%, coupling of oxidation with ATP synthesis increased by 1.5 times. Active phosphorylation of succinate was accelerated by adding isocitrate. NAD-dependent respiration was characterized by an increase in V_4 and V_{3-4} by 1.2 and 1.6 times, respectively, and V_3 rate tended to increase. ADP/O decreased by 17%. Malonate inhibited active phosphorylation by 26% compared to the intensity of this process in the test without SDH inhibition. V_{3-4}/V_4 ratio decreased in all metabolic states of mitochondria, the indices RS and RC were therefore lower than in intact animals.

Under conditions of experimental posthypoxic encephalopathy, LPO in the brain substantially increased (Table 2). Spontaneous and ascorbate-dependent MDA generation was accelerated by 2.1 times. The amount of conjugated dienes and Schiff bases increased by 2.4-2.5 times.

Thus, bioenergetic disorders during posthypoxic encephalopathy are characterized by monopolization of mitochondrial respiratory chain in the brain by succinate, a substrate for more kinetically favorable oxidation pathway of energy production. This is proved by the acceleration of oxidative phosphorylation during utilization of succinate and a simultaneous increase in its contribution to respiration during NADH-oxidase-dependent oxidation. SDH reaction to isocitrate addition attests to moderate degree of mitochondrial strain and their preserved ATP-synthesizing capacity. The status of mitochondrial energy production after hypoxic injury can be characterized as transition to a resistant phase of functioning [8].

Experimental therapy of posthypoxic encephalopathy with meadowsweet and valerian extracts improved respiratory function of brain mitochondria (Table 1). Respiration rate V_3 during oxidation of endogenous substrates increased by 20 and 17% after administration of *Filipendula* and valerian extracts, respectively, compared to the rate measured in non-treated rats subjected to brain hypoxia. ADP/O and RC tended to increase in this experiment. DM increased by 25 and 30% after treatment with *Filipendula* and valerian extracts, respectively.

In brain mitochondria of rats treated with meadowsweet extract, flavin adenine dinucleotide-dependent respiration was accompanied by acceleration of active phosphorylation (V_3) by 15% compared to the rate observed in posthypoxic encephalopathy. Treatment with valerian extract did not increase this parameter. ADP/O, RC, and RS remained the same as in animals with hypoxia not protected with phytopreparations. When SDH was activated with isocitrate, V_3 respiration rate increased by 15 and 11% in animals receiving meadowsweet and valerian extracts, respectively, compared to the rate measured in the absence of isocitrate. Oxidation of malate and glutamate was not accompanied by significant changes in

rates of phosphorylation. ADP/O increased (by 13%) only when extract of *Filipendula* was administered. In the test with utilization of NAD-dependent substrates against the background of inhibition of SDH with malonate, phosphorylation was decelerated by 17 and 11% after treatment with *Filipendula* and valerian extracts, respectively, compared to the value in mitochondrial suspension without inhibitor. Treatment with *Filipendula* extract increased ADP/O in NAD-oxidase oxidation pathway compared to this index in encephalopathy. After treatment with valerian extract, ADP/O increased slightly.

TABLE 1. Effect of *Filipendula vulgaris* and Valerian Extracts on Mitochondrial Respiration in Brain Homogenates from Rats with Posthypoxic Encephalopathy (*X*±*m*)

Parameters of mitochondrial respiration		Intact animals	Posthypoxic encephalopathy	Posthypoxic encephalopathy+	
				Filipendula extract	valerian extract
Oxidation of endogenous substrates	V_4	5.2±0.6	10.2±0.1*	10.1±0.3*	9.5±0.2*^{x}
	V_3	14.7±0.9	16.8±2.0	20.1±1.0*$^{+}$	19.6±0.7*
	V_{3-4}	4.6±0.2	8.1±0.4*	8.7±0.4*	10.0±0.5*^{x}
	ADP/O	2.8±0.2	2.3±0.2*	2.4±0.1*	2.5±0.1
	RS	2.4±0.1	1.6±0.1*	2.0±0.2*$^{+}$	2.1±0.1*$^{+}$
	RC	3.1±0.3	2.1±0.2*	2.3±0.1*	1.93±0.08*^{x}
Succinate oxidation	V_4	12.6±0.6	17.4±1.1*	18.6±1.0*	15.5±0.5*^{x}
	V_3	26.4±1.3	32.3±1.7*	37.1±0.6*$^{+}$	33.5±1.2*^{x}
	V_{3-4}	11.0±0.8	14.2±0.8*	18.9±1.9*	15.3±0.7*^{x}
	ADP/O	1.5±0.2	2.2±0.1*	2.2±0.1*	2.0±0.2*
	RS	2.3±0.3	2.0±0.2	2.3±0.1	2.2±0.1
	RC	2.7±0.3	2.0±0.2*	2.8±0.3^{+}	2.0±0.1*^{x}
Succinate oxidation in the presence of isocitrate	V_4	14.9±2.8	20.9±0.4*	21.3±0.1*	17.6±0.5*$^{+x}$
	V_3	30.8±4.7	38.6±0.6*	42.6±1.9*$^{+}$	37.1±1.6*^{x}
	V_{3-4}	12.8±3.0	16.2±0.3*	20.9±0.7*	18.2±0.9*$^{+x}$
	ADP/O	2.0±0.1	2.2±0.1*	2.3±0.1*	1.95±0.09^{x+}
	RS	2.0±0.1	1.7±0.1	2.3±0.1*$^{+}$	2.2±0.1^{+}
	RC	2.4±0.2	2.1±0.2	2.4±0.1*	2.1±0.2*
Oxidation of malate and glutamate	V_4	8.6±0.1	11.1±0.7*	8.4±1.6^{+}	12.1±0.6*^{x}
	V_3	23.4±1.1	24.8±0.9	26.8±1.2*	24.1±2.0
	V_{3-4}	9.2±0.4	14.5±1.3*	12.5±0.6*	13.4±1.1*
	ADP/O	2.9±0.2	2.4±0.1*	2.7±0.1^{+}	2.5±0.1*
	RS	2.8±0.2	2.27±0.06*	3.0±0.1^{+}	2.00±0.06*$^{+x}$
	RC	2.1±0.3	1.69±0.08	2.3±0.1^{+}	2.1±0.1^{+x}
Oxidation of malate and glutamate in the presence of malonate	V_4	10.2±0.3	14.2±0.1*	11.4±1.2^{+}	12.1±0.6*$^{+}$
	V_3	20.7±0.2	18.33±0.08*	23.8±0.3^{+}	20.1±1.3*$^{+x}$
	V_{3-4}	14.6±1.3	15.4±0.5	16.7±0.3*	11.4±1.1*$^{+x}$
	ADP/O	2.7±0.1	2.2±0.2*	2.9±0.1^{+}	2.4±0.1*^{x}
	RS	2.3±0.3	1.4±0.1*	1.9±0.1^{+}	1.8±0.2*
	RC	1.2±0.1	1.32±0.08	1.3±0.1	2.0±0.1*$^{+x}$

Note. Here and in Table 2: $p<0.05$ compared with *intact animals; $^{+}$posthypoxic encephalopathy; ^{x}administration of *Filipendula* extract. Rates of respiration (V_4, V_3, V_{3-4}) are expressed in ng-atom O_2/min/mg protein of mitochondria.

TABLE 2. Effect of Filipendula and Valerian Extracts on LPO in Brain Homogenate during Experimental Posthypoxic Encephalopathy (*X±m*)

Parameters of LPO intensity		Intact animals	Posthypoxic encephalopathy	Posthypoxic encephalopathy+	
				Filipendula extract	valerian extract
MDA, nmol/mg protein/min	spontaneous	0.19±0.02	0.39±0.01*	0.25±0.02*+	0.30±0.04*+
	ascorbate-dependent	0.45±0.03	0.96±0.02*	0.58±0.02*+	0.66±0.05*+x
Conjugated dienes, absorbance units/mg lipid		0.26±0.03	0.63±0.04*	0.42±0.04*+	0.51±0.02*+x
Schiff bases, absorbance units/mg lipid		1.04±0.06	2.6±0.1*	1.72±0.06*+	1.94±0.09*+x

Experimental phytotherapy inhibited LPO activated in the brain during posthypoxic encephalopathy (Table 2). Filipendula extract reduced the intensity of spontaneous and ascorbate dependent production of MDA by 36 and 40%; the content of conjugated dienes and Schiff bases decreased by 33 and 34%, respectively. The antioxidant effect of valerian extract was less pronounced.

Thus, the administration of Filipendula extract and, to a lesser degree, valerian extract caused regression of bioenergetic disturbances in the brain in experimental posthypoxic encephalopathy. Herbal anxiolytics activate the kinetics of energy production in the brain and increase oxidation-phosphorylation coupling and the rate of active flavin adenine dinucleotide- and NAD-dependent respiration. Experimental phytotherapy decreased the contribution of rapid oxidation of succinate to NAD-dependent respiration, which prevented exhaustion of the energy production system. Brain mitochondria from rats subjected to hypoxia continued functioning in the phase of resistance meeting the energy demands of neurons. Energotropic effect of *Filipendula* extract contributed to the mechanisms of its anti-anxiety action.

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