Mitochondrial Components in the Mechanism of Antihypoxic Effects of Baikal Scullcap Extract

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Antioxidant properties of baikalin, a flavonoid from baikal scullcap (*Scutellaria baikalensis Georgi*), depend on its ability to scavenge free radicals. In contrast to standard antioxidant ionol (butylated hydroxytoluene) baikal scullcap extract and baikalin possess cerebroprotective activity and stabilize energy metabolism in rat brain during hypoxia. Pharmacological effect of the extract is determined by the presence of a flavonoid. It is suggested that antihypoxic effect of baikalin depends on its ability to interact with mitochondrial NADH dehydrogenase.

Key Words: *brain mitochondria; hypoxia; flavonoid*

Lipid peroxidation (LPO) plays a significant role in the mechanisms of hypoxic damage to tissues. However, antihypoxic activity of the drugs does not correlate directly with their antioxidant properties. Thus, standard antioxidant ionol (butylated hydroxytoluene) possesses no antihypoxic properties. Recently, flavonoid-containing plant preparations attracted much attention as cytoprotectors during different pathological states associated with LPO activation. This is connected with recognized antioxidant properties of bioflavonoids [1], as well as with the possibility to reduce xenobiotic load to the organism. The presence of a quinone structure in the flavonoid molecule and its redox properties [1] prompted us to study the effect of flavonoid-containing preparations on the mitochondrial oxidation system.

We examined mitochondrial oxidation in rat brain during normobaric hypercapnic hypoxia after pretreatment with baikal scullcap extract (BSE) and its flavonoid baikalin [2].

MATERIALS AND METHODS

The study was carried out on 2-month-old male Wistar rats weighing 200-250 g (Laboratory of Experimental Biomedical Modeling, Tomsk Research Center).

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Normobaric hypercapnic hypoxia was modeled by placing the animals into a 5 liter glass air-tight vessel. BSE and baikalin (15, 60, 200, and 1000 mg) were injected intraventricularly 5 days and 1 h before the experiment. Survival time to the last agony inspiration was registered. The test drugs were compared with standard antihypoxant sodium hydroxybutyrate (200 mg/kg) [9] and antioxidants emoxypine (10 mg/kg) and ionol (15, 60, and 200 mg/kg).

Oxidative phosphorylation in brain mitochondria (MC) was estimated by polarography [4,5] in control animals after 2- and 3.5-h hypoxia (moderate and severe damage, respectively) and in rats pretreated with test drugs. Succinic acid (5×10^{-4} M) served as the oxidation substrate. The results were analyzed with Student's *t*-test.

The effect of test drugs on MC degradation was examined on the model of «aging» of brain homogenate [11]. To this end, homogenate was incubated for 40 min at room temperature and MC respiration was measured every 5 minutes. The state of organells was evaluated using an integral quantitative parameter of functional intactness (FI) [11], which allows to characterize quantitatively functional changes in organells as stable adaptive reactions ($1 \geq FI \geq 0.5$) and pathological shifts ($FI < 0.5$). FI=1.0 corresponds to intact MC.

Antiradical activity of test drugs was measured by the reaction with standard 1,1-diphenyl-2-picryl-hydrazyl radicals [10].

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RESULTS

Pretreatment with BSE and baikalin increased rat survival time in the air-tight chamber. Optimal doses of BSE and baikalin were 200 and 60 mg/kg, respectively, the latter corresponds to 30% concentration of this flavonoid in BSE [1]. Antihypoxic activity of BSE and flavonoid exceeded that of sodium hydroxybutyrate and emoxypine, while ionol showed no activity.

Examination of brain MC during succinate oxidation showed that moderate 2-h hypoxia activates energy metabolism against the background of low energy production. In particular, an increase in the MC respiration rate in all metabolic states, changes in the parameter at rest, and a decrease in oxidation-phosphorylation coupling were observed (Fig. 1).

Long-term hypoxia produced a more pronounced impairment of energy metabolism: MC respiration rate at rest and phosphorylation decreased, while resting respiration rate increased (Fig. 1). The revealed changes confirm our concept on different phases in hypoxia-induced low energy shift: initial activation of succinate-dependent MC respiration followed by its inhibition [11].

Pretreatment with BSE prevented hypoxia-induced impairment of energy production in the brain. After 2-h hypoxia, MC respiration rate before and after phosphorylation cycle remained unchanged and insignificantly increased during phosphorylation (ADP/O did not decrease). BSE administration prior to 3.5-h hypoxia also prevented the impairment of energy production, normalized MC respiration rate in all metabolic states, and stabilized oxidation-phosphorylation coupling (Fig. 1). The results suggest that the preparation preserves activity of the rapid metabolic cluster and possesses a membrane-stabilizing effect.

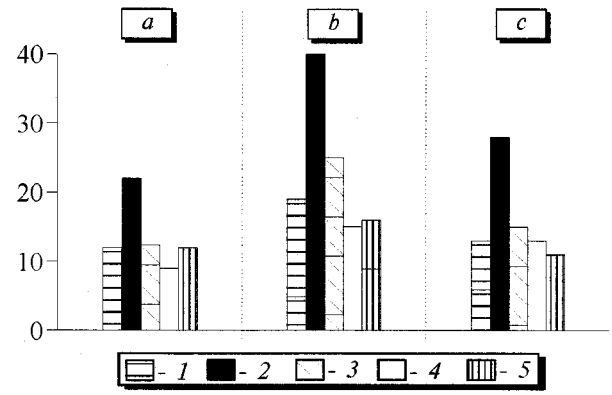

Fig. 1. Effect of baikal scullcap extract (BSE) on mitochondrial respiration rate before (*a*), after (*b*), and during (*c*) phosphorylation of added ADP during progressive hypoxia. Substrate of oxidation: succinic acid. *1*) control (ADP/O=1.8), *2*) moderate hypoxia (ADP/O=1.4), *3*) in the presence of BSE (ADP/O=2.0), *4*) severe hypoxia (ADP/O=1.4), and *5*) in the presence of BSE (ADP/O=1.8). Ordinate: ng-atom O_2/min/mg protein.

In vitro experiments showed that baikalin in a concentration of 10^{-6} M (which corresponds to the lowest brain content of neurotropic drugs administered in therapeutic doses) was comparable with ionol by the duration of its effect (20 min, FI>0.5; Table 1). Tenfold increase in flavonoid concentration did not prolong, but enhanced its effect.

The experiments with diphenylpicrylhydrazyl revealed higher antiradical activity of baikalin compared to ionol (Table 2), which suggests that the protective effect of BSE against hypoxia and MC aging is due to LPO inhibition. At the same time, antioxidant properties of baikalin and many other bioflavonoids [1] do not determine completely their antihypoxic effect, since ionol possesses no such effect. Antihypoxic activity of baikalin can be associated with its involve-

TABLE 1. Protective Effect of Baikalin and Ionol on Brain MC during Homogenate Aging (FI)

Time, min	Ionol, 10^{-6} M			Baikalin					
				10^{-6} M			10^{-5} M		
	control	experiment	Δ, %	control	experiment	Δ, %	control	experiment	Δ, %
10	0.47	0.78	66	0.50	0.67	34	0.54	0.87	61
13	0.38	0.70	84	0.45	0.60	33	0.39	0.73	87
20	0.30	0.55	83	0.38	0.50	32	0.33	0.53	61
30	0.21	0.42	100	0.29	0.37	28	0.29	0.42	45
36	0.15	0.35	133	0.27	0.27	0	0.27	0.27	0
40	0.13	0.28	115	0.20	0.20	0	0.21	0.21	0
60	0.12	0.20	67	0.15	0.15	0	0.16	0.16	0
70	0.12	0.12	0	0.15	0.15	0	0.16	0.16	0

TABLE 2. Antiradical Activity of Baikalin and Ionol

Drugs	Antiradical activity, %		
	0.5 sec	10 sec	10 min
Ionol, 10^{-5} M	5	20	47
Baikalin, 10^{-5} M	80	80	80

ment into intracellular redox reactions and membrane-stabilizing effect. Flavonoids exist in three forms: quinone, semiquinone, and phenol [1]. Therefore, they can switch the flow of reducing equivalents via tocopherol and ubiquinone for free radical quenching. This is indirectly confirmed by the ability of baikalin to reduce glutathione peroxidase [7], oxidize NADH (unpublished data) and agrees with our findings on the protective effect of baikalin on rapid metabolic MC cluster. Hydrophobic properties of baikalin molecule [1] imply its membrane compartmentilization. This can determine pecularities of its antioxidant and redox properties, in particular, its protective effect on MC against pathological reactions induced by hypoxia and aging. Our data show that baikalin prevents damage to the most active sites of rapid metabolic MC cluster (succinate oxidase reactions and aminotransferases).

Thus, BSE prevents hypoxia-induced impairment of energy metabolism in rat brain. Activity of the drug is probably based on its membranotropic effect associated with its direct interaction with NADH-dehydrogenase of the respiratory chain and pronounced antiradical activity.

REFERENCES

1. Zh. I. Abramova and G. I. Oksengendler, *Man and Antioxidant Drugs* [in Russian], Leningrad (1985).
2. E. D. Gol'dberg, A. M. Dygai, B. I. Litvinenko, *et al., Baikal Scullcap* [in Russian], Tomsk (1994).
3. G. G. Zhdanov, V. N. Nechaev, and M. L. Nodel', *Anest. Reanimatol.*, No. 4, 63-68 (1989).
4. M. N. Kondrashova and A. A. Ananenko, *Manual on Polarographic Study of Biological Oxidation* [in Russian], Moscow (1973), pp. 106-129.
5. M. N. Kondrashova, E. V. Grigorenko, A. M. Babskii, and V. A. Khazanov, *Molecular Mechanisms of Cell Homeostasis* [in Russian], Novosibirsk (1987), pp. 40-66.
6. L. D. Luk'yanova, *Advances in Scienses and Technology*, vol. 27 [in Russian], 5-26 (1991).
7. M. G. Matyash, *Extract of Baikal Scullcap as Immunocorrector during Antineoplastic Chemotherapy of Brain and Lung Cancer*, Abstr. of Cand. Med. Sci. Dissertation, Tomsk (1996).
8. F. Z. Meerson, *Pathogenesis and Prevention of Stress and Ischemic Damages to the Heart* [in Russian], Moscow (1984).
9. M. B. Plotnikov and E. A. Kobzeva, *Pharmacological Correction of Hypoxic States* [in Russian], Grodno (1991), pp. 461-462.
10. M. L. Tarakhovskii and T. V. Pochinok, *Khim. Farm. Zh.*, No. 5, 565-569 (1985).
11. V. A. Khazanov, *Role of Succinate Oxidation System in Brain Metabolism*, Abstract of Doct. Med. Sci. Dissertation, Tomsk (1993).