Effect of Several Flavonoid-Containing Plant Preparations on Activity of Mitochondrial ATP-Dependent Potassium Channel

G. D. Mironova*,, M. I. Shigaeva*,** N. V. Belosludtseva*, E. N. Gritsenko*, K. N. Belosludtsev*, E. L. Germanova***, and L. D. Lukyanova*****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 8, pp. 195-199, August, 2008
Original article submitted November 26, 2007

Flavonoid-containing plant preparations (water soluble extracts of *Pentaphylloides fruticosa* [Extralife], *Emblica officinalis Gaerth* [Amla], and *Bergenia crassifolia* [Bergenia]) produced a dose-dependent and tissue-specific effect on activity of mitochondrial ATP-dependent potassium channel. The effect of these preparations was biphasic (activation and inhibition). The activating effect of Extralife was one order of magnitude higher than that of Amla and Bergenia and was observed in a wider concentration range. The activating effect of preparations was abolished by inhibitors of the mitochondrial ATP-dependent potassium channel, which attested to specificity of their influence on mitochondrial channel. Under *in vivo* conditions, the antihypoxic effect of Extralife was partially abolished by mitochondrial ATP-dependent potassium channel inhibitor 5-hydroxydecanoate.

Key Words: *flavonoids; Pentaphylloides fruticosa; Emblica officinalis Gaerth; Bergenia crassifolia; mitochondrial ATP-dependent potassium channel*

Potassium plays an important physiological role in cell function. Intracellular and extracellular potassium exchange is realized via potassium channels on the cytoplasmic and mitochondrial membranes. The mitochondrial membrane plays a special role in the maintenance of mitochondrial structure [6] and tissue protection from ischemia [7]. K^+ influx into mitochondria is mainly provided by mitochondrial ATP-dependent potassium channel (mitoK_{ATP} channel). It is assumed that mitoK_{ATP} channel is closed or low effective in intact mitochondria due to high intracellular concentration of ATP, which serves as the inhibitor of this channel [3]. Opening of mitoK_{ATP} channel occurs under certain conditions, which is not necessarily associated with variations in intracellular ATP concentration.

Recent studies showed that opening of mitoK_{ATP} channel accompanies a variety of important physiological processes. For example, this channel is involved in the regulation of oxidative stress, apoptosis, adaptation of animals to extreme conditions, non-contractile thermogenesis, and cardioprotection [3]. Activation of this channel contributes to an increase in heart resistance to hypoxia during phases 1 and 2 of preconditioning [14]. Specific activators of mitoK_{ATP} channel (diazoxide) imitate the effect of preconditioning, while channel inhibitors suppress the immediate mechanism of adaptation to hypoxia under these conditions.

Our previous studies showed that uridine diphosphate is a potent metabolic activator of the channel [11]. Precursors of this compound also possess pronounced cardioprotective properties [8].

*Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino; **Pushchino State University; ***Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russia

0007-4888/08/1462-0229

Hence, mitoK_{ATP} channel activators hold much promise as cardioprotectors and antiarrhythmic drugs. However, the number of these drugs is very low. Therefore, the search for new compounds with these properties is an urgent problem.

Cardioprotective activity of flavonoids, bioactive polyphenol compounds of plant origin [5], is now intensively discussed. Polyfunctionality of flavonoids is related to the fact that metabolites of these compounds have a modulatory effect on activity of plant and animal enzymes [10]. Their effects are realized via signal pathways of phosphoinositide 3-kinase, Akt/protein kinase B (Akt/PKB), tyrosine kinase, protein kinase C, and mitogen-activated protein kinase. Inhibition or activation of these pathways violates phosphorylation of target molecules in cells and modulates gene expression. Adaptogenic activity of flavonoids serves as indirect evidence for this conclusion [2]. The quinone structure of flavonoids determines oxidation-reduction properties and high antioxidant activity of these compounds [15]. Previous studies showed that flavonoids directly interact with the mitochondrial respiratory chain [2] and exhibit antihypoxic activity [2] and antiapoptotic properties. They possess cardiotropic (vasodilatory), antiapoptotic, antihypoxic, and adaptogenic properties. Flavonoids probably modulate activity of potassium channels and, therefore, hold promise as cardioprotectors.

Here we studied the effect of three water-soluble flavonoid-containing plant preparations, Extralife (shrubby cinquefoil, *Pentaphylloides fruticosa*), Amla (*Emblica officinalis Gaerth*), and Bergenia (*Bergenia crassifolia*), on activity of mitoK_{ATP} channel. These preparations contain considerable amounts of phenols, flavonoids, and tannins and are characterized by a wide range of biological activity. For example, the preparation from shrubby cinquefoil contains considerable amounts of quercetin and exhibits high antioxidant, antihypoxic, and adaptogenic activity [2]. Bergenia plants include considerable amounts of flavonoid bergenan and exhibit cytotoxic, immunostimulatory, adhesive, nootropic, antioxidant, and antibacterial properties [13]. Amla enters the composition of a combined plant preparation Trifal [9]. Amla contains considerable amounts of tannins and is used for the therapy of diabetes, cognitive dysfunction, amnesia, radiation injury, Alzheimer's disease, ischemic disorders, and heart failure. These plants have high antioxidant and antibacterial activity [9].

MATERIALS AND METHODS

Isolated mitochondria from rat heart and liver were obtained by the standard method of differential centrifugation. Heart mitochondria were isolated in a medium containing 210 mM mannitol, 70 mM sucrose, 2 mM EGTA, 1 mg/ml bovine serum albumin (BSA), and 10 mM HEPES-NaOH (pH 7.4) and washed with the same medium without BSA and EGTA. The mitochondrial pellet was resuspended in washout medium (0.1 ml medium per gram tissue).

Liver mitochondria were isolated in a medium containing 210 mM mannitol, 70 mM sucrose, 1 mM EDTA, and 10 mM HEPES-NaOH (pH 7.4) and washed with a medium containing 210 mM mannitol, 70 mM sucrose, 0.05 mM EGTA, and 10 mM HEPES-NaOH (pH 7.4). The mitochondrial pellet was resuspended in the isolation medium not containing EDTA (0.1 ml medium per gram tissue).

Energy-dependent mitochondrial swelling (increase in static volume) due to ATP-dependent K^+ influx into mitochondria was estimated by light scatter. The mitochondrial incubation medium contained 50 mM KCl, 5 mM Na_2HPO_4, 5 μM cytochrome C, and 10 mM HEPES-NaOH (pH 7.4). Liver or heart mitochondria were put in the incubation medium (0.1-0.2 mg/ml). Mitochondrial swelling was induced by adding 5 mM succinate (substrate) to medium with 2 μM rotenone 1.5 min after the start of measurements. The measurements were performed on an UVIKON UV/VIS 923 spectrophotometer at 520 nm. Spectrophotometric parameters of mitochondrial swelling in the incubation medium without the test preparation served as the control.

Function of mitoK_{ATP} channel was also estimated from the rate of dinitrophenol-induced ATP-dependent K^+ efflux from mitochondria (reverse function of the channel) [1]. K^+ efflux from mitochondria in the medium without respiratory substrate and potassium was recorded after addition of oxidative phosphorylation uncoupling agent. K^+ efflux from mitochondria was induced by addition of 50 μM 2,4-dinitrophenol (DNP). The mitochondrial incubation medium contained 170 mM sucrose, 80 mM D-mannitol, 5 mM Na_2HPO_4, and 10 mM Tris-HCl (pH 7.4).

The role of mitoK_{ATP} channel in organism's resistance to a critical height (in altitude chamber) was evaluated on male outbred rats weighing 220-280 g and not resistant to hypoxic injury. The animals were "elevated" to a critical height of 11,000-11,500 m in a flow altitude chamber at 20-22°C (model of acute hypobaric hypoxia). The resistance to hypoxia was evaluated from two parameters: time to loss of posture (TLP) and lifespan (LS) at the critical height. TLP is the latency between ascent to a height of 11,500 m and the moment when the animal falls to the side and loses the ability to

maintain posture (min). LS is the latency between elevation to a height of 11,500 m and appearance of the 2nd agonal breath (min).

All flavonoid-containing preparations were presented by lyophilized and standardized water-soluble powder extracts.

For evaluation of the effect of Extralife on organism's resistance, the preparation in a dose of 20 mg/kg was injected intraperitoneally 30 min before exposure to simulated altitude in a pressure chamber. 5-Hydroxydecanoate (5-HD), an inhibitor of mitoK$_{ATP}$ channel, was injected intraperitoneally in a dose of 5 mg/kg 30 min before exposure to simulated altitude. During combined treatment, these preparations were administered in the same doses.

RESULTS

Flavonoid-containing preparations produced a dose-dependent biphasic effect on activity of mitoK$_{ATP}$ channel (energy-dependent mitochondrial swelling, Fig. 1). The test preparations in low concentrations increased the rate of potassium influx into mitochondria, which reflects activation (opening) of the channel. Addition of the test preparations in high concentrations to mitochondria was followed by the inhibition of this process. Activation of mitoK$_{ATP}$ channel was most pronounced after treatment with Extralife in low concentrations (0.005-1 mg/liter). Extralife in a concentration of 10-30 mg/liter inhibited mitoK$_{ATP}$ channel, which coincides with prooxidant [2] and toxic activity of this preparation.

Bergenia and Amla produced less pronounced activating effect, which was observed in a narrow range of concentrations (0.05 and 0.10 mg/liter, respectively, *vs*. 0.005 mg/liter for Extralife; Fig. 1). However, the concentration of these preparations inducing the inhibitory effect was similar to that of Extralife (10-30 mg/liter).

Hence, flavonoid-containing preparations differ in their ability to activate mitoK$_{ATP}$ channel. Extralife was most potent in this respect.

The test substances had a tissue-specific activating effect on mitoK$_{ATP}$ channel. The activating effect in liver mitochondria was less pronounced than in heart mitochondria. However, these effects manifested after treatment with the test substances in the same concentration (Fig. 1). Hence, functional significance of this channel differs in the liver and heart. Function of mitoK$_{ATP}$ channel is of particular importance for heart activity (especially during hypoxia) [12]. Moreover, the density of these channels in the heart is higher than in the liver [4].

The activating effect of flavonoid-containing preparations on mitoK$_{ATP}$ channel was abolished by specific channel inhibitor 5-HD (100 μM). It should be emphasized that the inhibitor had no effect on this channel (Table 1). Similar effect of the inhibitor was reported for Amla. Therefore, flavonoid-containing preparations serve as specific activators of mitoK$_{ATP}$ channel.

The effects of flavonoids on activity of mitoK$_{ATP}$ channel were studied by the method of DNP-induced K^+ efflux from mitochondria. The test preparations had a similar dose-dependent biphasic effect (Fig. 2). Extralife in low concentrations (0.01-0.50 mg/liter) most significantly increased K^+ efflux from mitochondria. Increasing the concentration of Extralife to 15 mg/liter or higher was accompanied by inhibition of K^+ efflux from mitochondria. Bergenia

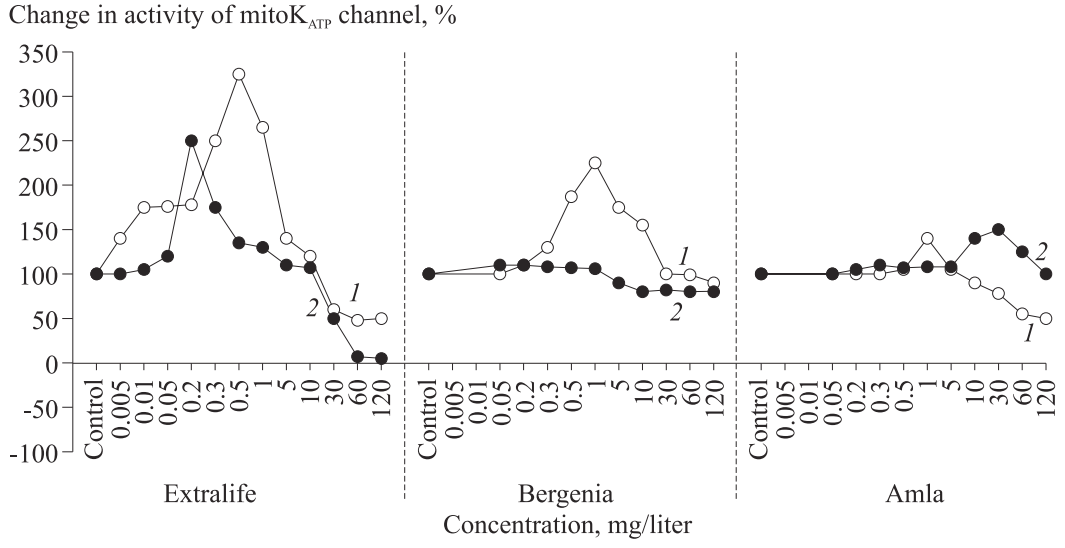

Fig. 1. Effect of flavonoid-containing preparations Extralife, Amla, and Bergenia on swelling of heart and liver mitochondria. Here and in Fig. 2: light symbols, heart mitochondria; dark symbols, liver mitochondria. Control, 100%. Oxidative substrate, succinate.

TABLE 1. 5-HD Abolishes the Accelerating Effect of Extralife and Bergenia on Energy-Dependent K^+ Influx into Rat Heart Mitochondria

Preparations	Rate of energy-dependent K^+ influx, d/mg protein/min	Change in the rate of energy-dependent K^+ influx (% of the control)
Control	0.125±0.010	0
Extralife, 0.3 mg/liter	0.250±0.019*	100
Extralife (0.3 mg/liter)+5-HD (100 μM)	0.126±0.010	1
Bergenia, 0.1 mg/liter	0.182±0.008*	46
Bergenia (0.1 mg/liter)+5-HD (100 μM)	0.128±0.015	2
5-HD, 100 μM	0.127±0.009	2

Note. *$p<0.01$ compared to the control.

had a less pronounced effect than Extralife. Amla was least potent in this respect. However, the range of concentrations of Bergenia and Amla inducing a biphasic effect was similar to that of Extralife. The activating effect of the test preparations on mitoK_{ATP} channel in liver mitochondria was much lower than in heart mitochondria. These data illustrate tissue-specific effect of preparations.

Activation of mitoK_{ATP} channel and increase in ATP-dependent K^+ transport in mitochondria are an essential stage, which initiates the adaptive response under extreme conditions (*e.g.*, during hypoxia). Activation of the channel should contribute to an increase in organism's resistance under extreme conditions. By contrast, inhibition of channel function can decrease the resistance under these conditions. In light of this, we studied the role of activation of mitoK_{ATP} channel by Extralife under *in vivo* conditions (ascent to a critical height of 11,500 m in the altitude chamber).

Injection of Extralife (20 mg/kg) 30 min before ascent to the critical height in the altitude chamber was followed by an increase in TLP and LS (by 7 and 8 times, respectively; Fig. 3). Hence, Extralife produces a potent protective (antihypoxic and adaptogenic) effect during acute hypoxia. Potassium channel inhibitor 5-HD partially abolished the effect of Extralife on organism's resistance. The inhibitor most significantly modified TLP. After combined treatment with 5-HD and Extralife, this parameter increased by only 2 times (not by 7 times). A relative decrease in TLP was 3.4 times (Fig. 3). Less significant variations in LS were observed after combined treatment with the inhibitor and Extralife. Under these conditions, LS increased by 6.5 times (not by 8 times, Fig. 3).

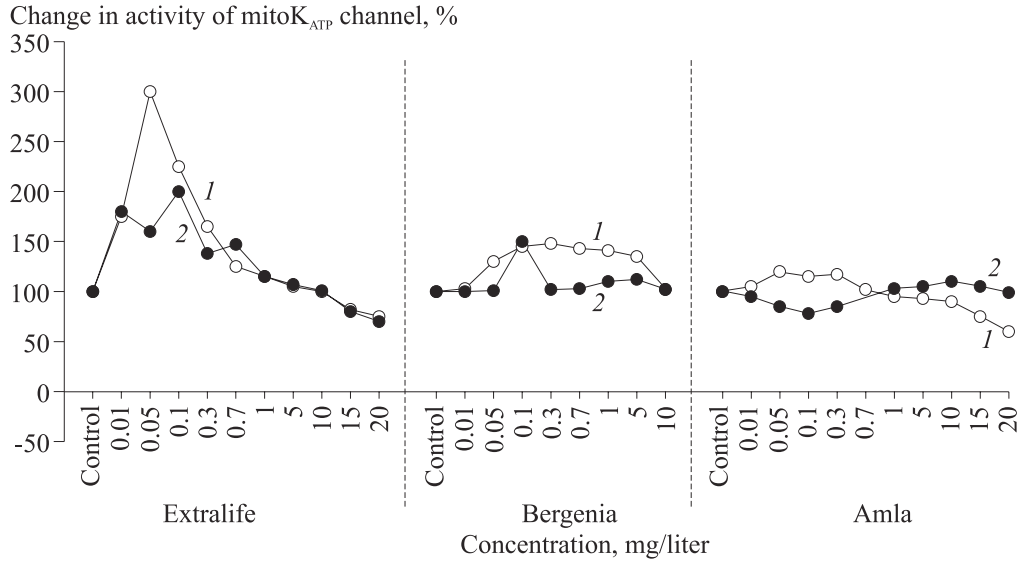

Fig. 2. Dependence of DNP-induced K^+ efflux from heart and liver mitochondria on the concentration of flavonoid-containing preparations Extralife, Amla, and Bergenia.

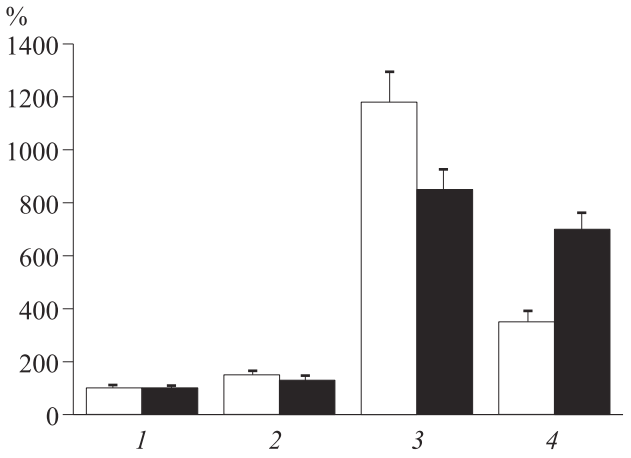

Fig. 3. Effect of a mitoK_{ATP} channel inhibitor 5-HD on parameters of the organism's resistance reflecting an antihypoxic effect of Extralife during normobaric hypoxia. Control (*1*); 5 mg/kg 5-HD (*2*); 20 mg/kg Extralife (*3*); 20 mg/kg Extralife+5 mg/kg 5-HD (*4*). Light bars, TLP; dark bars, LS. Control, 100%.

Our results indicate that systemic blockade of potassium channels abolished the antihypoxic and adaptogenic effect of Extralife. Variations in TLP were most significant. This parameter reflects adaptation under subcritical hypoxic conditions. However, inhibition of potassium channels with Extralife had little effect on LS, a parameter reflecting the state of functions responsible for reserve capacities and viability of the organism under extreme conditions of oxygen deficiency.

It can be hypothesized that the heart is a pharmacological target for the cardiotropic arrhythmogenic effect of Extralife during systemic hypoxia.

This work was supported by the Russian Foundation for Basic Research (grant No. 07-04-00759a) and International Scientific and Technical Center (grant No. 3301).

REFERENCES

1. O. V. Baranova, Yu. Yu. Skarga, A. E. Negoda, and G. D. Mironova, *Biokhimiya*, **65**, No. 2, 262-267 (2000).
2. L. D. Luk'yanova, E. L. Germanova, and A. I. Lysko, *Vestn. Ros. Akad. Med. Nauk*, No. 2, 55-62 (2007).
3. G. D. Mironova, E. V. Kachaeva, and A. T. Kopylov, *Ibid.*, No. 2, 34-50 (2007).
4. R. Bajgar, S. Steetharaman, A. J. Kowaltowski, *et al.*, *J. Biol. Chem.*, **276**, No. 36, 33,369-33,374 (2001).
5. J. W. Jr. Erdman, D. Balentine, L. Arab., *et al.*, *J. Nutr.*, **137**, No. 3, Suppl. 1, 718S-737S (2007).
6. K. D. Garlid, P. Paucek, V. Yarov-Yarovoy, *et al.*, *Circ. Res.*, **81**, No. 6, 1072-1082 (1997).
7. K. D. Garlid and P. Paucek, *Biochim. Biophys. Acta*, **1606**, Nos. 1-3, 23-41 (2003).
8. I. B. Krylova, E. V. Kachaeva, O. M. Rodionova, *et al.*, *Exp. Gerontol.*, **41**, No. 7, 697-703 (2006).
9. P. K. Mukherjee, *Drug Inf. J.*, **35**, 623-632 (2001).
10. E. Jr. Middleton, C. Kandaswami, and C. Theoharides, *Pharmacol. Rev.*, **52**, No. 4, 673-751 (2000).
11. G. D. Mironova, A. E. Negoda, B. S. Marinov, *et al.*, *J. Biol. Chem.*, **279**, No. 31, 32,562-32,568 (2004).
12. B. O'Rourke, *Circ. Res.*, **94**, No. 4, 420-432 (2004).
13. S. V. Popov, G. Y. Popova, S. Y. Nikolaeva, *et al.*, *Phytother. Res.*, **19**, No. 12, 1052-1056 (2005).
14. Y. Wang, M. Kudo, M. Xu, *et al.*, *J. Mol. Cell Cardiol.*, **33**, No. 11, 2037-2046 (2001).
15. R. J. Williams, J. P. Spencer, and C. Rice-Evans, *Free Radic. Biol. Med.*, **36**, No. 7, 838-849 (2004).