BIOPHYSICS AND BIOCHEMISTRY

Activity of Mitochondrial ATP-Dependent Potassium Channel in Animals with Different Resistance to Hypoxia before and after the Course of Hypoxic Training

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 1, pp. 30-35, January, 2011
Original article submitted July 6, 2010

Activity of mitochondrial ATP-dependent potassium channel in rats with high genetically determined resistance to hypoxia was higher than in sensitive animals. Adaptation of low resistant rats to hypoxia was accompanied by activation of the channel, facilitation of potassium recycling in mitochondria, and a decrease in the rate of H_2O_2 formation. Our results indicate that mitochondrial ATP-dependent potassium channel plays an important role in the delayed mechanisms of animal's adaptation to hypoxia.

Key Words: *hypoxia; tolerance; mitochondria; mitochondrial ATP-dependent potassium channel; reactive oxygen species*

Recent studies showed that ATP-dependent potassium channels of the cytoplasmic and mitochondrial membrane play a key role in the protection of the heart and brain from ischemic injury [3]. The antiarrhythmic effect is mainly associated with function of the cytoplasmic ATP-dependent potassium channel. Mitochondrial ATP-dependent potassium channel (mitoK_{ATP}) plays a greater role in the maintenance of mitochondrial structure [10,14], while K^+ influx into mitochondria is primarily provided by activity of mitoK_{ATP} [6]. It is believed that K^+ ions enter the matrix upon activation of this channel. This process is followed by swelling of mitochondria and increase in the static volume, which depends on channel function. Opening of the channel and mitochondrial swelling are probably followed by activation of another component of the potassium cycle (K^+/H^+ antiporter) specialized for K^+ release, which contributes to the maintenance of intracellular potassium homeostasis, stability, and regulation of mitochondrial matrix volume [6].

MitoK_{ATP} plays an important role in a variety of general physiological processes. The channel is involved in the regulation of oxidative stress and apoptosis, adaptation of animals to extreme conditions, nonshivering thermogenesis, and cardioprotection [3]. Activation of the channel improves heart resistance to hypoxia during the first and second phase

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0007-4888/11/1511-0025

of preconditioning [8]. During adaption to hypoxia, mitoK_{ATP} is more rapidly involved in the realization of immediate defense mechanisms and development of body tolerance (compared to the cytoplasmic channel) [8]. Opening of this channel is required for the cardioprotective effect of long-term intermittent hypoxia [4]. This process plays a role in the prevention of calcium overload during ischemia–reperfusion, which probably underlies the mechanism of cardioprotection [15].

Published data indicate that mitoK_{ATP} plays a role in the protection of the myocardium and other tissues from ischemic injury. However, the mechanism for the involvement of this channel in the development of hypoxia tolerance remains unknown. The role of mitoK_{ATP} in the formation of hypoxic resistance in animals with different individual hypoxia tolerance is poorly understood. There are contradictory results on the role of mitoK_{ATP} in the early and delayed mechanisms of adaptation [2,12].

Here we compared functional activity of mitoK_{ATP} in tissue of animals that differ in genetically determined hypoxia tolerance. The role of mitoK_{ATP} in adaptive mechanisms of the organism was evaluated. The course of intermittent normobaric hypoxia (INH) served as the model of long-term adaptation.

MATERIALS AND METHODS

Experiments were performed on male inbred albino rats (n=35) weighing 180-200 g. The animals differed in hypoxia tolerance. The study was conducted on hypoxia-resistant and hypoxia-sensitive rats (as described previously [2]). Sensitive rats were exposed to the course of INH. Daily training course of INH consisted of intermittent passive breathing with a hypoxic gas mixture (10% O_2, 5 min, constant pressure) and atmospheric air (20% O_2, 3 min). This procedure was repeated 6-7 times over 1 h. The training cycle was performed daily (5 times a week) for 4 weeks (long-term adaptation to INH under conditions of preconditioning). Activity of mitoK_{ATP} and formation of H_2O_2 were studied in mitochondria from the heart and liver. Control animals were not exposed to hypoxic training.

Mitochondria were isolated from the liver and heart of rats by the standard method of differential centrifugation. The final protein content in mitochondrial suspension from the liver and heart was 80-100 and 35-50 mg/ml, respectively.

The ATP-inhibited potassium transport in mitochondria was evaluated by the energy-dependent swelling of mitochondria in a hypotonic medium (50 mM HCl) [7] and K^+ efflux from mitochondria induced by an uncoupling agent dinitrophenol (DNP) in the presence of respiratory substrates. We used a K^+-selective electrode [1]. The rate of mitochondrial swelling was estimated from changes in absorption at 520 nm per 1 mg mitochondrial protein over 1 min.

H_2O_2 formation in mitochondria was studied in a peroxidase-containing system [5] using Amplex Red (staining agent, Molecular Probes). The amount of newly formed H_2O_2 was evaluated from the calibration curve with a standard solution of hydrogen peroxide. An aliquot of H_2O_2 (5 μl, 1-2 nmol hydrogen peroxide) was added to the incubation medium. The concentration of a standard solution of H_2O_2 was estimated from extinction E_{240}=43.6 cm^{-1}.

The amount of K^+ in mitochondria was evaluated with a selective potassium electrode after addition of

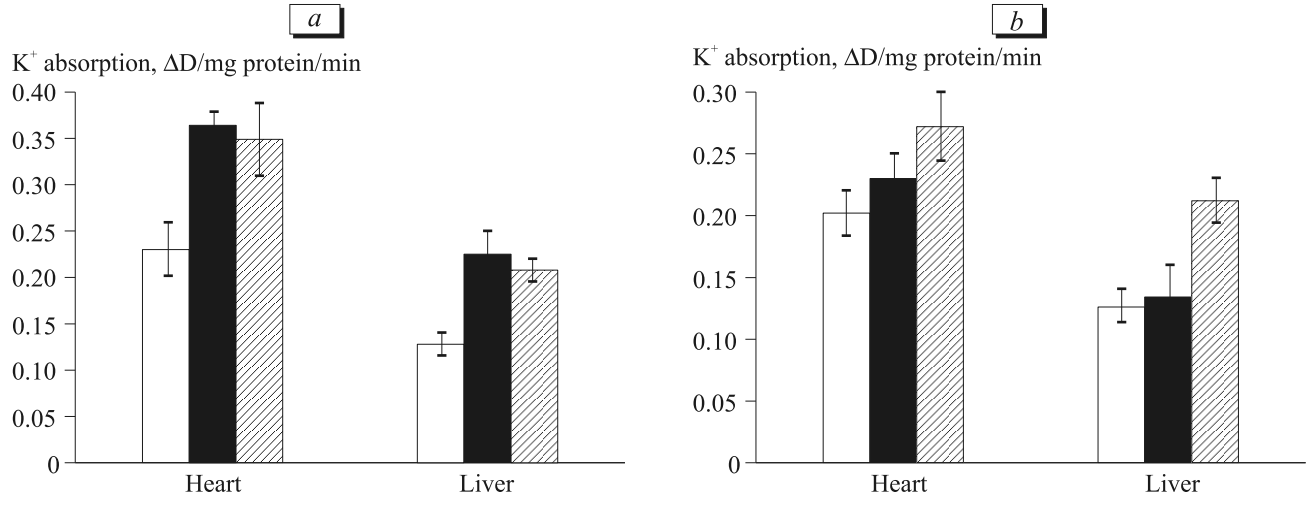

Fig. 1. Energy-dependent mitochondrial swelling in the heart and liver of rats with different resistance to hypoxia. Respiratory substrates: (*a*) glutamate (4 mM) and malate (1 mM); (*b*) succinate (5 mM) in the presence of rotenone (1 μM). The measurements are performed for 2-3 min. Here and in Fig. 2: light bars, hypoxia-sensitive rats; dark bars, hypoxia-resistant rats; shaded bars, sensitive rats after adaptation to hypoxia under conditions of INH.

detergent (0.05% Triton X-100) to the incubation medium [1].

RESULTS

The rate of potassium transport in mitochondria and functional activity of mitoK$_{ATP}$ were shown to differ in resistant and sensitive rats. The rate of ATP-dependent swelling of myocardial mitochondria during oxidation of NADH-dependent substrates in resistant rats was 1.5-fold higher than in sensitive animals; in the liver these values differed by 75% (Fig. 1). However, the described differences were less pronounced in experiments when succinate was used as the substrate (with rotenone).

Similar results were obtained on the model of DNP-induced K^+ efflux from mitochondria. This process depends on functional activity of mitoK$_{ATP}$ and is inhibited by ATP. The rate of DNP-induced K^+ efflux from mitochondria of the myocardium and liver in resistant rats was 1.5-fold higher than in heart mitochondria from sensitive animals (Fig. 2).

Similarly to ATP-dependent mitochondrial swelling, differences in the rate of potassium transport in intact mitochondria from resistant and sensitive rats during oxidation of NAD-dependent substrates were more pronounced than during succinate oxidation (Table 1). The data indicate that function of mitoK$_{ATP}$ under these conditions is related to activity of mitochondrial complex I, but not of complex II.

Potassium concentration in intact mitochondria from resistant rats was much lower than that in sensitive animals (Table 2). Stimulation of K^+ influx into mitochondria is accompanied by an increase in the rate of K^+ efflux, which probably results from activation of electroneutral K^+/H^+ exchange [6].

This hypothesis is confirmed by the results of our experiment. Long-term study of mitochondrial swelling demonstrated the influx and efflux of K^+ (Fig. 3). Two phases of mitochondrial swelling and contraction were observed over 30 min. These data illustrate synchronization of K^+ influx and efflux in some mitochondria. The effect was more pronounced in a hypotonic medium with 50 mM potassium. The rate of swelling was higher in isotonic medium (K^+ concentration 100-120 mM, data not shown). Variations in K^+ influx and efflux were less significant under these conditions. Our results are consistent with published data. The valinomycin-induced energy-dependent K^+ influx into mitochondria is accompanied by a rapid activation of electroneutral ion exchange for the proton [13]. Potassium recycling was suppressed by ATP-Mg^{2+}, which probably results from inhibition of K^+ influx (Fig. 3).

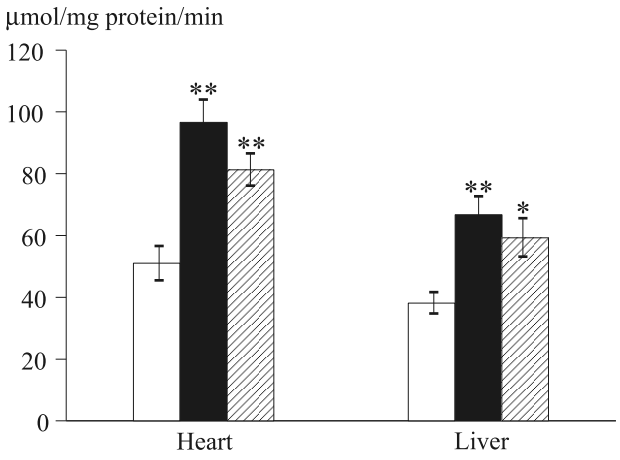

Fig. 2. Rate of DNP-induced K^+ efflux from mitochondria of the heart and liver in rats with different resistance to hypoxia. Respiratory substrate: glutamate (4 mM) and malate (1 mM). *p<0.05 and **p<0.01 compared to sensitive animals.

ATP inhibition constant ($K_{1/2}$) in myocardial and liver mitochondria was calculated in animals with different resistance to hypoxia. This index in resistant rats was 2-fold lower than in sensitive specimens. $K_{1/2}$ was estimated in mitochondria of the heart and liver from resistant (6.3 and 43.1, respectively) and sensitive animals (14.7 and 79.8, respectively).

MitoK$_{ATP}$ regulates H_2O_2 formation in mitochondria. Addition of ATP to mitochondria is followed by inhibition of this channel and increase in the rate of H_2O_2 formation, which is partially abolished by channel activator [5]. We showed that the rate of ATP-de-

TABLE 1. H_2O_2 Generation in Mitochondria of the Heart and Liver from Rats with Different Resistance to Hypoxia

Hypoxia tolerance	Rate of H_2O_2 generation, pmol/mg protein/min			
	heart		liver	
	-ATP	+ATP	-ATP	+ATP
Resistant	141±9	381±18	129±11	266±15
Sensitive	159±11	237±16	89±13	175±13

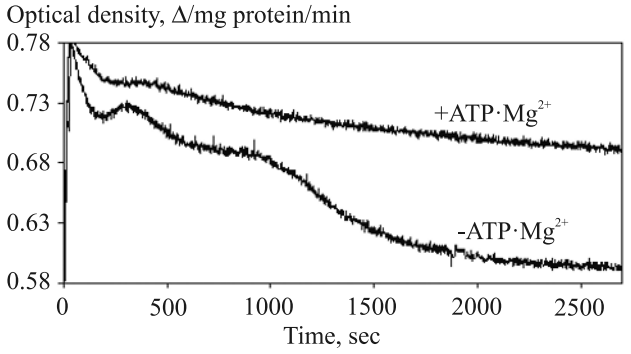

Fig. 3. Variations in the influx and efflux of K^+ in rat liver mitochondria under conditions of energy-dependent swelling in the presence of succinate (5 mM) and rotenone (1 μM) in the incubation medium with 50 mM KCl. The measurements are performed for 45 min.

pendent H_2O_2 generation in myocardial mitochondria from resistant animals increases by 3 times after addition of ATP in the presence of NAD-dependent substrates. This parameter was elevated only by 1.5 times in myocardial mitochondria from sensitive animals (Table 1). H_2O_2 generation in liver mitochondria also increased after adding ATP. However, no significant differences were found between resistant and sensitive rats. An ATP-induced increase in the rate of H_2O_2 generation in mitochondria from the heart and liver was observed in experiments with endogenous substrates (Table 3). However, the rate of this process sharply increased in the presence of NAD-dependent substrates. The rate of H_2O_2 generation in heart mitochondria from resistant animals was higher that that in sensitive specimens. Hence, this function of mitoK_{ATP} is well regulated in mitochondria of resistant rats.

Tissue-specific differences were found in activity of mitoK_{ATP}. The rate of mitochondrial swelling in the myocardium was 1.5-fold greater than in the liver. The observed differences are probably associated with higher activity of mitoK_{ATP} in cardiomyocytes [3] and specific features of energy synthesis in these tissues. The data are consistent with our finding that the rate of DNP-induced K^+ efflux in myocardial mitochondria is 1.5 times higher than in the liver (Fig. 2). $K_{1/2}$ for

TABLE 2. Potassium Concentration in Mitochondria of the Heart and Liver from Rats with Different Resistance to Hypoxia after Adaptation to Hypoxia

Hypoxia tolerance	K^+ concentration, μmol/mg protein	
	liver	heart
Resistant	88±6	112±5
Sensitive	60±4	81±6
Sensitive adapted	58.0±3.5	70±3

ATP inhibition in heart mitochondria was much lower than in the liver.

The rate of ATP-dependent H_2O_2 generation in heart mitochondria was much higher than in liver mitochondria (Table 1). An increase in the rate of H_2O_2 generation in mitochondria of the myocardium and liver after addition of ATP was observed in the absence of exogenous oxidation substrates and in the presence of NAD-dependent substrates (Table 3). H_2O_2 generation in mitochondria of the myocardium and liver increased sharply after addition of ATP and NAD-dependent substrates (but not of succinate), which indicates that this process depends on MPK I activity.

The existence of tissue-specific differences reflects higher intensity of K^+ transport in heart mitochondria compared to liver mitochondria. These data suggest that mitoK_{ATP} performs various functions in the studies types of tissues. mitoK_{ATP} plays an important role in contractile function of the heart, especially during hypoxia. It can be hypothesized that a greater rate of K^+ transport in the heart (compared to that in the liver) is related to a higher density of these channels.

Long-term adaptation of animals to various types of hypoxia contributes to the increase in their tolerance. The observed changes are particularly pronounced in sensitive specimens [2]. Our results are consistent with these data. The resistance of sensitive rats to hypoxia was shown to increase by 2-2.5 times after the course of INH. Hypoxia tolerance of resistant animals was reduced over the first week of training, but increased slightly in the follow-up period. Taking these data into account, a further study of potassium channels was conducted only on sensitive rats.

Adaptation of animals to INH for 3 weeks was accompanied by a significant increase in the rate of mitochondrial ATP-dependent K^+ transport in sensitive rats, which did not differ from that in resistant specimens (Figs. 1 and 2). Adaptation had a strong effect on the rate of mitochondrial K^+ transport in the liver and heart.

Our findings indicate that adaptation of animals to hypoxia is followed by mitochondrial contraction, but not by mitochondrial swelling. This conclusion is derived from a 30% decrease in K^+ concentration in mitochondria of the myocardium and liver from sensitive animals after adaptation to hypoxia. These data show that adaptation to hypoxia activates not only the influx, but also the efflux of K^+ from mitochondria (realized by the K^+/H^+ exchange system). Our results confirm the fact that activation of K^+ influx during adaptation to hypoxia is compensated by increased rate of K^+ efflux [5,6].

Therefore, mitoK_{ATP} plays a role in the delayed mechanisms of adaptation under conditions of long-term hypoxia. Potassium recycling in mitochondria

TABLE 3. H_2O_2 Generation in Heart Mitochondria from Rats with Different Resistance to Hypoxia in the Presence or Absence of Respiratory Substrates

Hypoxia tolerance	Rate of H_2O_2 generation, pmol/mg protein/min			
	without substrate		glutamate+malate	
	-ATP	+ATP	-ATP	+ATP
Resistant	43±2	145±7	141±6	381±18
Sensitive	65.0±1.5	178±10	159±11	237±16

is activated under these conditions. On the one hand, the resultant mild uncoupling maintains mitochondrial volume at a certain level. On the other hand, this process is accompanied by a decrease in the rate of reactive oxygen species generation [9]. The reduced accumulation of reactive oxygen species during activation of the potassium cycle probably underlies the mechanism for body adaptation to hypoxia and cardioprotective effect of channel-activating agents. The influence of mitoK_{ATP} on reactive oxygen species generation in mitochondria determines the physiological importance of this channel not only during ischemia, but also under other pathological conditions. The inhibition of reactive oxygen species accumulation in mitochondria upon activation of the potassium and calcium cycle holds much promise for the prevention and therapy of hypoxia consequences. This approach will be more efficient than the removal of peroxide radicals with antioxidants, which is extensively used in medical practice. It should be emphasized that long-term hypoxic preconditioning is accompanied not only by activation of mitoK_{ATP}, but also by the increased expression of this channel [11].

We conclude that mitoK_{ATP} plays an important role in adaptation of animals to hypoxia. Adaptation to hypoxia is accompanied by activation of mitoK_{ATP}, facilitation of potassium recycling in mitochondria, and decrease in the rate of H_2O_2 formation. These changes accompany the general economy of energy production.

This work was supported by the Russian Foundation for Basic Research (grant No. 10-04-009-20-a) and Program "Development of Scientific Potential of the Higher School" (grant No. 2.1.1/3840).

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