Effects of Succinic and Glutamic Acid Combination on Energy Metabolism in the Liver of Mice under Conditions of Hypoxia

K. Yu. Vasilyev, A. A. Kiselyova, and V. A. Khazanov

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Treatment of mice by a combination of succinic and glutamic acids prevented the metabolic disorders in the liver under conditions of normobaric hypoxia. In addition, the activity of the mitochondrial fast metabolic cluster remained intact and lipid peroxidation was limited.

Key Words: *hypoxia; liver mitochondria; succinic acid; glutamic acid*

Virtually any disease (respiratory, hepatic, renal, cardiovascular, hematological, status during narcosis and surgical intervention, intoxication, blood loss, and shock) is associated with hypoxia [2,3]. Development of antihypoxants (substances alleviating body reaction to hypoxia or preventing its development) is an important task of medicine.

The deficiency of ATP is the key component in the pathogenesis of hypoxia [6,7]. It seems that one of the most effective approaches to limitation of developing pathological reactions to hypoxia is direct modulation of energy formation processes in the mitochondria (MC) by agents modulating energy production processes and natural metabolites in MC [7]. Of these, succinic acid (SA) and glutamic acid (GA), whose antihypoxic activity is a known fact [4,5], deserve special attention. Glutamic acid plays an important role in reamination in MC of oxaloacetate (SDH inhibitor determining the activity of succinate-dependent system — the energy production system most resistant to hypoxia) [6], while SA activates the succinate oxidase system [5,7]. This suggests combined use of GA and SA in hypoxia.

Laboratory of Molecular Pharmacology with Pharmacokinetics Group, Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences, Tomsk, Russia. ***Address for correspondence:*** vkh@pharm.tsu.ru. V. A. Khazanov

We studied the effects of GA and SA combinations on energy metabolism and redox reactions in the liver of mice under conditions of hypoxia.

MATERIALS AND METHODS

Experiments were carried out on 3-month-old outbred male albino mice. Normobaric hypercapnic hypoxia was induced by placing the animals into a hermetic 200-ml flask. The life span of mice under conditions of hypoxia, LPO status, and liver MC function were evaluated. Hepatic energy production system and antihypoxic effects of the drugs were evaluated in mice exposed to hypoxia and removed from the flask before the last agonal inhalation. Our data indicate the development of lactate acidosis and accumulation of LPO products (which corresponds to severe hypoxic status and adaptive reaction exhaustion in the energy production system) by this moment of hypoxia. The animals received a mixture of SA and GA before hypoxia in equimolar antihypoxic and antitoxic daily doses of 50 and 78 mg/kg preventively intragastrically during 5 days [7]. Intact animals served as the control.

The function of liver MC was evaluated by spectrofluorometry by the level of pyridine nucleotide reduction in different metabolic states according to Chance [9]. In addition to the stationary

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levels of NADH fluorescence, the kinetic characteristics (V_3 velocity and Tr_3 time of NAD reduction in MC after addition of ADP into incubation medium to the concentration of 5×10^{-5} M) were evaluated, reflecting the proportion between delivery of reducing equivalents to NADH dehydrogenase and their oxidation by the respiratory chain [8]. Succinate, malate-glutamate mixture, their combination with SDH inhibitor malonate, and a combination of malate, glutamate, and amino-oxyacetate (aminotransferase inhibitor) served as the oxidation substrates (Fig. 1). Antioxidant effects of SA and GA were evaluated by spectrophotometry by accumulation of TBA-active LPO products in liver homogenate [1-3].

The significance of differences was evaluated by Mann—Whitney's nonparametrical test at 5% level of significance ($p<0.05$).

RESULTS

Succinate and malate-glutamate mixture, used as the substrates, accelerate NAD reduction in liver MC after addition of ADP into incubation medium in comparison with the parameters after endogenous substrate utilization (Fig. 1). Prevention of endogenous SA oxidation by means of SDH inhibitor (malonate) or transaminase inhibitor (amino-oxyacetate) inhibited the liver MC NAD reduction. These MC reactions to substrate loading and enzyme inhibitors indicate a significant relationship between the organelle function and oxidation of endogenous SA [6].

The life span of control mice exposed to hypoxia was 20.5±1.5 min. Hypoxia led to inhibition and prolongation of liver MC NAD reduction during oxidation of endogenous substrates in comparison with the control (Fig. 1). Presumably, these changes were caused by inhibition of NAD-dependent energy production and subsequent development of compensatory inhibition of SDH by oxaloacetate, produced during hyperactive oxidation of succinate [6]. Inhibition and prolongation of NAD reduction in comparison with the control were observed during oxidation of exogenous succinate in the liver MC of mice exposed to hypoxia. This confirms inhibition of succinate-dependent energy production in hypoxia and formation of SDH inhibition. Addition of malate-glutamate mixture to liver MC incubation medium did not modify the adaptive reaction of the energy production system of animals with hypoxia: the velocity of NAD reduction remained low and the duration of NAD reduction was long in comparison with intact animals. Analysis showed that inhibition of NAD reduction during utilization of malate-glutamate mixture under conditions of hypoxia in comparison with normal values was caused by a slower oxidation of endogenous SA, formed in oxaloacetate reamination reactions. These changes indicate the development of exhaustion stage in the MC energy production system under conditions of the hypoxia model used in the study.

Hypoxic exposure in our experiment led to an increase in the content of TBA-active LPO products in mouse liver (Fig. 2), this indicating activation of free radical formation and exhaustion of

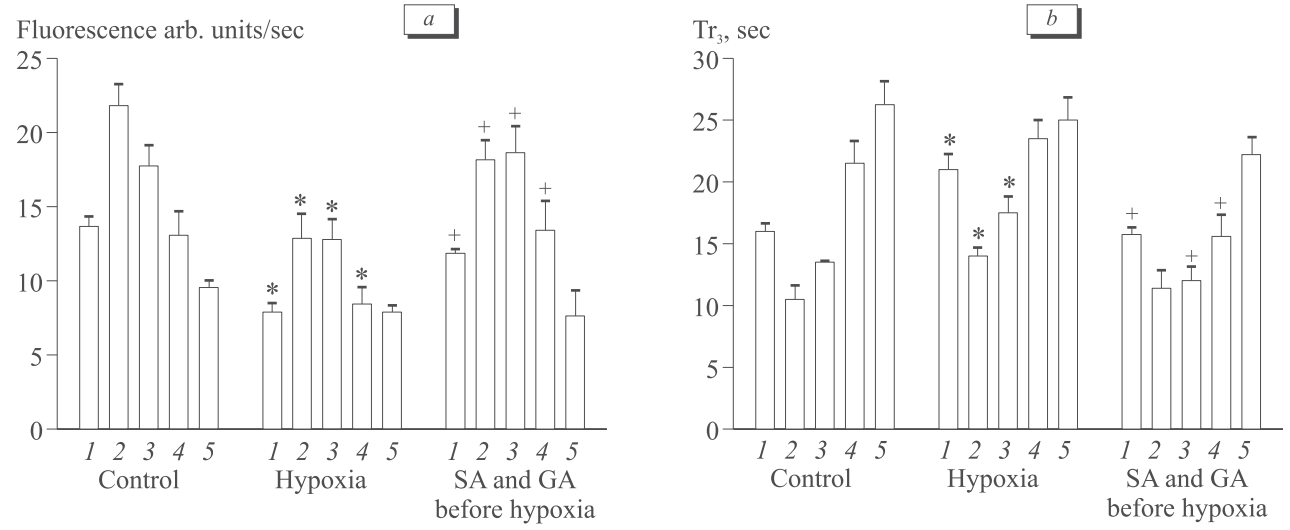

Fig. 1. Effects of SA and GA combination on the velocity (*a*) and time of NAD reduction (*b*) in the liver MC of mice exposed to hypoxia ($X\pm Sx$, n=8). *1*) endogenous substrates; *2*) SA, 5×10^{-3} M; *3*) malate and glutamate mixture, 3×10^{-3} M each; *4*) malate and glutamate mixture, 3×10^{-3} M each, +malonate, 2×10^{-3} M; *5*) malate and glutamate mixture, 3×10^{-3} M each, +amino-oxyacetate, 5×10^{-4} M. Here and in Fig. 2: $p<0.05$ *vs.* *control, $^{+}$hypoxia.

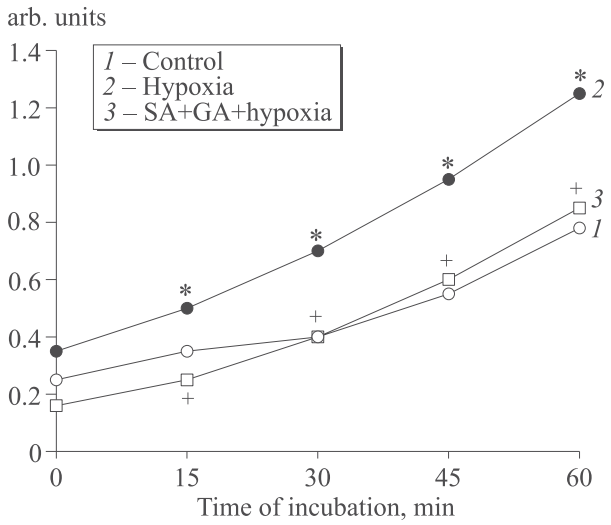

Fig. 2. Effect of SA and GA combination on accumulation of TBA-active products of ascorbate-dependent LPO during incubation of liver homogenate from mice exposed to hypoxia (n=8).

the antioxidant system potentialities [2,3]. Presumably, this was paralleled by modification of the physical and chemical characteristics of membrane lipids, membrane-bound proteins, and increase of proton conductivity of MC membranes [2-4], which suggests dissociation of oxidative phosphorylation [8].

Our data indicate realization of adaptive reactions in MC in response to hypoxia and other pathology. The course of these reactions conforms to the regularities of the common adaptation syndrome and are aimed at the maintenance of high level of energy supply and its prolongation. Urgent adaptation to slight hypoxia involves activation of energy metabolism via intensification of NAD-dependent substrates oxidation [7,8]. Under conditions of our experiment, augmenting hypoxia involves inhibtion of the MC respiratory chain electron transporting function [6], which leads to exhaustion of adaptation potentialities of organelles with progressive reduction of NAD-dependent energy production and suppression of oxidative phosphorylation. The factor limiting these processes is deficiency of NAD (oxidized transmitters of electrons). On the other hand, high degree of respiratory chain reduction activates the succinate oxidase oxidation pathway, which does not depend on NAD deficiency [5,6]. Hence, the kinetic advantages of SA oxidation in comparison with NAD-dependent substrates are realized at a certain values of oxygen partial pressure in tissues and the exhaustion of MC adaptive resources is prevented. The rapid substrate oxidation pathway is activated in MC, due to which ATP is produced by endogenous SA formation and oxidation (by-passing the slow Krebs' cycle reactions), thus providing the resistant status of energy production. Oxaloacetate inhibition of SDH under these conditions predominates over oxygen deficiency in the mechanism of macroerg production limitation [6]. This indicates high efficiency of measures aimed at prevention of the enzyme inhibition.

The SA and GA mixture exhibited an antihypoxic effect by increasing (by 20%) the mouse life span till the agonal inhalation (24.6±0.7 min) under conditions of normobaric hypercapnic hypoxia in comparison with the control animals. The MC function normalized in animals exposed to hypoxia. Among other things, the velocity of NAD reduction increased and the time of NAD reduction was shorter during oxidation of endogenous substrates, succinate, and NAD-dependent substrates, while the parameters of MC function approached the normal values (Fig. 1). Accumulation of LPO TBA-active products in liver tissue decreased, which fact indicated limitation of radical formation (Fig. 2). Presumably, these changes indicate reactivation of succinate- and NAD-dependent energy production processes, retention of the membranes integrity, and normalization of oxidative phosphorylation processes in MC under the effect of the SA and GA combination.

Succinic acid reactivates succinate-dependent oxidation through SDH activation [6,7]. Under conditions of hypoxia SA stimulates oxygen diffusion into cells, by which it restores NAD-dependent cell respiration. Glutamic acid under these conditions provides the delivery of endogenous SA by activating the rapid metabolic cluster reactions at the expense of oxaloacetate reamination and increase of coenzyme Q reduction.

Preventive treatment by SA and GA combination stimulated the function of the MC fast metabolic cluster, which was seen from an increase in the organelles sensitivity to NAD-dependent oxidation inhibitors (Fig. 1). Hence, a course of treatment by SA and GA prevented the development of hypoxic changes in the liver, preserved the resistant status of the MC energy production system. These results indicate the energy protective effect of mitochondrial substrates, their capacity to preserve the normal activities of NADH- and succinate-dependent systems. Presumably, limitation of lipid peroxides accumulation in the liver under conditions of hypoxia is due to the regulatory effect of SA and GA in MC, specifically, increased production of reduced glutathion as a result of retrograde transmission of electrons in the respiratory chain and limited formation of free radicals as a result of monopolization of energy production by succinate [6].

These data suggest the development of an antihypoxic agent based on succinic and glutamic acids.

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