Regulator of Energy Metabolism Protects the Myocardium during Pathological Processes

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Normalizing effect of energy metabolism regulator (succinic acid) on mitochondrial processes in rat myocardium during hypoxia, cardiac arrhythmia, and salicylate treatment was experimentally shown. A new alternative method for myocardial protection is proposed and its high efficiency in heart diseases of hypoxic and ischemic origin is validated.

Key Words: *cardiac protection; heart mitochondria; succinic acid; acetylsalicylic acid; arrhythmia; hypoxia*

Coronary disease developing as a result of coronary atherosclerosis is the leading cause of disability and mortality of population of capable age [7]. The progress of atherosclerosis in coronary arteries leads to a discrepancy between the level of coronary bloodflow and myocardial demands for oxygen and substrates associated with augmenting ischemia and hypoxia in cardiomyocytes, this leading to drastic changes in metabolism and energy supply [11]. Inhibition of ATP and creatinine phosphate synthesis associated with this condition leads to disorders in heart rhythm and myocardial contractility.

Drug therapy of myocardial ischemia consists in increasing O_2 delivery to the ischemic zone or reducing the O_2 demands of the myocardium. β-Adrenoreceptor blockers, nitrates, angiotensin-converting enzyme inhibitors, calcium antagonists, metabolic drugs, and antiaggregants are used for this purpose [7,8].

One of the main drugs for metabolic therapy of coronary disease is trimetazidine (TZ), a selective inhibitor of long-chain 3-ketoacyl-CoA-thiolase [8]. It inhibits β-oxidation of fatty acids without inhibition glycolysis, which creates metabolic prerequisites for lactate utilization and reduction of lactoacidosis in the ischemic myocardium. In addition, TZ partially reduces activity of phospholipase A_2 and LPO intensity, which in general leads to improvement of the myocardial function. However, TZ does not completely eliminate the hypoxic type of metabolism. Presumably, blockade of one of the most effective metabolic pathways of energy supply to the myocardium (lipid utilization) limits the potentialities of therapy for the diseases associated with the development of the deficiency of high-energy metabolites [11]. We proposed a principally new pharmacological approach to cardiac protection, consisting in removal of the metabolic limitations of ATP production in the ischemic myocardium without negative impact on the metabolism.

We proposed a new class of drugs regulating energy metabolism, optimizing energy supply processes, including those in the ischemic myocardium [11,13]. These drugs provide ATP production in cells at the expense of activity of the rapid metabolic cluster in mitochondria [12,13]. We previously showed that energy metabolism regulators reduced toxicity and increased the efficiency of acetylsalicylic acid (ASA) widely used in cardiology as an antiaggregant [2,7].

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We compared the effects of energy metabolism regulator amber antitox (AA) and TZ on mitochondrial processes in rat myocardium during hypoxia and cardiac arrhythmia and evaluated the effect of AA on energy metabolism in the myocardium of animals treated with ASA.

MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats (220-240 g). The animals received a preventive 5-day (daily orally) course of AA (Tomsk Pharmaceutical Factory) containing 0.2 g succinic acid in the antihypoxic and antitoxic dose of 50 mg/kg in conversion to succinic acid [10]; the last dose was given 1 h before hypoxia or arrhythmia. The reference group received a preventive 5-day course of TZ intragastrically in the antihypoxic dose of 20 mg/kg [3]. Controls received the solvent (1% starch gel).

Normobaric hypercapnic hypoxia was induced by placing the animals into a sealed 3-liter flask for 1.5 h. Functional activity of cardiac mitochondria was studied on day 1 after hypoxic injury. Experimental arrhythmia was induced by single intragastric dose of propranolol (200 mg/kg) [9]. Electrocardiogram was recorded in rats before and 5 min after propranolol dose, and then every 5 min over 40 min, in order to determine the latent time of the extrasystole development. Antioxidant activities of the test drugs were evaluated in the same animals by accumulation of MDA in heart homogenate after arrhythmogenic dose of propranolol. Basal MDA level and its changes during 1 h were evaluated [1]. Salicylate effects on energy production in the myocardium were studied by ASA treatment (250 mg/kg; $^1/_{10}$ LD_{50}). Group 1 animals received intragastric ASA daily for 7 days. Group 2 received ASA and AA (100 mg/kg in conversion to succinic acid) for 7 days.

Mitochondrial function in rat heart homogenate was evaluated by polarography on an LP-9 device using Clark type closed electrodes made at the laboratory [5]. The composition of the isolation medium was as follows: 0.3 M sucrose, 2×10^{-3} M Tris buffer, 0.01 M EDTA, 1 mg/ml BSA, 0.12 M KCl (pH 7.2) at 0°C. The composition of incubation medium was as follows: 0.3 M sucrose, 0.12 M KCl, 5×10^{-3} M KH_2PO_4, 0.01 M HEPES buffer, 1×10^{-3} M EDTA (pH 7.2) at 26°C. The rates of O_2 consumption by the mitochondria before, during, and after the cycle of phosphorylation of ADP added to a concentration of 1×10^{-4} (V_{4p}, V_3, and V_{4o}) and duration of phosphorylation were calculated. Flavin-dependent succinate (1×10^{-3} M) and NAD-dependent malate and glutamate (3×10^{-3} M each) served as the substrates. In order to detect the contribution of mitochondria to energy production during oxidation of NAD-dependent substrates by endogenous succinic acid, SDH inhibitor malonate (2×10^{-3} M) and aminotransferase inhibitor aminohydroxyacetate (5×10^{-3} M) were used. Coefficients of respiration stimulation (V_3/V_{4p}), respiratory regulation (V_3/V_{4o}), and conjugation of ADP/O oxidative phosphorylation were calculated.

RESULTS

Hypoxia accelerated oxidation of the rat heart mitochondrial endogenous substrates during ADP phosphorylation cycle, the efficiency of oxidative phosphorylation decreased (Fig. 1). This regularity was retained during oxidation of exogenous substrates. The estimated ADP/O value was reduced during succinic acid oxidation, while during oxidation of malate and glutamate mixture the rate of phosphorylating respiration and efficiency of oxidative phosphorylation were reduced. This can indicate uncoupling of the oxidative phosphorylation process in rat heart mitochondria under the effect of hypoxia. Inhibitory analysis indicated reduction of mitochondrial respiration sensitivity to SDH blocker malonate during oxidation of NAD-dependent substrates in the myocardium and of aminotransferases to aminohydroxyacetate in the studied hypoxic model, which reflected the developing inhibition of the rapid metabolic cluster [13]. Summary analysis of mitochondrial respiratory activity showed that reduction of the ADP/O value in the myocardium in hypoxia indicated uncoupling of oxidative phosphorylation, but not changed ratio of oxidized flavin- and NAD-dependent substrates (2 and 3 phosphorylation points, respectively) in favor of the former.

Preventive treatment with AA before hypoxia prolonged animal life span by 37%, while TZ did not change this parameter. The difference in the mechanisms of action of these drugs [8,12] suggests a relationship between antihypoxic effects and capacity of SA to maintain succinate-dependent energy production, less sensitive to O_2 deficiency than the NAD-dependent oxidation pathway.

The use of AA in hypoxia decelerated phosphorylating respiration of rat heart mitochondria during oxidation of endogenous substrates. During succinate oxidation *in vitro* AA promoted deceleration of the respiration rate before, during, and after ADP phosphorylation cycle, increasing significantly the ADP/O value (Fig. 1). The common trend of AA effect was retained during oxidation

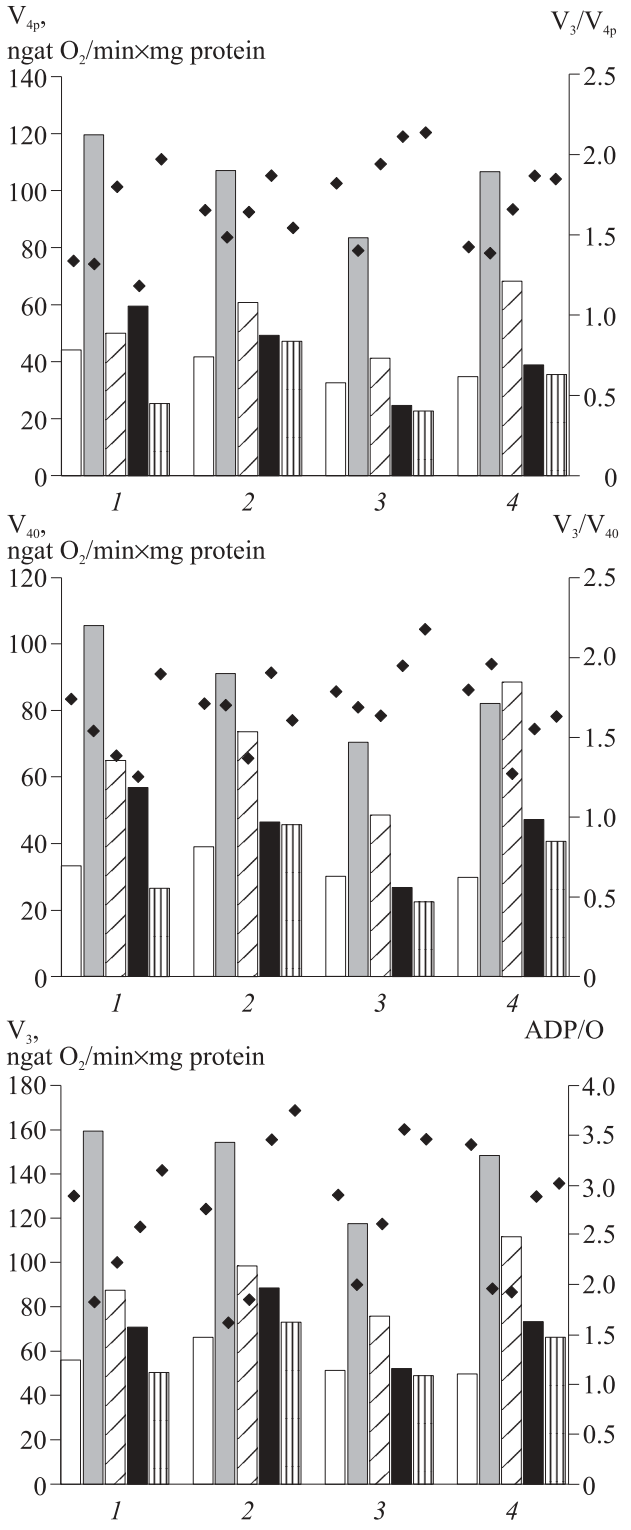

Fig. 1. Effects of AA and TZ on the function of the rat heart mitochondria in normobaric hypercapnic hypoxia. *1*) control; *2*) hypoxia; *3*) hypoxia+AA; *4*) hypoxia+TZ. Here and in Fig. 3: the studied parameters during oxidation of endogenous substrates (light bars), succinate (gray bars), malate and glutamate mixture (cross-hatched bars), malate, glutamate, and malonate (black bars), malate, glutamate, and aminohydroxyacetate (vertically hatched bars). Rhombi above show the right scale values, the bars show the left scale values.

of NAD-dependent substrates. The decrease in the respiration rates of the rat myocardial mitochondria before, during, and after ADP phosphorylation was associated with a significant increase in oxidative phosphorylation efficiency.

Energy deficiency forming during O_2 insufficiency manifested by increased mitochondrial respiration rates during oxidation of NAD-dependent substrates under conditions of oxidative phosphorylation uncoupling during transition from hypoxia to hyperoxic conditions of incubation [14]. Decompensated low-energy shift in hypoxia manifests in the myocardium by somewhat reduced rates of succinate oxidation by mitochondria in comparison with the control. It is a sign of overloading of the SDH inhibitor (oxaloacetate) elimination route in the presence of the substrate and O_2 excess in the incubation medium with mitochondria [5,15]. NAD-dependent substrates in saturating concentrations activate SDH and abolish enzyme inhibition, the rate of mitochondrial phosphorylating respiration in hypoxic animals surpasses that in the control. Acceleration of phosphorylating respiration during oxidation of endogenous substrates by mitochondria placed after hypoxic exposure into medium with O_2 excess seemed to reflect a sufficient level of substrate provision of cardiomyocytes under these conditions. Exogenous succinic acid energizes the organelles, transferring them into the reduced respiratory activity mode with maximum coupling of oxidative phosphorylation [12]. Mitochondrial respiration rate under the effect of AA during oxidation of endogenous and exogenous substrates was lower than in the control. Hence, AA exhibited an antihypoxic effect, normalizing cell respiration and oxidative phosphorylation processes in rat myocardium in experimental hypoxia. The mitochondria of animals treated with TZ were de-energized; this manifested most demonstratively during oxidation of NAD-dependent substrates (Fig. 1). Under the effect of TZ, succinate as the oxidation substrate energized the mitochondria *in vitro* and detected the signs of preceding energy deficiency (increased respiration rates in the presence of normal ADP/O ratio). Low respiration rates of these mitochondria in comparison with organelles in other groups of animals during oxidation of endogenous substrates and, vice versa, high rates during utilization of exogenous substrates suggest that high oxidative phosphorylation coefficient in the first case reflects de-energization of mitochondria, but not hypercoupled oxidative phosphorylation [12]. Judging from these data, antihypoxic effect of AA, optimizing the mitochondrial oxidation, is greater than that of TZ. Amber antitox really modulates the mitochondrial

processes by sensitization of SDH activity and modulation of energy production activity through its effects on specific receptors [6], without disordering the metabolism.

Propranolol treatment led to a 25.4% reduction of heart rate and caused extrasystoles in 30% rats. Treatment of intact rats with TS did not change heart rate, while AA reduced heart rate by 16%. No extrasystoles were detected in animals which received a 5-day course of AA 1 h before propranolol. Heart rate after propranolol treatment following AA course was within the normal range of values for rats. In contrast to AA, TZ promoted the development of propranolol-induced extrasystoles. Extrasystoles emerged in 50% of animals *vs.* 30% in the control and none in animals protected with AA.

Increased content of LPO products was detected in the myocardium of animals exposed to arrhythmia (Fig. 2). Amber antitox significantly limited the accumulation of MDA by the 30th min of incubation, this difference reaching the level of significance by the 60th min of homogenate incubation. Hence, succinic acid as a component of this drug exhibited antioxidant and cardioprotective effects in rats with experimental arrhythmia. By contrast, TZ exhibited a prooxidant effect (Fig. 2), augmenting the development of this pathology. It is obvious that the protective effect of AA in experimental arrhythmia with the ischemic component is more pronounced than that of TZ. Presumably, AA capacity to correct energy deficiency by optimizing the work of the rapid metabolic cluster in the myocardium [11] is more important for the maintenance of homeostasis in arrhythmia with characteristic ischemia than limitation of β-oxidation of fatty acids with TZ.

A course of ASA in a dose of 250 mg/kg led to metabolic shifts in the energy production system of rat myocardium, manifesting in inhibition of mitochondrial respiration (reduction of mitochondrial respiration rates during utilization of endogenous substrates before, during, and after ADP phosphorylation cycle in comparison with the control). This was paralleled by an increase ADP/O coefficient, presumably indicating predominant oxidation of NAD-dependent substrates and limitation of succinate-dependent energy production processes (Fig. 3), but not hyperenergization of the mitochondria, as there were no grounds for it. Similar changes were observed during utilization of exogenous substrates. Deceleration of O_2 consumption by the organelles before, during, and after ADP phosphorylation cycle during succinate oxidation by myocardial mitochondria was paralleled by reduction of the respiratory control value and an increase in ADP/O coefficient (Fig. 3). Oxidation of cardiac mitochondrial succinate in rats treated with ASA was also associated with prolongation of ADP phosphorylation time. These changes indicated reduced energization of organelles [12] (Fig. 3). This salicylate effect, inhibiting myocardial bioenergy, can have a negative impact on the ischemic heart metabolism.

A decrease in the phosphorylating respiration rate and prolongation of ADP phosphorylation in comparison with the control were noted in cardiac mitochondria in animals treated with ASA (Fig. 3). The decrease in respiration stimulation coefficient, paralleled by elevation of the ADP/O coefficient, indicates the kinetic pattern of energy deficiency in the cardiac mitochondria of rats treated with ASA [13]. On the other hand, inhibitory analysis using competitive SDH inhibitor (malonate) and aminotransferase inhibitor (aminohydroxyacetate) showed ASA-stimulated increase in the contribution of endogenous succinic acid oxidation to the respiratory activity of organelles during oxidation of NAD-dependent substrates. Presumably, this process is compensatory and reflects the specific feature of metabolic regulation of Krebs' cycle under conditions of ASA-modulated mitochondria exposed to the substrate and O_2 excess and, particularly, activation of the rapid metabolic cluster [4]. Salicylates are known to increase membrane permeability for H^+ [15], which promotes uncoupling of oxidative phosphorylation and de-energization of mitochondria. Presumably, the compensatory changes in mitochondrial energy production, induced by ASA, are caused by a short course of treatment with this drug.

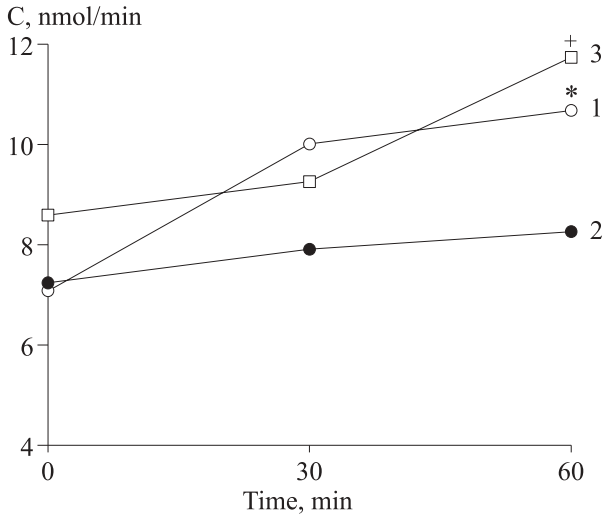

Fig. 2. Effects of AA and TZ on MDA content in rat heart homogenate in experimental arrhythmia. *1*) control; *2*) AA; *3*) TZ. *$p<0.05$ compared to the control; $^+p<0.05$ compared to the group treated with AA.

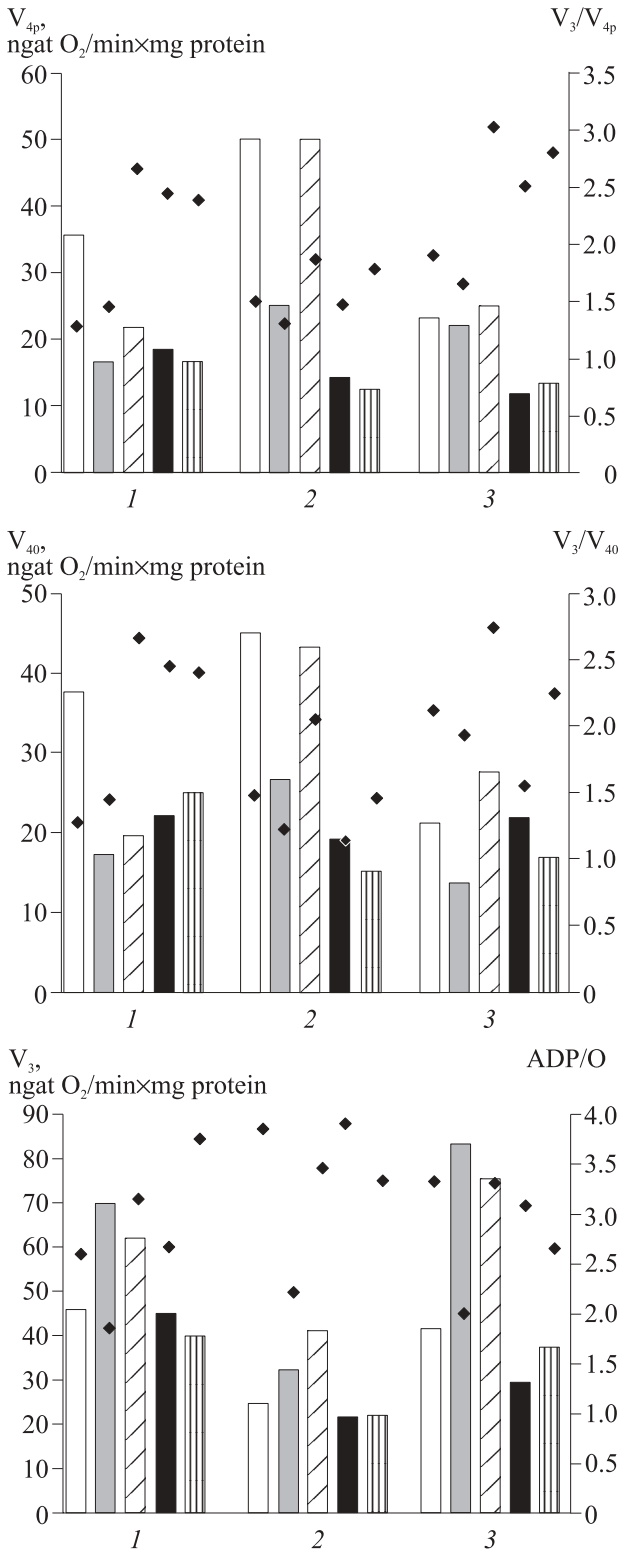

Fig. 3. Effect of AA on cardiac mitochondrial function in rats treated with ASA. *1*) control; *2*) ASA; *3*) AA+ASA.

Amber antitox promoted correction of ASA-induced negative metabolic shifts in rat heart mitochondria (Fig. 3). Normalization of mitochondrial respiration rates in all metabolic states during oxidation of endogenous substrates was observed in comparison with the values in animals treated with ASA alone. Respiration stimulation and respiratory control values increased, while the ADP/O ratio reduced towards the normal value in these animals, indicating normalization of succinate-dependent energy production. Succinate oxidation by heart mitochondria of rats treated with AA after ASA was associated with an appreciable increase in O_2 consumption rate before, during, and after ADP phosphorylation cycle and significant increase of the respiration stimulation and respiratory control coefficients in comparison with animals treated with ASA alone (Fig. 3). This confirms the hypothesis about ASA inhibition of the succinate-dependent route of substrate oxidation and its normalization under the effect of AA. Normalization of the ADP/O ratio indicates realization of the kinetic advantages of succinate oxidation in comparison with NAD-dependent substrates [12].

The rate of phosphorylating respiration and respiration stimulation during utilization of NAD-dependent substrates by heart mitochondria increased significantly in rats treated with AA after ASA in comparison with rats treated with ASA alone (Fig. 3). This indicates normalization of NAD-dependent respiration and oxidative phosphorylation in animals protected with AA. Inhibitory analysis using malonate and aminohydroxyacetate detected an appreciable increase of oxidation and production of endogenous succinic acid in comparison with animals treated with ASA, which indicates intense work of the rapid metabolic cluster. Hence, AA reduced the negative effect of salicylates on the myocardial bioenergy. The detected pharmacological effect is particularly important for the therapy of patients with cardiovascular ischemic diseases, who have to use salicylates for a long time, despite the risk of mitochondrial complications [15].

These data indicate high efficiency of cardioprotection of the myocardium under hypoxic conditions, cardiac arrhythmias, and prompt the use of salicylates in parallel with drugs regulating (but not blocking) mitochondrial processes in the myocardium. Energy metabolism regulators possess a wider spectrum of positive metabolic effects than fatty acid β-oxidation blockers and cause no metabolic disorders. These results demonstrate the possibility of more effective cardiac protection of coronary patients, particularly those who have to compensate for ischemic metabolism and use antiaggregant salicylates permanently.

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