Cardioprotective Effects of Trimetazidine and a Combination of Succinic and Malic Acids in Acute Myocardial Ischemia

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A mixture of mitochondrial substrates of succinic and malic acids more effectively than antihypoxant trimetazidine prevented functional and metabolic disorders in rat myocardium during acute ischemia: reduces *T* wave amplitude, *QT* interval, number and duration of arrhythmias, and restores oxidation-phosphorylation coupling.

Key Words: *acute myocardial ischemia; myocardial mitochondria; succinic acid; malic acid*

Coronary heart disease (CHD) is the leading cause of disability and mortality of capable population in Russia and all over the world. The key factor in the pathogenesis of CHD and development of ischemic arrhythmias is discrepancy between the level of coronary blood flow and oxygen demand in the myocardium. This results in the formation of hypoxic metabolism in cardiomyocytes, with suppressed aerobic synthesis of ATP and activation of glycolysis, accumulation of lactate, and uncoupling of oxidative phosphorylation [1,6].

Metabolic drugs improving the function of ischemic myocardium are an important component in the therapy for CHD. One of the main drugs of this series is trimetazidine (TR), an antihypoxant [2]. Partial blockade of lipid β-oxidation by TR leads to stimulation of glucose oxidation, which leads to a decrease in oxygen expenditures for ATP synthesis in cardiomyocytes, severity of acidosis, and intensity of LPO [7]. On the other hand, the antihypoxic effects of TR can be realized only in moderate hypoxia, while severe condition is associated with the development of regulatory limitation of lipid utilization, and TR is undesirable in this case.

We suppose that drug correction of energy imbalance developing in ischemia by optimization of energy formation processes in mitochondria can be more preferable than blockade of lipid β-oxidation. It can be achieved by using natural mitochondrial metabolites [4]. Among them succinic and malic acids, whose antihypoxic activities are proven, deserve special attention. The mechanism of their action is based on elimination of hypoxic metabolism under conditions of pronounced oxygen deficiency by reducing SDH inhibition and restoration of activity of fast metabolic cluster in mitochondria [7]. Reactivation of the fast metabolic cluster in this case not only eliminates lactacidosis, but also promotes β-oxidation of fatty acids in cardiomyocytes [5].

We studied the cardioprotective effects of a combination of succinic and malic acids in acute myocardial ischemia in rats.

MATERIALS AND METHODS

The study was carried out on 40 male Wistar rats (230-250 g). The animals received (intragastrically)

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a preventive course of a combination of succinic and malic acids in antihypoxic doses of 50 and 75 mg/kg, respectively, once daily for 5 days. Antihypoxant TR [2] served as the reference drug; it was injected by a preventive course (20 mg/kg/day during 5 days). Rats receiving the solvent (1% starch gel) served as the control. Normal values were measured in a group of intact rats. The animals were narcotized with sodium thiopental (40 mg/kg intraperitoneally). Acute myocardial ischemia was induced by 15-min occlusion of the left coronary artery under conditions of forced ventilation. ECG in standard lead II was recorded over 15 min of ischemia and 3 min of reperfusion. The amplitudes of *P*, *R*, *T* waves (mV), duration of *PQ*, *QRS*, *QT* (sec) intervals, and episodes of ventricular tachyarrhythmias (VTA) developing during ischemia were recorded. The severity of ischemic VTA was evaluated by the number of animals with VTA, time before the first VTA episode, and by the total duration of these episodes.

Functional state of cardiac mitochondria was evaluated by polarography as described previously [5]. Succinate (5×10^{-3} M) and NAD-dependent malate and glutamate (3×10^{-3} M each) served as the oxidation substrates. The contribution of endogenous succinic acid to mitochondrial energy production during oxidation of NAD-dependent substrates was evaluated using malonate, a competitive SDH inhibitor (2×10^{-3} M), and aminooxyacetate, aminotransferase inhibitor (5×10^{-4} M). The rates of oxygen consumption by mitochondria before (V_{4p}), during (V_3), and after (V_{4o}) 1×10^{-4} M ADP phosphorylation cycle, and ADP phosphorylation time (Tr) were calculated. Respiration stimulation ($RS=V_3/V_{4p}$), respiratory control ($RC=V_3/V_{4o}$), and oxidative phosphorylation coupling (ADP/O) coefficients were calculated for evaluation of energy status.

The significance of differences was evaluated using the nonparametric Mann—Whitney test and Fisher test at 5% level of significance ($p<0.05$).

RESULTS

The initial ECG parameters in animals of the studied groups were within the normal range for rats. In control rats, pronounced ECG changes (increase of *T* wave amplitude and decrease of *R* wave) indicating the formation of the zone of ischemia and damage in the myocardium developed from the 2nd minute of coronary occlusion (Fig. 1). Lengthening of *QT* interval in ischemia and persistence of this shift throughout the entire experiment indicated deceleration of excitation and conduction processes

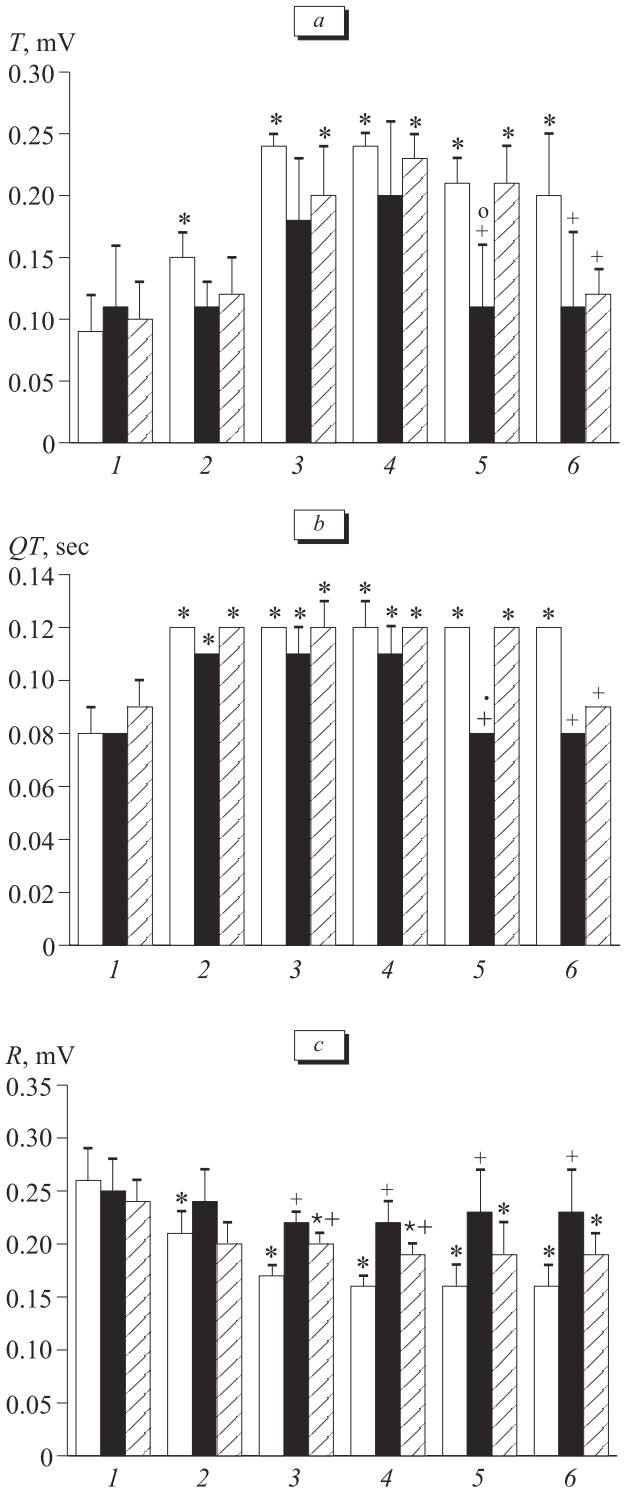

Fig. 1. Effects of the studied drugs on electrocardiographic parameters under conditions of myocardial ischemia/reperfusion in rats ($X\pm SD$; $n=10$). *a*) amplitude of *T* wave; *b*) length of *QT* interval; *c*) amplitude of *R* wave. Light bars: ischemia; dark bars: succinic and malic acids complex+ischemia; cross-hatched bars: TR+ischemia. *1*) initial values in the groups; *2*) ischemia, minutes 0-3; *3*) ischemia, minutes 3-7; *4*) ischemia min 7-15; *5*) reperfusion, minutes 0-1; *6*) reperfusion, minutes 1-3. $p<0.05$ compared to: *initial values in the groups, +values in ischemia, °TR+ischemia.

in the myocardium. *T* wave amplitude remained increased, while *R* wave amplitude remained low compared to the norm during the entire reperfusion period in the control. No appreciable shifts in the amplitudes of *T* and *R* waves in comparison with the initial values were noted in rats receiving the combination of succinic and malic acids (Fig. 1). The *QT* interval surpassed the initial values in ischemia, but as soon as during the first minute of reperfusion the duration of this interval returned to normal and remained at this level until the end of the experiment. In rats treated with TR, the *T* wave amplitude increased and *R* wave amplitude decreased starting from minutes 4.2±1.5 and 3.0±1.0 of ischemia, respectively (Fig. 1). Trimetazidine promoted the decrease in *T* wave amplitude only from the 2nd minute of reperfusion, while the amplitude of *R* wave remained low compared to normal by the end of ischemic period and throughout the entire reperfusion period (Fig. 1). The *QT* interval during ischemia in animals treated with TR was above the initial level and returned to normal only by the end of reperfusion.

The protective effect of the combination of succinic and malic acids was realized during the initial period of reperfusion, which was seen from a significant difference in the *T* wave amplitude in comparison with the control and TR group (Fig. 1). It seems that normalization of electrophysiological status of ischemic myocardium under the effect of succinic and malic acids mixture surpassed the therapeutic effect of TR.

A total of 90% control animals developed VTA. Summary duration of VTA episodes per animal was 2.38±0.39 min, of which 0.58±0.25 min were recorded during reperfusion. In rats treated with succinic and malic acids, the incidence of VTA decreased by 50%. The time before the onset of the first VTA episode was 50% longer than in the control, while the total duration of VTA episodes decreased by 29.4%; no manifestations of arrhythmias were recorded during reperfusion. In the TR group, summary activity of VTA did not differ from that in the control and was 2.33±0.35 min, of which 0.25±0.13 min were recorded during reperfusion. Trimetazidine did not modify the incidence of VTA (70%), but reduced the length of the period before its onset by 42.8%.

Hence, antiischemic and antiarrhythmic effects of the mixture of succinic and malic acids surpassed those of TR.

The rate of oxidation of endogenous substrate was reduced in the cardiac mitochondria of rats subjected to myocardial ischemia. This was paralleled by a pronounced inhibition of ADP phosphorylation cycle, which was seen from lower rates of phosphorylating respiration of organelles and longer ADP phosphorylation time compared to normal (Fig. 2). High RS, RC, and ADP/O values in this group seem to indicate the predominating oxidation of NAD-dependent substrates and inhibition of succinate-dependent energy production processes (predominance of electron flow in the respiratory chain through 3 phosphorylation points). Similar changes in the respiratory activity of myocardial homogenate mitochondria were observed during oxidation of exogenous succinate and NAD-dependent substrates: respiration rate of organelles decreased and ADP phosphorylation time increased (Fig. 2). Presumably, these shifts were caused by compensatory limitation of SDH hyperactivity. Pathological inhibition of SDH activity during some stages of hypoxia can lead to violation of metabolic regulation of mitochondrial respiration and cause deep suppression of the succinate-dependent energy provision, predominating in hypoxic metabolism [4]. Addition of β-hydroxybutyrate (SDH activator) to succinate-oxidizing mitochondria did not restore normal activity of the enzyme, which was seen from reduced (compared to normal) respiration rate of organelles (Fig. 2). Presumably, this indicates a drop of mitochondrial membrane potential, resultant from deep inhibition of SDH, failure of the organelles to maintain calcium homeostasis, and accumulation of free fatty acids [4]. Inhibitory analysis showed a reduction of the sensitivity of NAD-dependent respiration in ischemic myocardium to malonate (competitive SDH inhibitor) and aminooxyacetate (aminotransferase inhibitor), which indicates inhibition of the rapid mitochondrial metabolic cluster developing during the pathology.

Oxidation of endogenous substrates by mitochondria was more rapid in the group of rats treated with a mixture of succinic and malic acids before myocardial ischemia, with normalization of RC, ADP/O, and duration of ADP phosphorylation cycle (Fig. 2). Mitochondrial respiration rates, RS and RC values remained low during succinate utilization compared to normal, which was however paralleled by normalization of ADP/O and duration of ADP phosphorylation. During *in vitro* mitochondrial oxidation of succinate together with SDH activator β-hydroxybutyrate, the organelle respiration rate increased significantly, while ADP/O value decreased, which indicated recovery of succinate-dependent energy production processes. Utilization of NAD-dependent substrates was associated with an increase in mitochondrial organelle respiration rate, normalization of oxidative phosphorylation (time of ADP phosphorylation and ADP/O value),

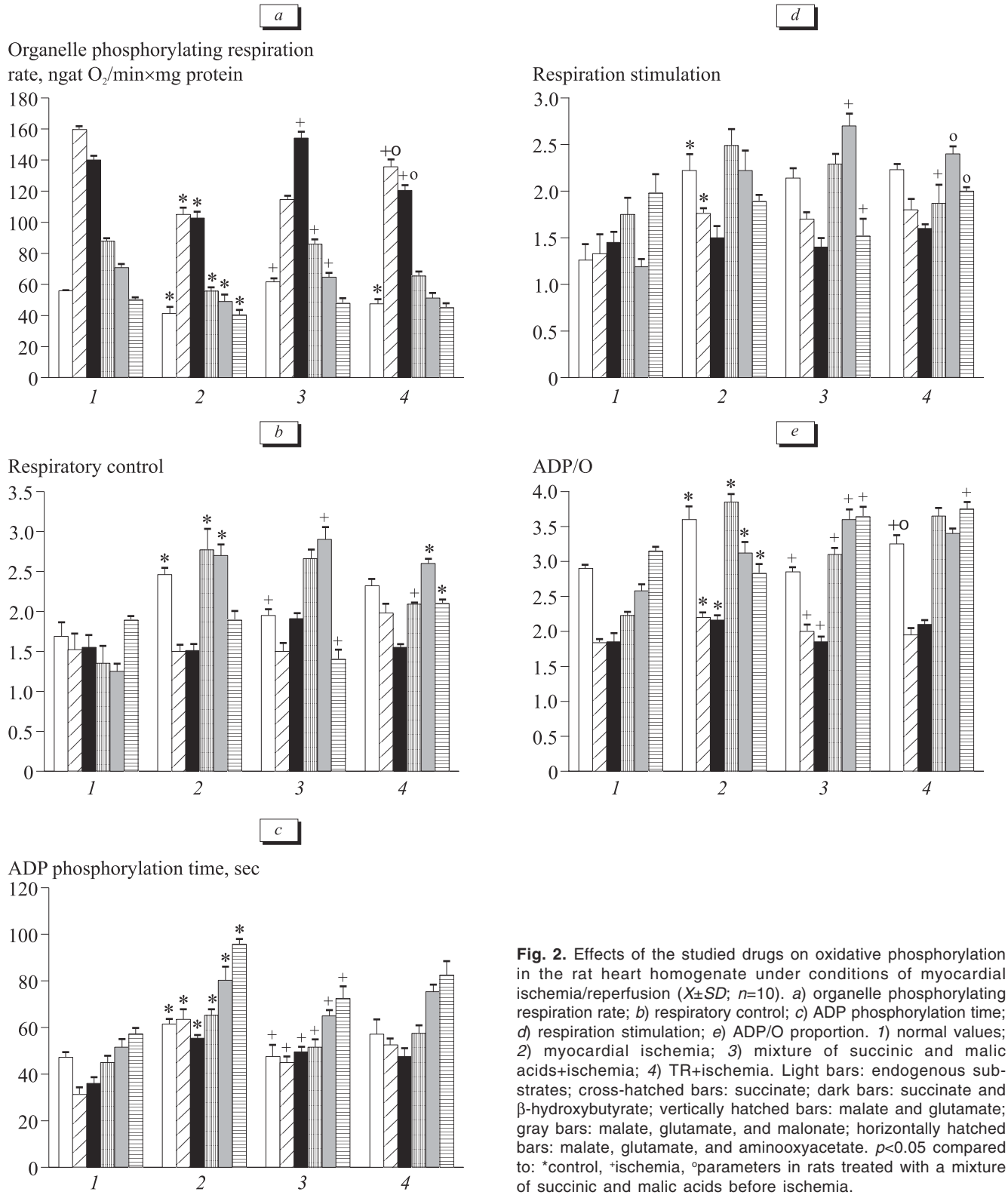

Fig. 2. Effects of the studied drugs on oxidative phosphorylation in the rat heart homogenate under conditions of myocardial ischemia/reperfusion ($X \pm SD$; n=10). *a*) organelle phosphorylating respiration rate; *b*) respiratory control; *c*) ADP phosphorylation time; *d*) respiration stimulation; *e*) ADP/O proportion. *1*) normal values; *2*) myocardial ischemia; *3*) mixture of succinic and malic acids+ischemia; *4*) TR+ischemia. Light bars: endogenous substrates; cross-hatched bars: succinate; dark bars: succinate and β-hydroxybutyrate; vertically hatched bars: malate and glutamate; gray bars: malate, glutamate, and malonate; horizontally hatched bars: malate, glutamate, and aminooxyacetate. p<0.05 compared to: *control, +ischemia, °parameters in rats treated with a mixture of succinic and malic acids before ischemia.

and also with recovery of sensitivity of organelles to malonate and aminooxyacetate, this indicating normalization of NAD-dependent oxidation processes and retention of activity of fast metabolic cluster in mitochondria. It seems that normalization of succinate-dependent oxidation, SDH activation, and reactivation of the Krebs cycle fast metabolic cluster play an important role in the mechanism of

cardioprotective effect of the combination of succinic and malic acids. This treatment seems to result in recovery of ATP and creatine phosphate synthesis in cardiomyocytes, normalization of redox homeostasis, reduction of lactacidosis in the ischemic zone [3], and preservation of myocardial function.

No normalization of energy production parameters were detected in the myocardial mitochondria of rats treated with TR. The mitochondrial organelle respiration rate during oxidation of endogenous substrates remained pathologically low in the presence of high ADP/O value and slow ADP phosphorylation cycle (Fig. 2). Succinate oxidation was paralleled by mitochondrial energization, which was seen from an increase in the organelle respiration rate in comparison with the group of ischemic rats. This reflects the known effect of succinic acid, realized *in vitro* in mitochondria, for which succinate serves as SDH activator. On the other hand, the parameters of oxidative phosphorylation of cardiac mitochondria did not normalize even in during combined oxidation of succinate *in vitro* together with SDH activator β-hydroxybutyrate. De-energization of myocardial mitochondria of rats treated with TR was most pronounced during oxidation of NAD-dependent substrates: the organelle respiration rates remained low in comparison with the normal, while the ADP/O and ADP phosphorylation time were higher than normally. Inhibitory analysis of NAD-dependent respiration revealed leveling of differences between experimental and ischemic groups of animals by organelle respiration rates, RS, RC and ADP phosphorylation time, this indicating inhibition of NAD-dependent oxidation.

Hence, the cardioprotective effect of a combination of succinic and malic acids regulating mitochondrial oxidation was higher than that of TR, a blocker of fatty acid β-oxidation. It seems that drug protection of the myocardium by optimization of mitochondrial oxidation in ischemia is more effective in comparison with cardioprotection by limitation of lipid oxidation.

These data demonstrate the efficiency of a combination of succinic and malic acids in practical cardiology for myocardial protection from ischemia.

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