Accelerated Utilization of Lactate under the Effect of Hypoxen after Intensive Exercise

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Administration of substrates of energy metabolism in combination with hypoxen (sulfur-containing oligoquinone) promoted the increase in blood lactate concentration during maximum exercise, accelerated lactate utilization, and reduced the lactate/pyruvate ratio during recovery. The *in vivo* effects are in line with hypoxen capacity to accelerate *in vitro* oxidation of exogenous NADH in mitochondria by the non-rotenone-dependent pathway realized with participation of cytochrome C.

Key Words: *hypoxen; lactate; exercise; mitochondria*

Discrepancy between oxygen delivery and intensification of functions during exercise is inevitably associated with the development of exercise hypoxia. Post-exercise or postischemic reoxygenation is the main constituent of posthypoxic damage and death of cells caused by the development of oxidative stress [3,12,14]. That is why antioxidants, specifically quinone derivatives, are now widely used for antihypoxic therapy [6,10-12].

We previously showed that hypoxen (sulfur-containing oligoquinone), a well-known antihypoxant [5,8] used in a wide range of concentrations, prevents generation and damaging effects of active oxygen forms (AOF), reduces energy dissipation at the level of complex I of the mitochondrial (MC) respiratory chain by improving the efficiency of oxidative phosphorylation in the most vulnerable site of the respiratory chain damaged by AOF. Today hypoxen is known as a drug used in athletic medicine for improving and stimulating the recovery of working capacity. Hypoxen appreciably accelerates recovery of many parameters, for example, it promotes elimination of underoxidized products from tissues after hemorrhage [1].

We studied possible mechanism of the effect of hypoxen in intensive exercise.

MATERIALS AND METHODS

The efficiency of hypoxen was evaluated in animals according to the protocol used for maintaining working capacity of athletes using this drug in combination with energy metabolism substrates. Experimental animals were divided into 3 groups: controls (receiving water); experimental group 1 (substrate mixture) and experimental group 2 (substrate mixture with hypoxen). The substrate mixture contained carbonic acids (20% succinic, 20% glutamic, 7% fumaric, 25% ascorbic, and 48% glucose). The total dose of the substrate mixture was 300 μg/g, which was equivalent to the mean dose for humans multiplied by coefficient 6.5 in accordance with the recommendation of the Pharmacological Committee of Ministry of Health of the Russian Federation for drug trials on mice [7]. Supplemented substrate mixture contained 12.28% hypoxen. Hence, the dose of hypoxen received with the substrate mixture was 36.8 μg/g. With consideration for coefficient 6.5 for mice, this dose was

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equivalent to the minimum dose of hypoxen for humans (5 mg/kg) [6]. The study was carried out on 68 male albino SD mice (25-30 g). Exercise started 20 min after oral water (control), substrate mixture without hypoxen (group 1), or substrate mixture with hypoxen (group 2) administered into the stomach (5 μl/g) through a tube. Two hours before exercise (running), the animals were deprived of fodder. Four days before the test, the animals were trained to run on a treadmill moving at a velocity of 32 cm/sec. A needle metal arrester was used to make the animals run at a preset velocity. Testing was carried out in two modes: after moderate exercise (3-min running at 32 cm/sec) and after maximum exercise (7-min running at 44 cm/sec) in experimental groups of 7 animals each and 6 controls. The animals refused to run after maximum exercise.

Glucose, lactate, and pyruvate concentrations were measured in the blood collected after decapitation. Glucose, lactate, and pyruvate were evaluated by the enzymatic method in protein-free blood extracts by changes in the NAD(P)H fluorescence, measured at λ=340 nm, in Bergmeyer's conjugated reactions [9].

Mitochondria were isolated from the liver of Wistar rats (200-250 g) by differential centrifugation [2]. The isolation medium contained 0.3 M sucrose, 10 mM HEPES (pH 7.4), and 1 mM EGTA. The mitochondria were washed and suspended in isolation medium without EGTA to a final concentration of 60-80 mg/ml. Suspension of native MC with respiratory control >4.0 was stored on ice. Mitochondria with destroyed external membrane

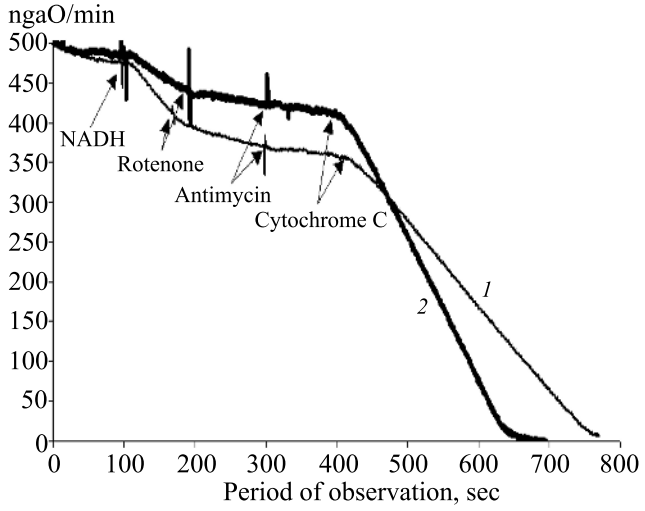

Fig. 1. Stimulation of oxygen consumption by damaged MC from rat liver during oxidation of exogenous NADH under the effect of hypoxen. *1*) control (witout hypoxen); *2*) hypoxen. Final concentration of hypoxen 10.7 μg/mg MC protein.

were obtained after defrosting of the suspension frozen at -20°C. Oxygen consumption during oxidation of exogenous NADH in MC was recorded by the polarographic method using a closed oxygen Clark electrode in a 1-ml thermostat cell at 27°C with constant agitation. The composition of the incubation medium was as follows: 155 mM sucrose, 50 mM KCl, 3 mM KH_2PO_4, 10 mM HEPES (pH 7.4), 1 mM $MgSO_4$ with NADH (1 mM), rotenone (2 μM), antimicin A (2 μM), and cytochrome C (1-8 μM).

Oxygen consumption recording was started during addition of MC.

The following reagents were used: NADH, NADPH, NAD, lactate dehydrogenase, pyruvate kinase, glucose-6-phosphate dehydrogenase (G-6-PDH), glutamate pyruvate transaminase (GPT) (all from Sigma), Tris-HCl and ATP (Serva), and hexokinase (Fluka).

RESULTS

In controls, lactate/pyruvate ratio after moderate exercise (3-min running) increased from 8.0±0.8 to 17.7±3.3 (n=6; p<0.02), the content of lactate increased from 1.7±0.3 to 3.9±1.1 mM and of glucose from 7.6±0.3 to 9.4±1.0 mM. After maximum exercise (7-min running), the lactate/pyruvate ratio reached 24.1±4.7 (p<0.01), lactate content reached 6.2±0.8 mM (n=6; p<0.02), while glucose level did not change (9.3±1.1 mM). After 15-min rest following the maximum exercise, the concentration of lactate decreased significantly (3.4±0.8 mM; p<0.02), while the lactate/pyruvate ratio virtually did not change (22.0±6.9) and glucose concentration was still increasing and reached 10.5±1.4 mM (p<0.05 in comparison with its level at rest). Hence, lactate concentration decreased by 2.80±0.51 mM during 15 min of rest (p<0.01). The substrate mixture without hypoxen had virtually no effect on the parameters in moderate or maximum exercise and at rest.

The parameters after moderate exercise in animals treated with the substrate mixture with hypoxen differed negligibly from those in the control and group 1. Lactate level increased to 3.3±0.8 mM, lactate/pyruvate ratio reached 16.2±4.6 mM, and glucose concentration increased to 10.0±1.6 mM. Substrate mixture with hypoxen administered to animals after maximum exercise led to a still greater increase in lactate level (8.9±0.4 mM; n=7; p<0.1) and lactate/pyruvate ratio (28.4±2.3; n=7; p<0.01) in comparison with the levels in other groups. However, after 15-min rest following the maximum exercise, the level of lactate dropped to 3.6±0.9 mM in animals treated with the substrate mixture with

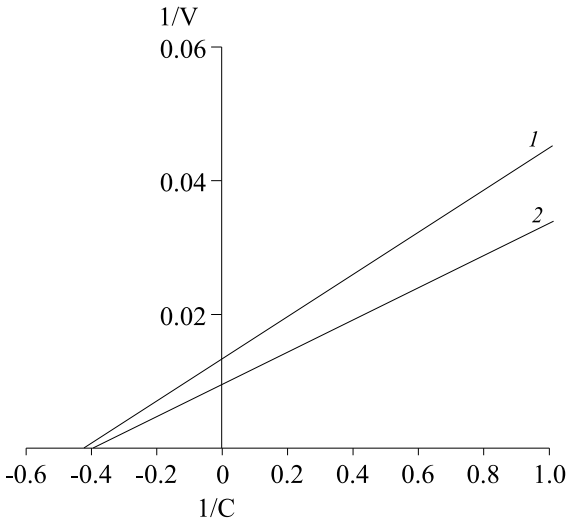

Fig. 2. Changed Lineweaver—Burk curve under the effect of hypoxen during oxidation of NADH by the rat liver damaged MC in the presence of cytochrome C in concentrations of 1-8 μM. *1*) control; *2*) hypoxen, 8-18 μg/mg MC protein. The data are presented as the mean of 7 independent experiments ($p<0.01$).

hypoxen. Lactate concentration decreased by 5.3± 0.8 mM within 15 min ($p<0.5$ in comparison with the rate of lactate concentration decrease in other groups), lactate/pyruvate ratio decreased to 20.8±3.5 mM (by 7.6±1.3 mM within 15 min; $p<0.1$ *vs.* the rate of this ratio decrease in other groups).

Hence, substrate mixture with hypoxen promoted greater increase in lactate concentration and of pyruvate/lactate ratio during maximum exercise and significantly accelerated the recovery (reduction of lactate concentration and of lactate/pyruvate ratio during rest after maximum exercise).

We hypothesized that rapid recovery of lactate/pyruvate ratio was due to more intensive oxidation of cytosol NADH in MC, its level stechiometrically corresponding to the increment of lactate (product of glycolysis activated during exercise hypoxia).

Exogenous NADH is not oxidized in intact liver MC [13]; activation of the external pathway of NADH oxidation is triggered by swelling and damage to the MC external membrane. These changes in MC structure are observed in hypoxia and reoxygenation. Damage to outer MC membrane was inflicted by single freezing and thawing, which provided unlimited oxidation of extramitochondrial NADH (Fig. 1). Violation of the outer membrane integrity caused no appreciable injuries in the respiratory chain located in the MC inner membrane, which was seen from persisting inhibitory effects of rotenone and antimycin A during oxidation of NAD-dependent substrates and NADH. Hypoxen added to the incubation medium in a concentration of 9-12 μg/mg MC protein reduced the rate of NADH oxidation by 30% ($p<0.05$) in the respiratory chain, located in inner MC membrane (rotenone-sensitive pathway) and activated the external pathway of NADH oxidation, stimulated by addition of 6 μM cytochrome C in the presence of rotenone and antimycin A. This was paralleled by 25% increase in oxygen consumption: from 56.2±3.2 to 70.1±4.6 ngat/min/g protein ($n=9$; $p<0.001$).

Kinetic analysis showed that hypoxen in a wide range of concentrations did not modify MC affinity for cytochrome C essential for the realization of rotenone-dependent oxidation of extramitochondrial NADH, but increased the maximum rate of oxygen consumption by this pathway (V_{max}) from 71.8±3.5 to 106.4±5.7 ngat/min/mg MC protein ($p<0.001$; Fig. 2). Hypoxen virtually did not influence K_m for cytochrome C (2.3±0.2 without hypoxen and 2.5± 0.2 with hypoxen). This effect of hypoxen can be due to its incorporation into transporting system of reducing equivalents between NADH and cytochrome C and/or between cytochrome C and cytochromoxidase.

Increased blood level of lactate and lactate/pyruvate ratio after administration of substrate mixture with hypoxen can be determined by more rapid washout of metabolites from the working muscle under the effect of hypoxen. Significant increase in NADH oxidation rate by the non-rotenone-dependent pathway activated during the posthypoxic period can contribute to the process of accelerated lactate oxidation.

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