Donor of Nitric Oxide Improves, while NO-Synthase Inhibitor Impairs Resistance and Adaptation to Strenuous Physical Exercise

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It is shown that NO donor (dinitrosyl iron complexes) 1.5-fold improves, while NO-synthase blocker (Nω-nitro-L-arginine) 1.5-fold impairs the resistance to strenuous exercise in experimental animals. Animals adapted to physical exercise swim 22.1±2.0 min, while control (nonadapted) animals only 13.6±1.8 min. Administration of NO donor during adaptation prolongs swimming 1.6-fold in comparison with adaptation and 2.6-fold in comparison with the control. Inhibitor of NO-synthase completely abolishes adaptation to physical exercise. Our findings demonstrate the involvement of NO into mechanisms of organism's resistance to physical load and the possibility of modulating physical capacity and adaptation to strenuous physical exercise.

Key Words: *physical exercise; adaptation; nitric oxide; NO-synthase inhibitor; donor of nitric oxide*

Adaptation to physical exercise (APE) is a principal component of training programs for athletes, aviators, space pilots, and other professions associated with hard physical work under usual and stress conditions. Moreover, APE is currently used as an effective prophylactic and therapeutic approach in different pathologies [2]. Therefore, modulation of APE formation is an important problem. When analyzing this problem we proceeded from three assumption.

1. Moderate physical exercise is accompanied by enhanced release of catecholamines and stress-related hormones, activation of free-radical oxidation, adequate stimulation of synthesis and utilization of macroergic compounds, and enhanced Ca^{2+} transport in skeletal muscles and other organs involved into muscle strain [3]. However, long-term and/or exhaustive physical exercise promotes realization of adrenotoxic effects, excessive activation of free-radical processes, and depletion of energy resources in muscles and other organs [2]. This leads to impossibility of performing physical work and sometimes to death.

2. APE requires some rearrangements in neurohumoral and local cell regulatory systems [2,3]. These changes restrict stress-induced damage always accompanying physical exercise [2] and improve blood supply [4] and oxygen transport [13], stimulate ATP synthesis [6] and gene expression for contractile proteins [9] and antioxidant enzymes [7], and promote accumulation of stress-defense HSP70 proteins [8] in organs and tissues responsible for adaptation. This results in adequate stimulation of energy production and cell protection against damage caused by strenuous exercise.

3. Formation of stable APE occurs against the background of increased content of nitric oxide (NO) [1]. NO participates in neuronal transmission [5] and restriction of catecholamine release in the central nervous system [11]. Moreover, NO plays an im-

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portant role in regulation of vascular tone [10]. It has been recently found that NO is involved into regulation of gene expression of antioxidant enzymes [12] and HSP70 [9].

Analysis of changes accompanying APE and physiological effects of NO suggests that NO can be involved into processes determining organism's resistance and adaptation to strenuous exercise.

Our study was aimed at verification of this hypothesis. In light of this, we studied the effect of NO donor and NO-synthase inhibitor on organism's resistance to physical exercise and formation of APE.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 220-250 g.

Resistance to strenuous physical exercise was assessed by the duration of swimming (min) with a load (3% body weight) to complete exhaustion and death. Each animal was tested individually in a 40-liter tank (80 cm depth).

APE was performed in the same daytime as follows: the animals were trained to swim at 22°C water temperature for 3 consecutive days (10, 15, and 20 min, respectively) with a load (1% body weight) fixed to the tail root.

Efficiency of APE was assessed 24 h after the last swimming session from prolongation of swimming compared with the control.

Appropriateness of the used test for organisms's resistance to strenuous physical exercise was proven in special experimental series.

Dinitrosyl iron complexes (DNIC) used as NO donors [12] were injected into the caudal vein in a dose of 200 μg/kg 24 h prior to testing or 24 h prior to each swimming session during adaptation. The NO-synthase blocker Nω-nitro-L-arginine (L-NNA, Sigma) was injected intraperitoneally in a dose of 50 mg/kg 1 h prior to testing and 1 h prior to each swimming session during adaptation.

Significance of differences was assessed using the Student's *t* test.

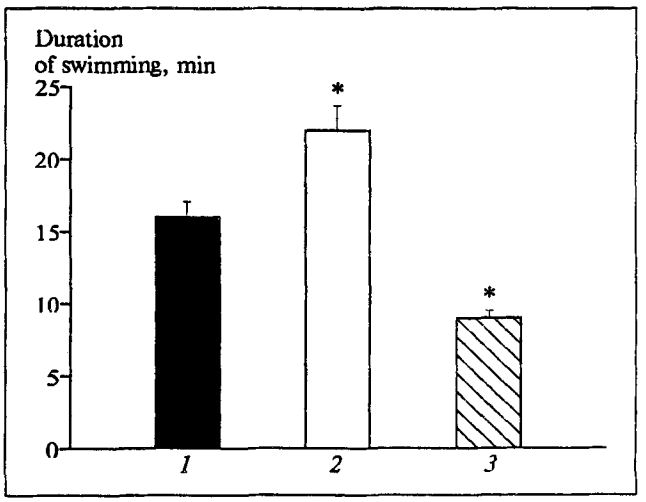

Fig. 1. Effect of nitric oxide donor (DNIC) and NO-synthase blocker (L-NNA) on organism's resistance to physical exercise. *1*) control (n=11); *2*) DNIC (200 μg/kg, n=6); *3*) L-NNA (50 mg/kg, n=6). Here and in Fig. 2: *p<0.05 compared with the control.

RESULTS

In our experimental model, three factors acted upon a rat: strenuous physical exercise (swimming with a load), stress caused by physical exercise and unusual environment and frequent forced submersion into water, and cold water (22°C). As seen from Table 1,

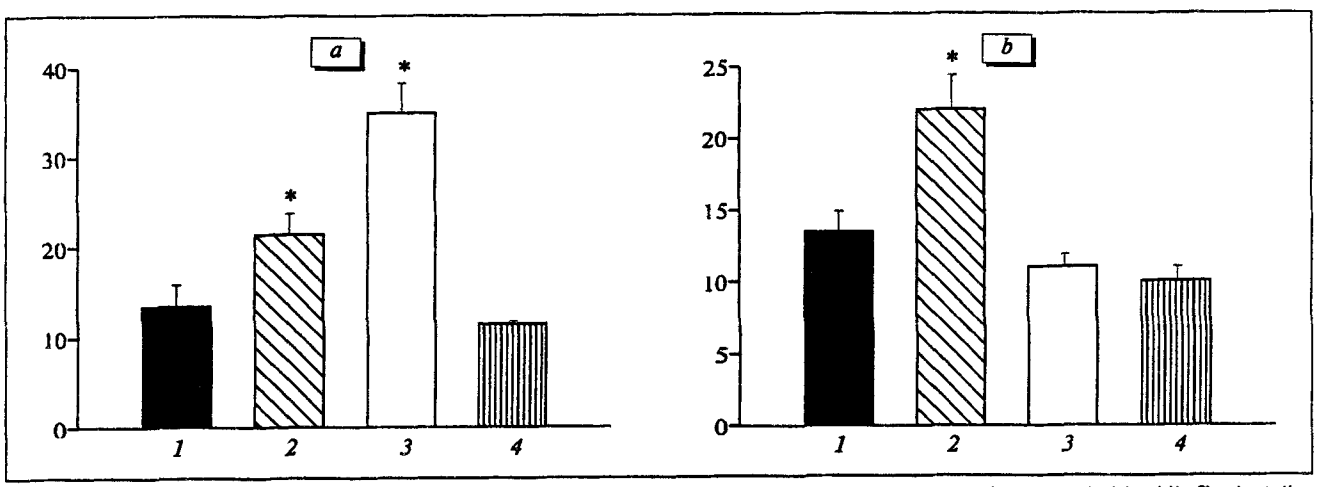

Fig. 2. Effect of nitric oxide donor(DNIC) and NO-synthase blocker (L-NNA) on adaptation to physical exercise. *1*) control (n=11); *2*) adaptation (n=14); *a*: 3) adaptation+DNIC (200 μg/kg, n=7); 4) DNIC (200 μg/kg, n=6); *b*: *3*) adaptation+L-NNA (50 mg/kg, n=8); 4) L-NNA (50 mg/kg, n=7). Ordinate: duration of swimming, min.

TABLE 1. Effect of Physical Load on the Duration of Swimming under Different Experimental Conditions (*M±m*)

Experimental conditions				Duration of swimming to exhaustion
factors during test	temperature of water, °C	depth, cm	load, % of body weight	
Physical load, cold water, stress	22	80	0	>8 h
	22	80	3	19.0±2.1 min
	22	80	5	10.0±1.1 min
	22	80	10	4.0±0.5 min
	22	80	12	2.5±0.5 min
	22	80	15	1.5±0.4 min
Cold water, stress	22	25	0	>24 h
Physical load, stress	32	80	0	>8 h

TABLE 2. Effect of APE Conditions on Organism's Resistance to Strenuous Physical Load (*M±m*)

Conditions of adaptation to physical exercise at 22°C			Duration of test swimming, min
factors during APE	depth, cm	load, % of body weight	
Control (nonadapted) rats			26.0±1.7
Moderate physical exercise, cold water, stress	80	1	60.2±5.0**
Minimal physical exercise, cold water, stress	80	0	41.0±3.4**
No physical exercise, cold water, stress	25	0	17.9±1.6*

Note. *$p<0.05$, **$p<0.01$ compared with the control.

in the absence of physical load animal swam more than 24 h, while an increase in the load weight to 15% body weight reduced the duration of swimming to 1.5 min.

Thus, under our experimental conditions the duration of swimming depended on physical load; hence, this test is an adequate method for assessing organism's resistance to physical exercise.

Apart from physical load, the animal during APE was subjected to stress and cold water. The data in Table 2 suggest that prolongation of swimming and adaptation to physical exercise depended primarily on periodic moderate physical exercise, but not on periodic stress and exposure to cold water.

The NO donor (DNIC) 1.5-fold increased, while the inhibitor of NO-synthase (L-NNA) 1.5 reduced the duration of swimming (Fig. 1). Thus, modulation of NO production affects the ability to perform physical work in experimental animals.

NO is also involved into APE. Figure 2 shows that adaptation prolongs the duration of swimming (22.1±2.0 *vs.* 13.6±1.8 min in the control, $p<0.05$) DNIC administered during adaptation increased the duration of swimming to 35.7±2.8 min, which 1.6- and 2.6-fold surpassed this parameter in adapted untreated and control animals, respectively (Fig. 2, *a*).

In contrast to the NO donor, the inhibitor of NO-synthase completely abolished the formation of APE (Fig. 2, *b*).

Thus, our findings suggest that organism's resistance and adaptation to physical exercise is modulated by regulation of NO production.

The contribution of different NO-dependent mechanisms is to be evaluated. However, it can be assumed that NO is involved into mechanisms of organism's resistance to physical exercise and its adaptative capacity can be regulated through modulation of NO generation systems.

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Effect of Repeated Cold Stress on Intensity of Lipid Peroxidation and Tissue Antioxidant System

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Repeated cold stress performed in a cold-tempering mode reduces lipid peroxidation and activates tissue antioxidant system.

Key Words: *stress; adaptation; antioxidants*

In the middle latitudes, all living organisms including man are often exposed to cold stress. Repeated cooling in a cold-tempering mode promotes adaptation processes, which allows us to use it in medical practice. It is often difficult to reveal the onset of the adaptation stage accompanied by improvement of organism's resistance. The state of biomembranes, in particular, the intensity of lipid peroxidation (LPO) can serve as an indicator of this stage.

The aim of the present study was to measure the intensity of LPO and the state of tissue antioxidant system in homoiothermal animals in repeated cold exposure.

MATERIALS AND METHODS

Experiments were carried out on 3-4-month random-bred albino rats. The animals were daily exposed to -5°C for 3 h during 20-25 days. During the first 4-5 sessions, body temperature decreased by 0.5-1°C and then returned to normal. Intact animals served as the control.

Intensity of LPO was measured by the content of malonic dialdehyde [15]. Total antioxidant activity (AOA) was assessed by inhibition of peroxidation of linolenic acid in the presence of tissue homogenates and blood serum [5]. Activity of hydrophilic antioxidants was determined by measuring the inhibition constant for oxidation of sodium 2,6-dichlorophenolindophenol on air in the presence of aqueous tissue extracts [13]. Activities of superoxide dismutase (SOD) and catalase in homogenates were measured at 25°C after sedimentation of mitochondria. Catalase activity was measured as described elsewhere [7], concentration of H_2O_2 was calculated using the calibration curve. Activity of SOD in tissue was assessed from inhibition of reduction of nitroblue tetrasolium [14]. The enzyme was preliminary purified from ballast proteins by adding 1 ml chloroform:methanol mixture (2:1) and few drops of KH_2PO_4 [4].

RESULTS

Unlike single cold exposure [9], repeated cold stress considerably suppressed LPO in the majority of studied organs (Table 1). This is consistent with published data on the dynamics of LPO in different types of stress [3,8,10] and suggest that LPO activation probably accompanies only early stages of stress. Tempering exposures to moderate stress, in particular, to cold is characterized by stabilization of LPO processes, hence the intensity of LPO can serve as an indicator of the adaptation stage of stress.

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