Metabolic Effects of Trekrezan during Adaptation of Rats to Intermittent Hypoxic Hypoxia

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The animals were adapted to intermittent hypoxic hypoxia in a flow pressure chamber for 3 days. Each one-day training session consisted of 4 elevations to an altitude of 6000 m for 20 min (15 m/sec, 20-min intervals between assents). Trekrezan (25 mg/kg intraperitoneally) was injected immediately after the end of daily training over 3 days. We showed that trekrezan increased the degree of adaptive metabolic changes in the brain, heart, and liver of rats during adaptation to hypoxic hypoxia.

Key Words: *intermittent hypoxic hypoxia; energy metabolism; trekrezan*

Adaptive capacities of the organism should be increased to maintain homeostasis during continuous exposure to the anthropogenic hypoxic factor. The rate and completeness of adaptive reactions depend not only on the severity and duration of hypoxia, but also on potential capacities and individual resistance of the organism to hypoxia [5]. The typological functional and metabolic differences in natural resistance determine the metabolic response to hypoxia in rats with high and low resistance to hypoxia. Energy deficiency, metabolic acidosis, lipid peroxidation (LPO), and inhibition of the antioxidant system in highly resistant (HR) rats with potent compensatory mechanisms are less significant than in low resistant (LR) specimens. Physiological nonmedicinal and pharmacological methods are used to increase the general nonspecific resistance during adaptation to hypoxia for the prevention and therapy of various diseases. Intermittent hypoxic training is the most effective physiological method, which allows us to adjust the ratio of the strength and duration of each hypoxic stimulus to the length of respiratory pauses and total time of hypoxia [4].

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However, adaptation to hypoxic training is a long-term process [8]. Combined exposure to high-altitude training and administration of pharmacological drugs producing rapid stimulatory effect on adaptive processes can increase and strengthen the effectiveness of physiological procedures increasing the resistance to hypoxia and stabilizing the adaptive response (including that in hypoxia-trained subjects) [7]. Trekrezan (triethylammonium salt of 2-methylphenoxyacetic acid) has antihypoxic, actoprotective, and immunostimulatory properties and, therefore, complies with these requirements [1,3,9].

Here we studied the effect of trekrezan on energy metabolism in rats during adaptation to intermittent hypoxic hypoxia.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats (n=80) weighing 160-180 g. They were divided into 4 groups of 20 animals each (HR rats, n=10; LR rats, n=10). Group 1 included intact animals. Group 2 rats were exposed to acute hypoxia at an altitude of 6000 m for 30 min. Group 3 consisted of trained animals. Trained rats of group 4 received trekrezan. Intact HR and LR rats served as the control for group 2 animals. HR and LR rats of group 2 served as the control for the corresponding

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subgroups of group 3. HR and LR rats of group 3 served as the control for HR and LR animals of group 4, respectively. All control animals received an equivalent volume of physiological saline.

The severity of metabolic changes was estimated from the content of lactate, pyruvate [6], and creatine phosphate [11] and energy charge of the adenylate system [10] in tissues of the brain, heart, and liver frozen in liquid nitrogen. The content of free adenine nucleotides ATP, ADP, and AMP was measured by ascending thin-layer chromatography and scanning on a MPF-4 spectrofluorometer (Hitachi) [2].

The results were analyzed by Student's *t* test.

RESULTS

Energy metabolism is the key process determining general resistance of animals to hypoxia and its improvement during adaptation. Intermittent hypoxic hypoxia training was accompanied by significant changes in energy metabolism in vital organs of HR and LR rats (Tables 1 and 2).

Lactate concentration in the brain and heart of all rats was lower than in animals exposed to acute hypoxia (*i.e.* by 20% in the brain). Lactate concentration in the liver of HR and LR animals decreased by 45 and 25%, respectively ($p<0.05$). Pyruvate concentration in the brain increased by 50%. Pyruvate concentration in the heart of HR and LR animals increased by 48 and 64%, respectively. Pyruvate concentration in the liver of HR and LR rats was higher than in animals exposed to acute hypoxia (by 96 and 183%, respectively). Variations in the concentration of lactate and pyruvate during intermittent hypoxic training were followed by a decrease in the concentration ratio, which reflects reduction of lactate acidosis.

Intermittent hypoxic training was accompanied by less significant changes in the amount of macroergic phosphates. Creatine phosphate concentration in the brain of HR and LR rats was higher than in animals exposed to acute hypoxia (by 45 and 173%, respectively). Creatine phosphate concentration in the heart of these animals increased by 41 and 96%, respectively ($p<0.05$). ATP concentration in the brain of HR and LR rats was higher than in animals exposed to acute hypoxia (by 114 and 65%, respectively). In HR and LR animals, ATP concentration in the heart increased by 47 and 68%, respectively and in the liver by 77 and 50%, respectively. At the same time, the concentrations of ADP and AMP in tissues of these rats decreased.

Changes in the concentration of adenine nucleotides during intermittent hypoxic training led to more pronounced increase in the energy charge in all organs of trained rats (compared to the group of acute hypoxia, Fig. 1).

Post-training changes in vital organs reflect transition of energy metabolism to a functional state adequate for intermittent hypoxia. However, energy supply in animals during hypoxic training was lower than in intact rats.

Administration of trekrezan to animals during intermittent hypoxic training was accompanied by more pronounced metabolic changes. The directionality of these changes was similar in all organs. The signs of lactate acidosis were not observed after trekrezan administration. In HR and LR rats, lactate concentration in the brain decreased by 37 and 55%, respectively, in the myocardium by 20 and 13%, respectively, and in the liver by 48 and 28%, respectively ($p<0.05$). Moreover, HR and LR rats had increased pyruvate concentration in the brain (by 46 and 61%, respectively), heart (by 24 and 22%, respectively), and liver (by 40 and 126%, respectively). Variations in the concentration of lactate and pyruvate were accompanied by a significant decrease in the ratio of these compounds. Carbohydrate metabolism in treated rats did not differ from that in intact animals.

Administration of trekrezan to rats during intermittent hypoxic training prevented depletion of macroergic phosphates, which was revealed in animals subjected to hypoxic training and not receiving this preparation. Experiments with HR and LR rats revealed increased creatine phosphate concentration in the brain (by 36 and 51%, respectively) and heart (by 20 and 31%, respectively, $p<0.05$). In HR and LR rats, ATP concentration increased in the brain (by 35 and 56%, respec-

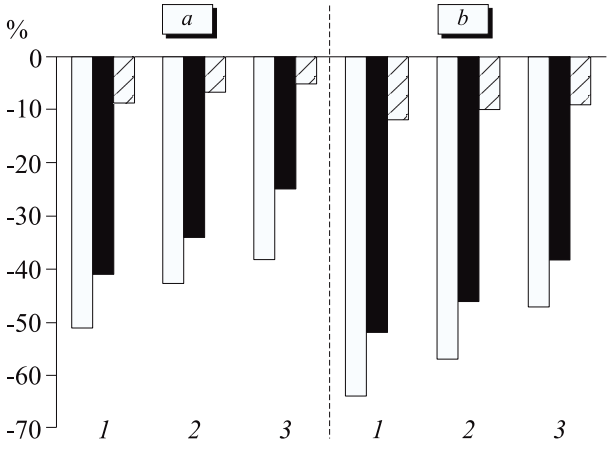

Fig. 1. Energy charge of the adenyl nucleotide system in the brain (*1*), heart (*2*), and liver (*3*) of rats HR (*a*) and LR to acute hypoxia (*b*). Light bars, acute hypoxia; dark bars, adaptation to hypoxic hypoxia; shaded bars, administration of trekrezan during training in hypoxic hypoxia.

TABLE 1. Effect of Trekrezan on Concentration of Lactate and Pyruvate in Organs of Hypoxic Hypoxia-Trained Rats (*M*±*m*)

Parameter	Group	Brain		Heart		Liver	
		HR	LR	HR	LR	HR	LR
Lactate, μmol/g	1	2.67±0.24	3.550±0.093	4.64±0.22	5.55±0.22	2.17±0.22	4.55±0.22
	2	7.65±0.22*	8.94±0.21*	7.33±0.29*	8.35±0.29*	6.23±0.21*	7.64±0.29*
	3	6.12±0.21*+	7.14±0.17*+	5.77±0.26*+	6.66±0.26*+	3.45±0.23*+	5.75±0.26*
	4	3.85±0.17*	3.21±0.19*+	4.59±0.29+o	5.78±0.29+o	1.78±0.22+o	4.12±0.29+
Pyruvate, μmol/g	1	0.44±0.01	0.35±0.02	0.44±0.02	0.24±0.02	1.26±0.03	0.84±0.02
	2	0.16±0.01*	0.12±0.01*	0.22±0.02*	0.11±0.02*	0.45±0.02*	0.12±0.02*
	3	0.24±0.02*+	0.18±0.02*+	0.33±0.03*+	0.18±0.03*+	0.88±0.03*+	0.34±0.03*
	4	0.35±0.02*	0.29±0.02*+	0.41±0.02+o	0.22±0.02+o	1.23±0.01+o	0.77±0.02+o

Note. Here and in Table 2: $p<0.05$: *compared to group 1; +compared to group 2; °compared to group 3.

TABLE 2. Effect of Trekrezan on Content of Macroergic Phosphates in Organs of Hypoxic Hypoxia-Trained Rats (*M*±*m*)

Parameter	Group	Brain		Heart		Liver	
		HR	LR	HR	LR	HR	LR
Creatine phosphate, μmol/g	1	5.22±0.21	4.26±0.11	6.47±0.22	5.15±0.22	—	—
	2	2.14±0.17*	1.15±0.12*	3.07±0.21*	1.95±0.21*	—	—
	3	3.11±0.16*+	2.72±0.14*+	5.22±0.19*+	3.83±0.19*+	—	—
	4	4.22±0.16*	4.12±0.14*+	6.24±0.19+o	5.02±0.16+o	—	—
ATP, μmol/g	1	3.97±0.13	3.82±0.13	5.73±0.26	4.31±0.26	3.43±0.14	2.51±0.16
	2	1.13±0.12*	1.24±0.13*	2.24±0.28*	1.72±0.28*	1.12±0.12*	1.13±0.18*
	3	2.42±0.11*+	2.04±0.12*+	3.29±0.22*+	2.89±0.22*+	1.98±0.13*+	1.69±0.12*+
	4	3.26±0.14*	3.18±0.15*+	5.59±0.21+o	3.89±0.21+o	2.96±0.12+o	2.49±0.11+o
ADP, μmol/g	1	0.51±0.02	0.60±0.02	0.80±0.02	0.82±0.02	0.47±0.03	0.60±0.02
	2	0.78±0.03*	0.99±0.03*	1.69±0.03*	2.11±0.03*	0.85±0.04*	1.02±0.03*
	3	0.69±0.03*+	0.89±0.03*+	0.99±0.02*+	1.13±0.02*+	0.75±0.02*+	0.85±0.02*+
	4	0.57±0.01+o	0.68±0.01+o	0.85±0.02+o	0.92±0.0+o	0.54±0.02+o	0.65±0.02+o
AMP, μmol/g	1	0.34±0.02	0.47±0.02	0.55±0.03	0.68±0.03	0.45±0.03	0.57±0.03
	2	0.77±0.03*	0.95±0.03*	0.99±0.04*	1.12±0.04*	0.83±0.04*	1.09±0.04*
	3	0.57±0.02*+	0.77±0.02*+	0.76±0.03*+	0.87±0.03*+	0.68±0.02*+	0.83±0.03*+
	4	0.42±0.01+o	0.54±0.01+o	0.64±0.02+o	0.72±0.02+o	0.51±0.02+o	0.64±0.02+o

tively), heart (by 70 and 35%, respectively), and liver (by 50 and 47%, respectively, $p<0.05$). HR and LR rats were characterized by a significant decrease in ADP concentration in the brain (by 18 and 24%, respectively), heart (by 11 and 19%, respectively), and liver (by 28 and 24%, respectively). AMP concentration decreased in the brain (by 26 and 30%, respectively), heart (by 26 and 17%, respectively), and liver (by 25 and 23%, respectively, $p<0.05$) of HR and LR rats. The concentration of macroergic phosphates and energy charge of adenine nucleotides in trekrezan-receiving rats did not differ from those in intact animals.

A close relationship exists between the impairment of energy metabolism and activation of LPO. It can be hypothesized that stabilization of energy metabolism after trekrezan administration is due to the antioxidant effect of this compound [3]. However, the mechanisms of energy-stabilizing activity of trekrezan require further investigations.

Our results indicate that combined exposure to high-altitude training and trekrezan treatment potentiates metabolic changes in organs of rats after intermittent hypoxic training. The pool of major carbohydrate substrates and macroergic phosphates and energy charge of the adenyl system in vital

organs are preserved against the background of trekrezan treatment. Trekrezan is a promising drug accelerating adaptation to hypoxic hypoxia.

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