PHYSIOLOGY

Effect of Intermittent Hypobaric Hypoxia on the Maximal Hydraulic Conductivity of the Circulatory Bed, Capillarization, and Size of Muscle Fibers in Rats

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Training in hypoxia promotes a decrease of the structural component of the resistance of the circulatory bed, which depends on two factors, namely, the density of the vascular network and the internal diameter of the individual arteriole. Changes of the density of the vascular network of the skeletal muscles may be due both to the generation of new vessels and to a decrease of the size of the muscle fibers. There is evidence of the generation of new capillaries in the skeletal muscles in hypoxia [1,6,8]. However, since the middle of the 80s contradictory data have begun to appear, casting doubt on the induction of angiogenesis in skeletal muscle by hypoxia [3,7,9,11, 12,14,16]. Thus, the nature of the effect of hypobaric oxygenation on skeletal muscle has remained unclear.

The aim of the present investigation was to study the morphological changes in muscles induced by hypobaric hypoxia under different durations of exposure.

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MATERIALS AND METHODS

The experiment was carried out on 6-7-month-old rats and lasted for 2 weeks. One group of rats remained intact for the control (n=12, the first group). The second group was held in a pressure chamber for 18-20 h per day (n=12) and the third group for 2 h per day (n=14). The rarefaction of the air was maintained equivalent to an altitude of 5000 m, the temperature was 20°C, and ventilation was 9 liters of air per minute. The structural component of circulatory resistance was analyzed in the vascular bed of the hind portion of the body. The maximal hydraulic conductivity of the vascular system was assessed by perfusion experiments. The vascular bed was perfused with Tirode solution through a cannula introduced in the abdominal aorta, in a regime of stabilized delivery from a peristaltic pump (LKB, Sweden). Since the vessels are maximally dilated under perfusion with saline, the perfusion pressure recorded at the inlet of the cannula was in direct proportion to the value of the structural component of the resistance. Thus, according to the perfusion pressure for different outputs, the structural component of the resistance was determined in the different groups of animals. For the morphological and

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histochemical examination, m. extensor hallucius proprius was taken (n=5, first group; n=7, second group; n=5, third group) and frozen in liquid nitrogen. Then serial transverse sections with a thickness of 10 μ were performed in a cryostat at -20°C. The sections were stained to reveal the myofibrillar ATPase (with preincubation, pH=4.35 [5]) and NADH-tetrazolium reductase (NADH-TR) [10]. Capillaries were visualized by the histochemical reaction to alkaline phosphatase after Gomori [10], and the activity of the enzymes was cytophotometrically assessed with an MPV2 microscope-photometer. The area of the cross section (ACS) of the muscle fibers (MF) of the first (MFI) and the second (MFII) types was determined using an ASM68K image-analyzing system. The number of capillaries per fiber and their density per mm² of an ACS were estimated.

RESULTS

It was established that intermittent hypobaric hypoxia resulted in a decrease of the structural component of resistance by 20-25% in the second group and by 17-20% in the third group (Fig. 1). The other data are listed in Table 1. The number of capillaries per fiber in the rats of all groups did not differ significantly from each other, although a trend toward a decrease of this index compared to the control was noted in the second group. The density of capillaries was also similar in all groups. The ACS of MFI was reliably lower in the animals of the second group as compared to the control. There was no significant difference between the groups in the ACS of MFI or in the NADH-TR activity.

The nature and degree of the morphofunctional changes of the microcirculatory bed subjected to the hypoxic factor depend on the time of exposure to oxygen deficiency in the organism. We obtained a decrease of the maximal hydraulic conductivity in the second and third groups, but a reduction of the exposure to hypobaric hypoxia to 2 h/day versus 20 h/day (i.e., 10-fold) practically did not affect the degree of decrease of the structural component

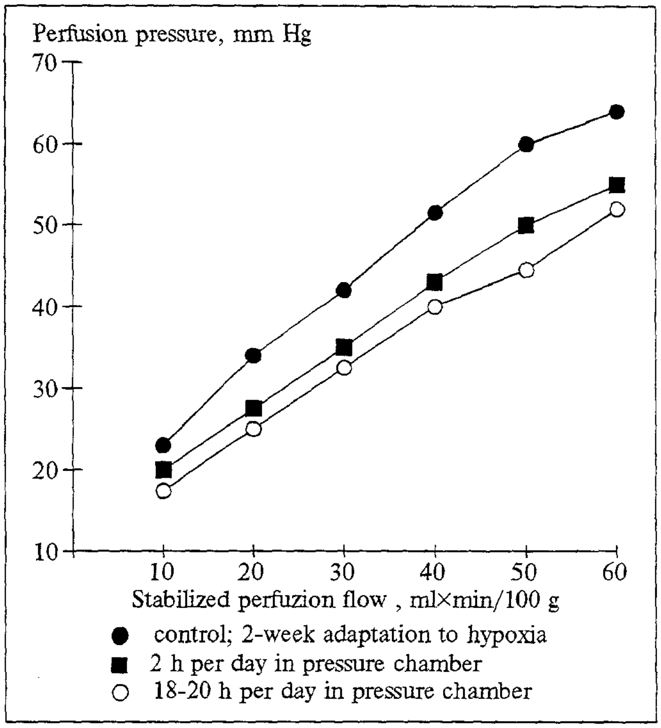

Fig. 1. The structural component of vascular resistance in rats subjected to hypoxia.

of the vascular resistance. These findings are consistent with the experimental results of Adair and co-workers [2]. However, histochemical examination of the muscle tissue did not reveal an increase of the new capillaries per MF in any group. Similar results were obtained previously [3,7,9,11,12,14,16]. Hoppeler emphasizes the fact that the evidence about the generation of new capillaries in animals is contradictory, while exposure of human skeletal muscle tissue to hypoxia does not result in a proliferation of capillaries or even promotes a reduction of their number [7,9,11]. However, a two-week physical training against the background of hypoxia may induce an increase of the number of capillaries per MF, whereas such an early beginning of capillary generation is not obtained under normal conditions [13]. A three-week training against the background of normobaric hypoxia also stimulates the generation of capillaries in man. Thus , hypoxia promotes the generation of capillaries to a

TABLE 1. Parameters of Capillarization and Structural–Metabolic Parameters of MF in Rats under Normal and Hypobaric Oxygen Pressure in the Inspiratory Air ($M \pm m$).

Group of animals	Capillary/fiber	Capillary/mm²	ACS, μ²		Activity of NADH-TR, OD	
			MFI	MFII	FBI	MFII
1st (control)	0.73±0.09	505±80	779±49	1307±49	0.46±0.02	0.22±0.01
2nd (20 h)	0.62±0.05	442±33	611±44*	1418±142	0.48±0.06	0.22±0.02
3rd (2 h)	0.77±0.05	434±39	818±74	1176±141	0.41±0.04	0.20±0.01

Note. * significance ($p<0.05$) of differences in comparison with 1st group. OD = optical density.

higher degree in active muscle than in resting muscles. The oxygen demand of resting muscle is evidently so modest [4] that a low partial pressure of oxygen, equivalent to that at an altitude of 5000 m, is not a significant stimulus for capillary growth. The capacity of muscle tissue for increasing the number of capillaries in hypoxia may be specific, depending on age or species. That is why the probability of revealing the features of angiogenesis in hypoxia would be higher with the use of younger experimental animals.

The MFI sizes changed only in rats exposed to hypoxia lasting 20 h. The same phenomena have been observed in people who have spent a long time at a high altitude [9,11], and in athletes, who undergoing endurance training where intermittent hypoxia is suffered as a matter of course [17]. It is of interest that these changes take place only in MFI, which are characterized by a higher reduction potential and are mainly involved in aerobic performance. A decrease of MF size may be related to a decrease of the rate of protein synthesis in animal muscles exposed to hypobaric hypoxia over 20 h [15]. Aa a result of the decrease of MF size, the density of capillaries is often increased, which may shorten the diffusion distance for oxygen from the capillaries to the mitochondria. In our case the structural changes were not so pronounced due to the short-term exposure to hypoxia.

The activity of the mitochondrial oxidative enzymes was not affected by the low partial pressure of oxygen in the inspiratory air, attesting that the oxygen uptake by the mitochondria remained unchanged.

Thus, hypoxic training, either intermittent or continuous, affects the morphofunctional characteristics of muscles as soon as during the first 2 weeks, but the degree of this effect may vary. Whereas during intermittent training, changes in the architectonics of the circulatory bed were obtained only due to a structural dilatation of the resistive vessels, but not to the generation of new capillaries, continuous exposure to hypoxia results also in a decrease of MFI size.

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