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Impact of Twelve-Day Combined Exposure to Hypobaric Hypoxia and Physical Exercise on Structural and Metabolic Characteristics of Skeletal Muscle in Rats

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This study, in which rats were exposed on 12 successive days to hypoxia in combination with exercise on a treadmill, showed that a reduction in partial oxygen pressure leads to a decrease in the magnitude of the structural component of vascular resistance rather than to improvement in the system of oxygen utilization, and that such combined exposure may cause alterations in protein synthesis and result in early stimulation of capillary growth in muscles, as well as elicit differential changes of enzyme activity in different types of muscle fibers.

Key Words: *hypoxia; physical exercise; muscle fibers; capillaries; enzymes*

It is currently thought that the main strategy of muscle tissue adaptation to reduced partial oxygen pressure is directed at maintaining the concentration of adenosine triphosphoric acid (ATP) at the appropriate level [7]. This can be achieved in two ways: by raising the activity of oxidative enzymes with a resultant increase of metabolic influx into the muscle fibers because of a rise in the concentration gradient of oxygen and substrates [8], or by making oxygen more accessible to the fibers through increased capillarization and the consequent decrease of the diffusion distance for oxygen inside the fibers. It has also been suggested that the local hypoxia arising during physical activity may be one of the major stimuli triggering the development of adaptive changes in skeletal muscle tissue.

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The purpose of the present study was to examine structural and enzymatic changes in skeletal muscle in response to hypobaric hypoxia and to hypobaric hypoxia combined with physical exercise.

MATERIALS AND METHODS

A total of 38 male Wistar rats aged 1.5-2 months were used. They were divided into four groups. Group 1 rats (n=7; body weight 246±12 g) were exposed to intermittent hypoxia in a pressure chamber for 3.5-4 h daily (at 20°C) at a pressure corresponding to an altitude of 5000 m above sea level and at a ventilation rate of 9 liters/min, followed 1.5 h later by running on a treadmill at a speed of 30 m/min for 35 min/day. Group 2 rats (n=13; body weight 252±10 g) were subjected only to treadmill running as described above, while group 3 rats (n=7; body weight 261±11 g) were only exposed to hypoxia under the same conditions as group 1. Group 4 rats (n=11; body weight 237±7 g) served as intact controls.

The rats were taken for technical manipulations not earlier than 24 h after being exposed to hypoxia and/or treadmill exercise. They were anesthetized with Nembutal (20 mg/kg intraperitoneally) and the structural component of the resistance offered by the vasculature of their right hind limb was analyzed. The maximal hydraulic conductivity of the vascular system was evaluated in perfusion tests. For these tests, the vascular bed was perfused, via a cannula inserted into the right iliac artery, with Tyrode's salt solution at flow rates stabilized by a peristaltic pump (LKB). (Before the tests, a single dose of sodium nitroprusside in a concentration of 50 mg/kg was injected through the cannula.) Because perfusion with a salt solution causes blood vessels to dilate to the maximum [4], the perfusion pressure recorded at the cannula's entry is directly proportional to the structural component of vascular resistance; accordingly, perfusion pressure values at different flow rates indicated the magnitude of this component in our tests.

The perfusion data were used to calculate the ratio of flow rate to the recorded perfusion pressure that characterized the maximal hydraulic conductivity of the vascular system in the hind limb. For histochemical assays, muscle tissue samples from the lateral gastrocnemius muscle were frozen in liquid nitrogen, and serial 10-μ cross sections were then prepared in a cryostat at -20°C and stained for cytochrome-C oxidase, succinate dehydrogenase, and α-glycerophosphate dehydrogenase (α-GPDH) after to Logda [1] (the sections to be compared were stained simultaneously). Enzyme activities were estimated by end-point cytophotometry in the core of individual type IIa and IIb fibers at 570 nm using a microscope-photometer (Reichert) and were expressed in optical density units of diformazan granules. The fibers were revealed by staining for myofibrillar ATPase after incubation of the sections at pH 4.6 [6]. Cross-sectional areas of the muscle fibers were determined with an ASM-68K image analysis system (Leitz Wetzlar). Capillaries were detected by staining for microfibrillar ATPase at pH 4.0 [14]. The number of capillaries per fiber and their number per mm^2 of cross-sectional area were counted.

The statistical significance of differences between the control and test groups was estimated by Student's t test.

RESULTS

The mean maximal hydraulic conductivity of the hind limb's vascular system in group 2 was 1.30± ±0.20 arb. units and did not differ from that in the control group (1.30±0.07 arb. units), whereas in groups 1 and 3 it was significantly increased to 1.68±0.16 (p<0.05) and 1.70±0.06 arb. units (p<0.01), respectively, at a pressure of 65 mm Hg. Because *m. gastrocnemius lateralis* is for the most part a white muscle, the number of type I fibers was too small for the results obtained for these fibers to be discussed. The cross-sectional area of IIb fibers was increased by 13% in group 1, which was the only group where the number of capillaries per fiber was also increased (by 14%). Capillary density remained unchanged in all three test groups. In group 1, α-GPDH activity increased by 29% in IIb fibers and cytochrome-C oxidase activity by 20% in IIa fibers. Morphological and histochemical data are presented in Table 1.

It is widely believed that the oxidative capability of muscular tissue must be increased if the metabolic influx into muscle fibers is to be improved [8]. We found, however, that the activity of the oxidative enzymes succinate dehydrogenase and cytochrome-C oxidase remained unchanged in the rats exposed to hypoxia alone (Table 1, group 3). Hochachka *et al.* also noted in 1992 that the activity of oxidative enzymes in persons living at of high altitudes is no different from that in a control group of subjects living at sea level [7]. On the other hand, Green *et al.* found in acclimation experiments depressed levels of citrate synthase and succinate dehydrogenase activity (by 30%) in subjects after 40 days of exposure to hypoxia in a pressure chamber [5]. This result is in accord with those obtained by the same authors for seven

Table 1. Characteristics of Skeletal Muscle Fibers of Types IIa and IIb from Rats ($M \pm m$)

Parameter		Group			
		1	2	3	4
Cross-sectional area, μ^2	IIa	1135±66	1020±46	1063±49	1026±41
	IIb	1838±64*	1618±68	1634±116	1629±64
Succinate dehydrogenase	IIa	0.53±0.02	0.54±0.03	0.48±0.02	0.48±0.05
	IIb	0.23±0.03	0.24±0.02	0.22±0.01	0.20±0.04
Cytochrome-C oxidase	IIa	0.53±0.02	0.52±0.02	0.41±0.04	0.44±0.04
	IIb	0.26±0.03	0.26±0.03	0.22±0.03	0.25±0.04
α-GPDH	IIa	0.58±0.04	0.51±0.04	0.47±0.05	0.56±0.04
	IIb	0.66±0.05*	0.59±0.05	0.49±0.05	0.51±0.05
No. of capillaries per fiber		1.71±0.01**	1.60±0.05	1.47±0.03	1.50±0.04
No. of capillaries per mm^2		728±48	831±34	688±49	747±40

Note. *$p<0.05$, **$p<0.01$ in comparison with the control group.

mountaineers who had climbed Mount Everest [5] and has been confirmed by a study of a larger sample [9] in which the volume fraction of mitochondria was found to have decreased in skeletal muscle under hypoxia. There is a positive correlation between the activity of mitochondrial enzymes and mitochondrial volume density [11], and a decrease in the latter may therefore be interpreted as an indication of reduced oxidative potential of the tissue concerned. Thus, instead of the postulated rise, the activity of oxidative enzymes actually remains unchanged or even falls under hypoxia. Taken together, the evidence at hand strongly suggests that the utilization of oxygen and substrates is not intensified under hypoxic conditions unless these lead to increased oxygen demand.

A second assumption of the hypoxic hypothesis rests on August Krogh's 1919 postulate that oxygen homeostasis is regulated through maintenance of optimal architectonics of the vascular bed. However, we found little or no change in the number of capillaries per muscle fiber in group 3 (Table 1). Similarly, no differences in capillary numbers in skeletal muscles were found either between sherpas from Nepal and people living at sea level [7] or between animals living at a high altitude (3800 m) and at sea level [13]. Examination of mountaineers, however, revealed decreased capillary numbers in their muscles [10]. The hypothesis that new capillaries are formed in skeletal muscle tissue under hypoxia has not been debated in recent years. Practically all of the studies mentioned above showed increased capillary density per mm^2 tissue as a result of decreased cross-sectional areas of the muscle fibers. In our study, muscle fibers did not change in size in group 3, so that the capillary density also remained at the pre-exposure level in this group (Table 1), which agrees with our earlier findings [2]. Admittedly, the duration of hypoxia in our study was not sufficient to elicit structural changes. However, the perfusion of muscles with Tyrode's solution showed a decrease in the structural component of vascular resistance. Changes in resistance vessels in response to a fall in partial oxygen pressure are likely to occur before the time when changes in tissue morphology or oxygen utilization become demonstrable.

Thus, hypoxia may lead in some instances to changes in the system of oxygen cascade in skeletal muscle, but the direction of such changes is not always in accord with the widespread notion that tissues become adapted to reduced partial oxygen pressure in the air.

Adaptation to physical activity is also accompanied by alterations in the circulatory and structural/metabolic characteristics of muscles. Rats exercised on the treadmill after being exposed to hypoxia showed greater increases in enzyme activity than did rats exposed to physical exercise alone (Table 1), but these changes occurred differentially in the two types of muscle fibers. A significant elevation of α-GPDH activity was recorded for the fast glycolytic fibers, which may be an indication of activated anaerobic glycolysis with a consequent substantial augmentation of ATP production. Type IIb fibers have lower activity of mitochondrial enzymes initially and their potential for aerobic ATP synthesis is therefore also lower. Adaptation of this kind may help maintain ATP synthesis at a particular level when the oxygen regime changes. The fast oxidative fibers, on the contrary, show a tendency toward a greater rise in mitochondrial enzyme activity, and this may lead to a higher partial oxygen pressure gradient in the area between capillaries and mitochondria and to better oxygen recovery. It may be concluded that hypoxia com-

bined with physical exercise is likely to dictate different strategies of adaptation by muscle fibers of different types. The exposure to hypoxia followed by treadmill exercising (group 1) resulted in a 12% increase in the size of IIb fibers. When physical exercise is performed under normoxic conditions, such changes in fiber size are usually recorded later, although they have been reported to occur in athletes after just 2 weeks of training at high altitude [12]. Similar results were obtained by Desplanshes [8]. It has also been shown that both hypoxia and exercise affect the rate of protein synthesis [15].

In the group exposed to hypoxia in combination with treadmill exercising (group 1), the number of capillaries increased, but their density did not because of the increase in the cross-sectional areas of muscle fibers. Reported data on changes in capillary numbers resulting from physical exercise in the presence of hypoxia are rather contradictory, although, as noted by many workers, exercise is conducive to capillary formation [3]. In the studies referred to above, capillary growth with a concomitant increase in fiber cross-sectional area was observed after just 2-3 weeks of exercising under hypoxic conditions [8-12].

Maximal hydraulic conductivity increased in group 1 to the same extent as in group 3, whereas in group 2 (the one subjected only to treadmill running) it differed little from that in the control group. Presumably, the increase in maximal hydraulic conductivity caused by hypoxia will result in greater hyperemia during exercise. Hudlicka suggested that hyperemia is a factor essential for capillary growth [16].

To sum up, a decrease in partial oxygen pressure primarily leads to increased maximal hydraulic conductivity of blood vessels rather than to enhanced oxidative potential of the muscle(s) concerned. Exposure of animals to hypoxia in combination with physical exercise may result in early stimulation of capillary growth in the muscles, as well as in a greater increase in the size of type IIb muscle fibers and in their glycolytic potential as compared to animals subjected to exercise alone.

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