Adaptations in skeletal muscle capillarity following changes in oxygen supply and changes in oxygen demands

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Summary. The effects of changes in oxygen supply and oxygen demands on fiber cross-sectional areas, capillary densities and capillary to fiber ratios were determined in three skeletal muscles of rat. The muscles examined were the vastus lateralis, soleus, and diaphragm. Reduced oxygen supply was produced by subjecting rats to ambient hypoxia, and increased oxygen demands were produced by subjecting rats to low ambient temperatures or treatment with thyroxin. Capillaries were visualized by injecting fluorescent dyes into the circulation. Muscles were quick frozen at resting lengths to preserve normal fiber geometry and were subsequently sectioned on a cryostat. All of the muscles sampled from animals in the experimental groups had elevated capillary densities. However, capillary to fiber ratios were not increased significantly in any muscle, for any experimental condition. Thus, all of the observed differences in capillarity were due to changes in the intrinsic rate of muscle fiber growth. Further, the relations of capillary density and capillary to fiber ratio to fiber area were the same as those obtained during normal maturation, suggesting that capillary growth is closely linked to the intrinsic rate of fiber growth.

Key words: Microvasculature – Capillary growth – Cold – Hypoxia – Thyroxin

Introduction

It is widely held that capillary growth is an important part of the adaptive mechanisms which compensate increased oxygen demands and decreased oxygen supply (see reviews by Banchero 1987; Hudlicka 1984). However, recent studies (Boutellier et al. 1983; Hoppeler et al. 1990) on human subjects acclimated to high altitude have failed to find evidence of capillary growth, suggesting that the topic may be more complex than previously thought. Unfortunately, few studies involving human subjects have dealt with the relationships of capillary growth and changes in oxygen supply or changes in oxygen demands. This is undoubtedly due to the difficulty of obtaining subjects and to the limited breadth of experimental treatments that can be applied directly to humans.

Interestingly, however, the results obtained for human subjects are consistent with reports based on animal experiments (Sillau and Banchero 1977; Snyder et al. 1985) and model analyses (Snyder 1988, 1990), all of which have suggested that there may be a limited potential for improving oxygen flux between blood and tissues by adding more capillaries to the microvascular bed. The similarities in the results obtained by these diverse approaches further suggest that there may be some features or relationships between capillarity and the potential for tissue gas conductances that are common to mammals in general, and that studies using animal models may provide important insights into the adaptive mechanisms to be anticipated in humans.

In the present study we have examined the effects of changes in both oxygen supply and oxygen demand on capillarity and capillary growth in skeletal muscle of rats. Changes in oxygen supply are produced by subjecting experimental animals to ambient hypoxia because this variable is still considered the most likely stimulus for capillary growth (Hudlicka 1984). Changes in oxygen demands are brought about by subjecting experimental animals to low ambient temperature or by administering thyroid hormone to experimental animals. Both of these experimental treatments have been reported to promote capillary growth in skeletal muscle (Capo and Sillau 1983; Sillau et al. 1980), but the mechanisms involved may be somewhat different in each case.

To minimize differences in capillarity produced by normal growth, body masses of experimental and control animals are carefully matched. Further, problems associated with tissue preparation are avoided by quick freezing muscles at resting length, and we report our re-
sults against muscle fiber area in order to control for differences in the intrinsic rates of both microvascular growth and muscle fiber growth. Finally, we have quantified two elements of capillarity – capillary numerical density and capillary to fiber ratio. We measured capillary density because this element of tissue capillarity is most frequently used to quantify tissue gas conductances (Banchero 1987; Hudlicka 1985; Snyder 1990). Capillary to fiber ratio was measured because this element of tissue capillarity is the only direct measure of capillary growth (Snyder et al. 1985; Snyder 1988).

Methods

Male Sprague-Dawley rats (n = 48) were used in the present study. Because the experiments could not be completed all in a single time interval, two general groups of rats, each with its own control group, were used. The two control groups were raised at the same time and under the same conditions as their respective experimental counterparts, excepting for the specified conditions of the experiment as described below. Using this approach, the average body mass of each control group could be selected to closely match the average body mass for each of the experimental groups. From the initial stock of rats, animals were divided at random between control and experimental groups. Further, where experimental treatment of the animals altered growth rates, e.g., hypoxia and cold, we adjusted the times when experimental versus control animals were sacrificed to avoid significant differences in body mass. Where experimental procedures involved surgery, animals were anesthetized with sodium pentobarbital (35 mg·kg⁻¹).

Experimental group 1: hyperthyroidism. Ten male rats were injected intraperitoneally every other day for 5 weeks with 350 μg·kg⁻¹ 3,3',5-triiodothyronine (T₃) (T2752, Sigma, St. Louis, Mo.). The injection medium was prepared by dissolving T₃ in minimal amounts of 100 mmol·L⁻¹ sodium carbonate followed by addition of an equal amount of 100 mmol·L⁻¹ sodium bicarbonate. This solution was diluted to the appropriate concentration of T₃ with 0.9% normal saline. Control animals were injected with the same amount of a sodium carbonate/sodium bicarbonate solution in normal saline.

After 4 weeks of T₃ injections, resting rates of oxygen consumption were measured for individual control and experimental rats. Each rat was placed in a cylindrical metabolism chamber consisting of a clear plastic tube (length 0.25 m, diameter 0.08 m) with removable single-hole rubber stoppers in each open end. The holes in the stoppers were baffled and were fitted with tubing to allow for continuous airflow. Airflow through the chamber was kept constant at 8.3 ml·s⁻¹ STPD with a mass flow controller (model FC280, Tylan, Carson, Calif., USA). Air entering and leaving the metabolism chamber was passed through columns of anhydrous calcium sulfate (Drierite, Hammond, Xenia, Ohio) and sodium hydroxide (Ascarte II, Thomas Scientific, Swedesboro, N. J.) to absorb water vapor and carbon dioxide, respectively. Air from the excurrent absorbing columns was passed through an oxygen analyzer (model 5-3A, Applied Electrochemistry, Sunnyvale, Calif., USA) connected to a microcomputer via an analog-to-digital converter. Rates of oxygen consumption were calculated from the equations of Depocas and Hart (1957) as modified by Withers (1977) to take into consideration the positioning of the water vapor and carbon dioxide absorbents. Temperature in the animal chamber was monitored with a thermocouple (BAT-12, Withers, Ltd., 2 Blake Parade, Witham, Essex, England) and maintained constant by submerging the chamber in a water bath. The water bath was covered to prevent visual contact between animal and individuals in the laboratory. After an initial 60-min equilibration period, the concentrations of oxygen in the incident air and in the excurrent air were recorded for 1 min every 6 min for the next 3 h. From these values, rates of oxygen consumption at STPD were calculated. The values reported in the results section are the averages of the minimum rates of oxygen consumption that were obtained for five consecutive 1-min intervals.

Experimental group 2: hypoxia. Six experimental animals were housed two per cage and kept in a hypobaric hypoxia chamber for 6 weeks at 46.7 kPa BP, 9.7 kPa inspired oxygen pressure, roughly equal to the oxygen pressure expected at an elevation of 6100 m.

Air flow through the chamber was maintained with an Atlantic Fluidics water-sealed vacuum pump and was set at a minimum of 31 l/min per animal by adjusting an excurrent air bleeder valve. Over a 2-day period, the air flow into the chamber was damped until chamber pressure was stable at 46.7 kPa. The pressure was set by adjusting a valve on the excurrent port against the one on the excurrent port so that pressure inside the chamber could be adjusted while keeping total airflow through the chamber constant. The chamber was pressurized once per week so that the experimental animals could be provided fresh cages, bedding, food, and water. The ten rats in the control group were housed two animals per cage at 84 kPa BP, 18 kPa inspired oxygen pressure. All animals were weighed at least once a week.

Experimental group 3: cold. Ten rats were exposed to low ambient temperatures [mean (SEM), 5.0 (0.5) °C] for 8 weeks. To minimize the effects of huddling, animals were kept two per cage with enough bedding to cover the floor, but not enough bedding to form a nest. Control animals for this group were the same as for the animals in the hypoxic chamber.

Tissue sampling. Following anesthesia, blood samples, taken from the tip of the tail, were collected into three heparin-coated hematocrit tubes. The samples were centrifuged for 15 min and the blood hematocrit determined. Corrections for plasma trapping were not made. Capillaries were identified by fluorescent dyes injected into the central circulation, after the methods of Snyder et al. (1989). In the present study, rats were dissected to expose the right jugular vein which was then injected with 250 mg·kg⁻¹ fluorescein isothiocyanate bound to albumin (FITC-albumin, A9771, Sigma). The FITC-albumin was allowed to circulate for 5 min. The animal was given 50 μl saturated pentobarbital into the jugular vein to stop the circulation. The heart, without atria, was rapidly dissected out, blotted to remove surface moisture, and weighed. The hindlimb was pinned in its resting position, and the soleus and vastus lateralis muscles were dissected free of connective tissue. The ends of each muscle were marked with fine pins and the distances between the marks measured. The muscles were then cut free at the tendons of their origins and insertions, trimmed along their lengths to roughly 3 mm on a side, mounted on a muscle clamp, coated with an embedding compound (OCT, Miles Inc, Elkhart, Indiana, USA), stretched to their original resting lengths and quick-frozen for 15 s in isopentane cooled with liquid nitrogen. The diaphragm was marked in a manner similar to the two skeletal muscles, dissected free and trimmed, then stretched to its original length and frozen. All frozen muscles were stored at −85°C until used.

From each block of frozen muscle, several 10-μm-thick transverse sections were cut in a cryostat at −24°C. Total capillary bed was visualized by examining sections under a fluorescence microscope (Leitz Diaplan, Leica Inc, Deerfield, Illinois, USA) with a 100-W mercury light source and a 25 × water immersion objective. Sections were ep-illuminated through a 470-nm primary filter, a 510-nm dichroic interference mirror and a 535-nm barrier filter. Digitized images of seven fields measuring 0.3444 mm², selected at random from each section, were captured with a video camera (Dage ITM CCD-72) driven by an image processing hardware (Windham Hannaway series 150) coupled to a microcomputer (Silicon Graphics, Mountain View, Calif., USA).

As a check to ensure that the fluorescent dye marked all of the capillaries, selected sections were prepared by a histochemical treatment for endothelial cell specific ATPase to visualize capillary...