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Full Length Research Paper

Morphology and histochemistry of primary flight muscles in *Rhinolophus mehelyii*

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Two primary flight muscles of *Rhinolophus mehelyii* were studied using morphological and histochemical analysis. Two fast-twitch fiber types are histochemically identified in pectoralis muscles of *R. mehelyii*. These were classified as type IIa and type IIb according to glycine-calcium-formalin preincubation staining protocol for myosin ATPase. The primary flight muscles, serratus ventralis included type I, type IIa and type IIb fibers. Type I fibers were highly oxidative, as stained dark for NADH-TR. Type IIa fibers exhibited relatively weak staining properties for NADH-TR and SDH, indicating an intermediate oxidative capacity. Type IIb fibers showed low oxidative capacity, as indicated by weak reaction for NADH-TR.

Key words: *Rhinolophus mehelyii*, skeletal muscles, ATPase, NADH-TR, SDH, histochemical tests.

INTRODUCTION

Rhinolophus mehelyi (Mehely's Horseshoe Bat) is a species of bat in the Rhinolophidae family found in Eastern Europe and parts of the Middle East. The bat is medium-sized for a member of the *Rhinolophus* genus, with pale lips and grey-brown ears and flight membranes. The bat is cave-dwelling, preferring areas of limestone with nearby water. It has been known to nest in caves with other horseshoe bats, hanging free on the cave roof. The bat emerges at dusk, hunting low over the ground on warm hillsides and also among bushes and trees, praying on moths and other insects (Siemers and Ivanova, 2004). Flight is energetically more expensive than walking, running, or swimming. In bats, the only mammals capable of true flight, metabolic expenditure increases approximately 18-fold over the resting level during flight (Carpenter, 1975, 1985; Thomas, 1975). The chiropteran wing bat cycle requires that muscle perform contraction-relaxation cycles as frequently as 15 Hz (Hermanson and Foehring, 1988). These highest metabolic rates recorded from flying bats are essentially the same as those predicted for flying birds of the same body masses, but are from 2.5 to 3.0 times greater than the highest metabolic rates of which similar size exercising terrestrial mammals appear capable (Thomas,1975).

The highest maximal aerobic powers in mammals have been reported for flying bats (Thomas, 1975), which have peak oxygen consumption 2.5 - 3 times greater than those for running mammals with similar body mass (Pasquis et al., 1970; Thomas, 1975).

Histochemistry is a useful indicator of muscle function. Mammalian skeletal muscle fibers have been classified in type I, type IIa and type IIb fibers. Type I fibers are identified by a slow contraction time and a high resistance to fatigue. Structurally, they have a small motor neuron and fiber diameter, a high mitochondrial and capillary density, and a high myoglobin content. Energetically, they have a low supply of creative phosphate, a low glycogen content. Functionally, type I fibers are used for aerobic activities requiring low-level force production (Šoic-Vranic et al., 2005).

Type II fibers are identified by a quick contraction time and a low resistance to fatigue. Type II fibers are further divided into type IIa and type IIb fibers. Type IIa fibers have amoderate resistance to fatigue and represent a transition between the two extremes of the type I and type IIb fibers. Structurally, type IIa fibers have a large motor neuron and fiber diameter, a high mitochondrial density, a medium capillary density, and a medium myoglobin content. They have both a high glicolytic and oxidative enzyme activity (Barany, 1967).

Type IIb fibers are very sensitive to fatigue and are used for short anaerobic, high force production activities.

These fibers are also capable of producing more power than type I fibers. Like the type IIa fibers, type IIb fibers have a large motor neuron and fiber diameter, but a low mitochondrial and capillary density and myoglobin content. The purpose of the present investigation was to determine the fiber diameter and the fiber composition of pectoralis muscle and serratus ventralis in the *R. mehelyii.*

MATERIALS AND METHODS

Eight *R. mehelyii* were used in this study (four males and four females ranging in weight from 11 to 24 g). The muscles studied include the primary flight muscles: pectoralis, serratus ventralis.
All animals were killed with an overdose of sodium pentabarbital administered intraperitoneally. Muscles were dissected free, cleaned of excess fascia, blotted dry and weighed. Their proximal and distal portions were mounted in gum tragacanth on cork, quick-frozen by immersionin isopentane cooled to about -160°C, sealed in plastic bags, and stored at -20°C. Transverse serial sections (10 – 12 µm thickness) were obtained with a freezing cryostat (Hermanson and Foehring, 1988).

Sections from each muscle were stained with nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) to assess oxidative capacity (Novikoff et al., 1961). The method of Nachlas et al. (1957) was used demonstrate succinate dehydrogenase (SDH) activity. Sections of each muscle were stained using myosin adenosine triphosphatase (mATPase) (Tunnel and Hart, 1977).

Fiber diameters were estimated for each fiber type in each muscle by measuring minimum fiber diameters of 300 fibers (100 fibers drawn from each of tree regions within a muscle) with a calibrated eyepiece mounted the microscope (Armstrong, 1982).

RESULTS

The pectoralis was the largest muscle present in *R. mehelyii*. The right and left pectoralis comprised about 5.02% of total body mass. Two fast-twitch fiber types are histochemically identified in pectoralis muscle. These were classified as type IIa and type IIb according to glycine-calcium-formalin preincubation staining protocol for myosin ATPase. Type IIa fibers stain intermediate intensity, and type IIb fibers stain darkest (Figure 1). Type IIa fibers had a mean diameter of 30.7 µm. Fiber ratios of *R. mehelyii* for the pectoral muscle are 74% for fiber type IIa and 26% for fiber type IIb.

Type IIa fibers exhibited relatively intense staining properties for NADH-TR and SDH, suggesting an intermediate oxidative capacity. Formazan granules in the NADH-TR stain formed a ring around the outer edge of the fibers (Figure 2). Type IIb fibers had a mean diameter of 41.3 µm. Within the pectoralis, type IIb fibers were significantly larger than type IIa fibers. Type IIb fibers were low oxidative, as indicated by light reaction for NADH-TR and SDH (Figure 3).

The mass of the serratus ventralis muscle constituted 3.45% of the total body mass. Sections of serratus ven-

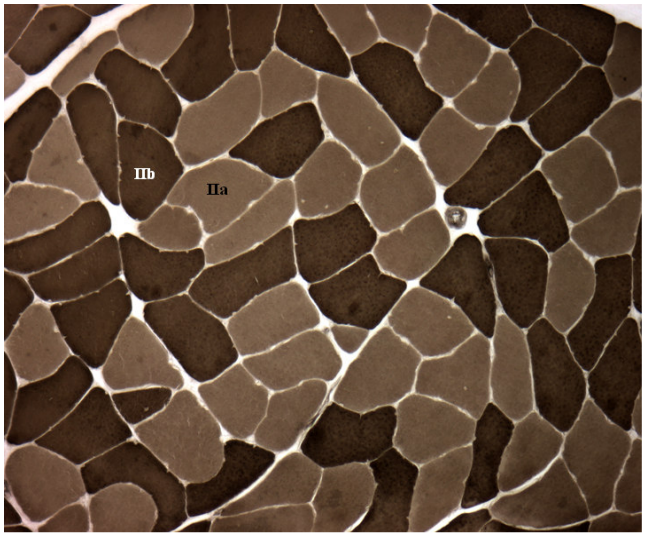

Figure 1. Pectoralis muscle section from *R. mehelyi*. ATPase stain. X1000.

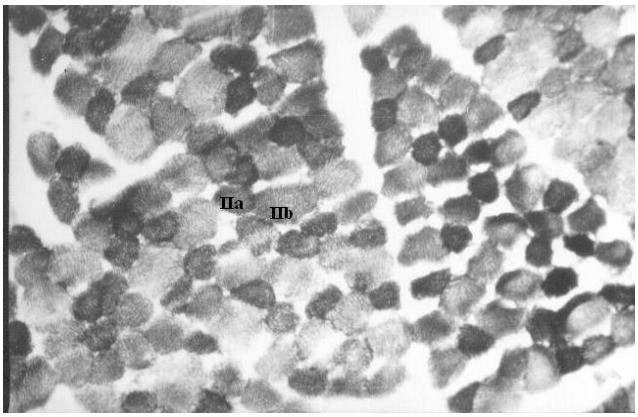

Figure 2. Transversal section of *R. mehelyi* pectoralis muscle stained for NADH. X500.

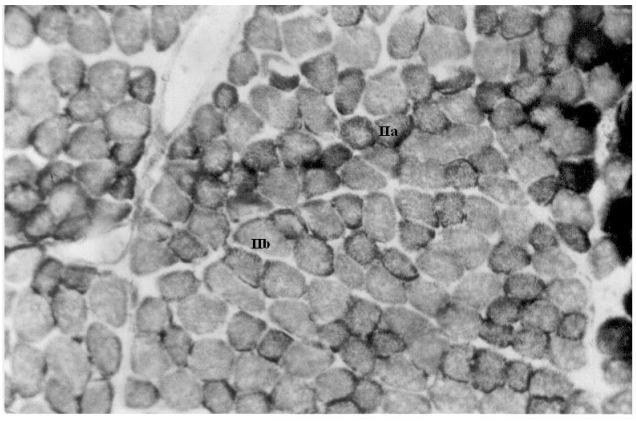

Figure 3. Transversal section of *R. mehelyi* pectoralis muscle stained for SDH. X500.

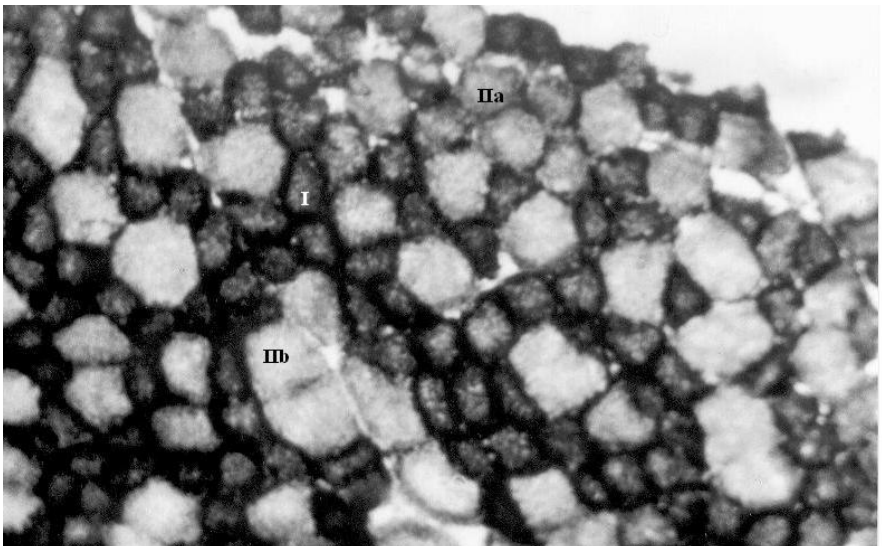

Figure 4. *R. mehelyi* serratus ventralis muscle stained for NADH. X500.

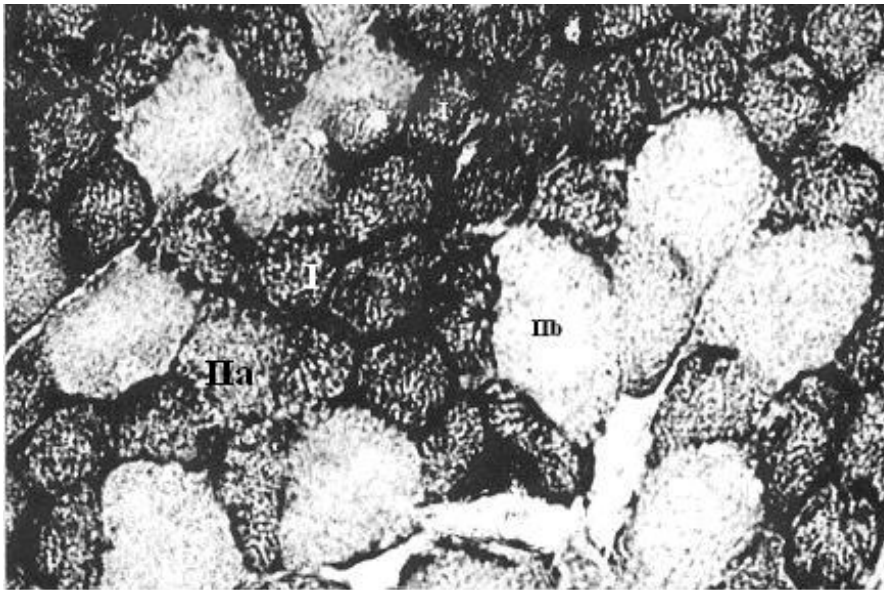

Figure 5. Transversal section of *R. mehelyi* serratus ventralis muscle stained for SDH. X1000.

tralis muscle preincubated in glycine-calcium-formalin solution followed by myosin ATPase procedure yielded three fiber types. Type IIa fibers stain intermediate intensity, and type IIb fibers stain darkest. Fiber ratios of *R. mehelyii* for the serratus ventralis muscle are 29% for fiber type I and 53% for fiber type IIa and 12% for fiber type IIb.

Type I fibers had a mean diameter of 20.5 µm. Type I fibers were highly oxidative, as stained darkly for NADH-TR (Figure 4) and SDH. Type IIa fibers (mean diameters 29.8 µm) were larger than type I fibers. The type IIa fibers displayed moderately to strong when stained for NADH-TR and SDH. The type IIb fibers had a mean diameter of 38.4 µm. Tupe IIb fibers were characterized by low activities of SDH (Figure 5) and NADH-TR. Formazan granules were evenly distributed throughout the fibers.

DISCUSSION

The two pectoralis in *R. mehelyii* comprised about 5.02% of total body mass. The right and left pectoralis muscles

in *Myotis lucifugus* comprised about 6.5% of total body mass (Foehring and Hermanson, 1984). In some bats the pectoralis muscles may compose up to 9% of total body weight (Vaughan, 1970 a,b), whereas in quadrupedal mammals these muscles typically make up about 1% of body mass. Vaughan (1959) reported that the pectoralis muscle in *Eumops perotil* is about 4 times heavier than the next largest muscle, the posterior portion of serratus arterior.

The mass of the serratus ventralis muscles constituted 3.45% of the total body mass in *R. mehelyii*. In *Artibeus jamaicensis* the serratus ventralis muscle comprised about 1.6% of total body mass (Hermanson and Altenbach, 1985). In *Myotis myotis* the serratus ventralis muscles comprised about 3.57 % of total body mass (Cebesoy and Ayvalı, 2003). In *Tadarida brasiliensis* the serratus ventralis was 2% (Foehring and Hermanson, 1984).

Type IIb fiber have a larger diameter than IIa fiber in pectoralis and serratus ventralis muscles that we studied. But in *A. jamaicensis* type IIa fibers have a larger diameter than type IIb in all muscles (Hermanson and Foehring, 1988). In several mammals, it has been shown that type I and type IIa fibers have smaller than type IIb fibers, a factor that increases cell surface area and thus may thereby facilitate diffusion of oxygen or energy sources (Hermanson and Foehring, 1988). In *R. mehelyii* flight muscles type IIa and type I fibers exhibit equally high oxidative potential based on their equal staining intensities for NADH and these fibers are relatively small in diameter. The size differences in the force-fibers might reflect differences in the force-generating potential or recruitment patterns of each type (Saltin and Gollnick, 1983). Thus *R. mehelyii* type IIb fibers, with their larger diameters, may be capable of producing larger increments of force than the IIa fibers.

The structural and metabolic properties of bats pectoralis muscle fibers reflect the exceptional maximal oxygen consumption of these animals (Thomas, 1975), and possibly represent the ultimate adaptation of a mammalian locomotory muscle for aerobic generation of muscular power. Peak metabolic power in bats is inversely related to body mass (Armstrong et al., 1977).
Among bats, the pectoralis muscles, during the wing's downward movement, are particularly well adapted to the formation of power (Vaughan, 1970a,b) and makes it possibly for a lot of vertical planes to be lifted (Norberg, 1976). During the wing movement, the pectoralis muscles enables the major components to be pushed forward not only in the course of outward wing movement but also in course of inward wing movement (Hermanson and Altenbach, 1985). During the flight, the main muscles for the downward movement are pectoralis and serratus ventralis muscles. These muscles differ from others in terms of the large quantity of type IIa fibers. Type IIa fibers (FO) are fast oxidative fibers. These fibers provides the formation of the power that is needed during the flight and fast movement (Foehring and Hermanson, 1984). The present study, which was carried out with *R. mehelyii,* indicates that the serratus ventralis and pectoralis muscles have a higher percentage of type IIa fibers (FO) as opposed to other fibers.

Taking into consideration the structural and metabolic qualities of pectoralis muscle fibers of the bats, it becomes obvious that these animals have a considerable amount of oxygen consumption. This study revealed that in the pectoralis muscle fibers of bats there are much more type IIa fibers, that is to say, the fast oxidative (FO) fibers, than type IIb fibers, that is to say, fast glicolitic (FG) fibers. This provides further proof of high level of oxygen consumption in the pectoralis muscles.

Saltin and Gollnick (1983) demonstrated that under circumstances where the specific contraction is equal, the generation of power is usually in proportion to the area of microfibers. In this way, type IIb fibers of *R. mehelyii* are more influential in the formation of power increase as compared to type IIa fibers, since type IIb fibers are wider in diameter than those of type IIa.

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