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Histochemical Determination of Muscle Fiber Types in Primary Flight Muscles of *Miniopterus schreibersi*

Suna Cebesoy and Mehmet Karakas
Department of Biology, Faculty of Science, Ankara University, 06100 Ankara, Turkey

Abstract: Two primary flight muscles of *Miniopterus schreibersi* were studied using morphological and histochemical analysis. All animals were killed with an overdose of sodium pentabarbital administered intraperitoneally. Muscles were dissected free, cleaned of excess fasia, 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. Transverse serial sections (10-12 μm thickness) were obtained with a freezing cryostat sections from each muscle were stained with Nicotinamide Adenine Dinucleotide Tetrazolium Reductase (NADH-TR) to assess oxidative capacity. The method was used demonstate Succinate Dehydrogenase (SDH) activity. Sections of each muscle were stained using myosin Adenosine Triphosphatase (mATPase). Two fast twitch fiber types are histochemically identified in pectoralis muscles of *M. schreibersi*. These were classified as type IIa and b according to glycine-calcium-formalin preincubation staining protocol for myosin ATPase. The primary flight muscles, serratus ventalis included type I, IIa and b fibers. Type I fibers in serratus ventralis were highly oxidative as stained darkly for NADH-TR. All type IIa fibers exhibited relatively intense staining properties for NADH-TR and SDH suggesting a intermediate oxidative capacity. In *M. schreibersi* primary flight muscles, type IIb fibers were low oxidative as indicated by light reaction for NADH-TR. Fiber ratios of *M.schreibersi* for the pectoral muscle are fiber type IIa 87% and fiber type IIb 13%. Fiber ratios for *serratus ventralis* are fiber type I 14%, fiber type IIa 70% and fiber type IIb 16%.

Key words: *Miniopterus schreibersi*, skeletal muscles, ATPase, NADH-TR, SDH, histochemical tests

INTRODUCTION

Miniopterus schreibersi is commonly known as long-fingered bat. It grows up to 46.5~56.6 mm with a wingspan of 40.75~50 mm. It has characteristically long wings and square-shaped ears. Many caves serve as common habitat for this species. It does not migrate much throughout its longevity and shows a strong tendency for group behaviors. It produces single offspring in July and August and provides care in a group-wise manner with other individuals. It hibernates in a cave from November to February in the following year (Park and Lee, 2009). *Miniopterus schreibersi* is nocturnal. These bats spend the day in their roosts and come out just after sunset. They spend most of the night foraging and return to their caves the next morning (Grzimek, 1990). Their flight has been described as rapid and jerky (Nowak, 1999). This species may also be highly gregarious.

As it is known, among such activities as walking, running, flying and swimming, flying is theone which requires the highest energy. Among mammals, bats are the only species that can truly fly. While flying these mammals generate 18 times as much metabolic force as they do while resting (Carpenter, 1985). In order for bats to fly with each wingbeat cycle, their muscles contract and expand at a minimum rate of 15 Hz (Hermanson and Altenbach, 1985). In addition, it has been stated that bats are a species with a high aerobic capacity among mammals (Thomas, 1975). Compared to those running mammals with a similar body size, bats use 2.5-3 times as much oxygen (Thomas, 1975; Pasquis *et al*., 1970). The fibrillar composition of the flight muscles of bats are well adapted to this enormous metabolic potential. The primary downstroke muscles that create the lift and forward propulsion in bats are the pectoral muscles (Vaughan, 1970a, b) therefore, these muscles show a lot more expertise than the secondary muscles. As a result, bat muscles provide a suitable model for studies on the muscle tissues of mammals.

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.

Corresponding Author: Suna Cebesoy, Department of Biology, Faculty of Science, Ankara University, 06100 Ankara, Turkey

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 (Soic-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 b fibers. Type IIa fibers have amoderate resistance to fatigue and represent a transition between the two extremes of the type I and 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 *Miniopterus schreibersi.*

MATERIALS AND METHODS

Eight *Miniopterus schreibersi* were used in this study (four males and four females ranging in weight from 11-24 g. The muscles studied include the primary flight muscles: pectoralis and 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 of each muscle were stained using myosin Adenosine Triphosphatase (mATPase) (Tunnel and Hart, 1977). The method of Nachlas *et al*. (1957) was used demonstrate Succinate Dehydrogenase (SDH) activity. Sections from each muscle were stained with Nicotinamide Adenine Dinucleotide Tetrazolium Reductase (NADH-TR) to assess oxidative capacity (Novikoff *et al*., 1961).

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 AND DISCUSSION

The pectoralis was the largest muscle present in *M. schreibersi*. The right and left pectoralis comprised about 4.08% 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 (Fig. 1). Type IIa fibers had a mean diameter of 33 μm. Fiber ratios of *M. Schreibersi* for the pectoral muscle are 87% for fiber type IIa and 13% 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 (Fig. 2). Type IIb fibers had a mean diameter of 43 μm. Within the pectoralis, type IIb fibers were significantly larger than type IIa fibers. Type IIb fibers

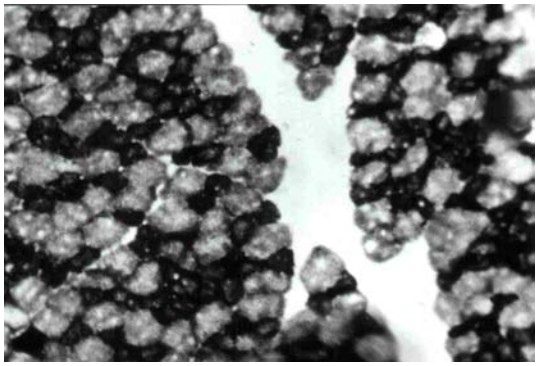

Fig. 1: Pectoralis muscle section from *M. schreibersi* ATPase stain; x500

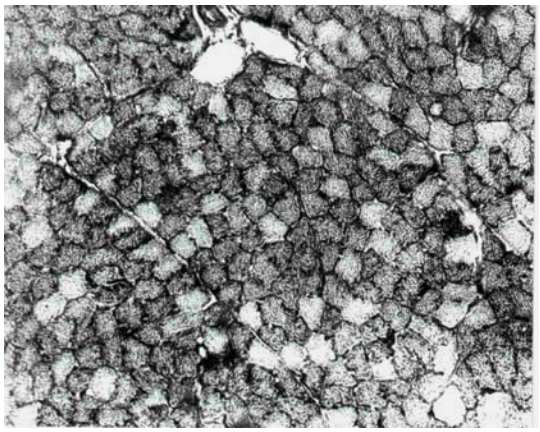

Fig. 2: Transversal section of pectoralis muscle stained for NADH; x500

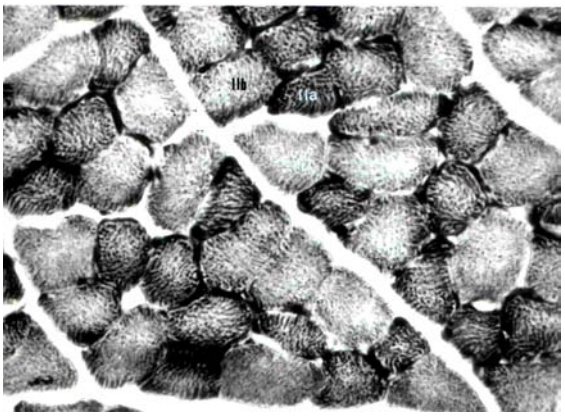

Fig. 3: Transversal section of pectoralis muscle stained for SDH; x1000

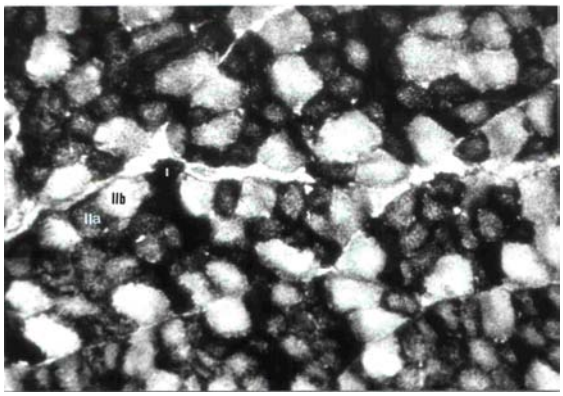

Fig. 4: Serratus ventralis muscle stained for NADH; x500

were low oxidative as indicated by light reaction for NADH-TR and SDH (Fig. 3). The mass of the serratus ventralis muscle constituted 2.9% of the total body mass. Sections of serratus ventralis 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 *M. schreibersi* for the serratus ventralis muscle are 14% for fiber type I and 70% for fiber type IIa and 16% for fiber type IIb.

Type I fibers had a mean diameter of 21.6 μm. Type I fibers were highly oxidative as stained darkly for NADH-TR (Fig. 4) and SDH. Type IIa fibers (mean diameters 32.5 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 43.9 μm. Tupe IIb fibers were characterized by low activities of SDH (Fig. 5) and NADH-TR. Formazan granules were evenly distributed throughout the fibers.

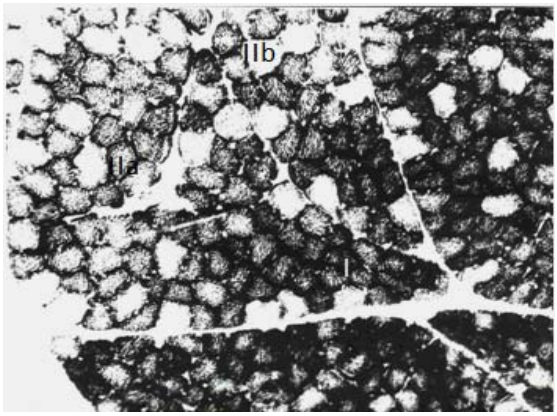

Fig. 5: Transversal section of serratus ventralis muscle stained for SDH; x500

In *M. schreibersi*, two pectoral muscles account for 4,08% of the total body weight. In *Myotis lucifugus* this percentage is 4% for left and right pectoral muscles (Amstrong *et al*., 1977). Yet in *Tadarida brasiliensis* the ratio of the two pectoral muscles to the total body weight is 6.5% (Foehring and Hermanson, 1984). while it is 4.7% in another species called *Artibeus jamaisensis* (Hermanson and Foehring, 1988). In *Rhinolophus mehelyii*, the right and left pectoralis comprised about 5.02% of total body mass (Cebesoy, 2009). In some species, the pectoral muscles may account for >9% of the total body weight (Vaughan, 1970a, b). However, it has been found out that these muscles constitute only 1% of the total body weight in four-legged mammals or quadrupeds (Amstrong *et al*., 1977). Vaughan (1959) has stated that the pectoral muscle in *Eumops perotis* weighs four times as much as another wide muscle called serratus anterior (Amstrong *et al*., 1977).

It has been calculated that in *M. schreibersi*, the serratus ventralis muscle makes up 3.57% of the total body weight whereas it makes up 1.6% of the total weight in *Artibeus jamaicensis* (Hermanson and Foehring, 1988). Foehring and Hermanson (1984) have maintained that serratus ventralis muscles constitute 2% of the total body weight in *T. brasiliensis*. In *Myotis myotis* the serratus ventralis muscles comprised about 3.57% of total body mass (Cebesoy and Ayvali, 2003). On the other hand, it can be clearly seen that *M. schreibersi* has twice as much serratus ventralis muscle when compared to the other two previously mentioned species.

Pectoral muscles in bats adjust themselves especially to the creation of force when the wing beats downwards which is the basic movement in flight (Vaughan, 1970a, b) and thus enables many vertical planes to be lifted upwards (Norberg, 1976). During wingbeat, pectoral

muscles enable the major components to be pushed and thrusted in pushing the wings outwards and rotating them inwards (Hermanson and Altenbach, 1985).

Of the bats included in the study, *M. schreibersi* is quite a fast flier. In bats that fly fast, fibers that contract fast outnumber the other types of fiber. Flight muscles require approximately a frequency of 60 m sec^{-1} in order to complete their contraction-expansion cyle and it becomes obvious that the fibers that are involved need to have the property of the ability to contract fast (Close, 1972; Burke and Edgerton, 1975). Since, fast fibers can adjust themselves to concentric dynamic contractions, pectoral muscles in bats are not important in maintaining posture (Armstrong *et al*., 1977). Goldspink *et al.* (1970) have asserted that fibers that contract fast are more effective and efficient for isotonic concentration than the fibers that metabolically contract slowly. In fast fibers, force reaches its peak more quickly than it does in slow fibers (Close, 1972; Burke and Edgerton, 1975). The properties of the fibers in the pectoral muscles of bats show better adjustment to the special requirements that emerge during flight.

When the structural and metabolic properties of pectoral muscle fibers of bats are taken into consideration, it becomes clear that these animals use a lot of oxygen. In this study, it has been detected that in the pectoral muscles of *M. schreibersi* type IIa (OF) fibers, i.e., fast oxidative fibers are much higher in number than type IIb (GF) fibers, i.e., fast glycolytic fibers. This is another proof of high levels of oxygen consumption in the pectoral muscles.

In bats, during flight, the primary downstroke muscles are pectoral and serratus ventralis muscles. In these muscles, type II a (OF) fibers are notably high in number. Type II a fibers are fast oxidative fibers. These fibers enable rapid movement and the generation of the force required to fly (Foehring and Hermanson, 1984). In this study, too, conducted on *M. schreibersi*, it has been noted that type IIa (OF) fibers in pectoral and serratus ventralis muscles have a higher percentage compared to other fibers.

CONCLUSION

In this study, the fiber types of the primary flight muscles of *M. schreibersi* have been histochemically identified through the use of ATPase ve NADH dyes. Two types of fibers have been found in pectoral muscles and according to the classification of Brooke and Keiser they have been named type IIa and b. Through, the use of ATPase and NADH, three different types of fiber have been detected in the Serratus ventralis muscle and again based on the classification of Brooke and Kaiser (1970) they have been classified as type I, IIa and b. In the primary flight muscles of *Artibeus jameisensi*, Hermanson and Foehring (1988) have distinguished two types of fibers that histochemically contract fast which they have classified as type II a and b with the use of myosin ATPase staining in acidic preincubation. Although, in this study fast fibers such as Hermanson and Foehring (1988) have also been classified as type IIa and b, IIa and b fibers of *A. jameisensis* are not identical to the type IIa and b fibers of *M. schreibersi.* Researchers have stated that type IIa fibers exhibit a glycolytic potential and type IIb fibers show a strong reaction to NADH. Similarly, they have also mentioned that type IIa fibers have a greater diameter than that of type IIb fibers. Conversely in the bats used in this study however, type IIa fibers have shown a strong reaction when stained with NADH and type IIb fibers have shown a weak reaction. In both flight muscles that have been examined, it has been observed that type IIb fibers are larger than type Iia fibers. It has been reported that type IIb fibers have a greater diameter due to fast and strong contractions (Guyton and Hall, 1996). Respectively, Foehring and Hermenson (1984) have stated that of the the three primary and two secondary flight muscles of *T. brasiliensis* in the pectoral muscle there are FO fibers and in serratus ventralis there are SO and FO fibers. The type I fibers of *M. schreibersi* correspond to the fibers of *T. brasiliensis* and again, the type Iia fibers of *M. schreibersi* correspond to the FO fibers of *T. brasiliensis*.

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