Morphological Characteristic and Functional Organization of The Iliofibularis Muscle of The Lizard Stellio Stellio Brachydactyla

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Abstract

The anatomy of the iliobibularis muscle of the lizard, Stellio stellio brachydactyla has been studied by light microscopy and transmission electron microscopy. Three fiber types were observed: 1) fast-twitch- glycolytic (FG) -large (210μm diam.) fibers; 2) fast-twitch-oxidative glycolytic (FOG)-smaller (135μm) fibers; and 3) tonic -small (81μm) fibers. The white and red regions of the muscle show a variable distribution of the three distinct fiber types which differ in their innervation and ultrastructural properties. The histochemical profile of fiber types was examined based on alkaline myofibrillar ATPase (m-ATPase), succinic dehydrogenase(SDH), and α - glycerophosphate dehydrogenase (α—GPDH). The white region is composed of FG that stain dark for m-ATPase, moderate for α—GPDH and light for SDH, while the red region is composed of both FOG and tonic fibers. FOG fibers stain dark for m-ATPase and SDH but moderate for α—GPDH, whereas tonic fibers stain light for m-ATPase, moderate for α—GPDH and dark for SDH.

Quantitative measurements demonstrated that the cross-sectional area of the white region of the iliobibularis is 72%, four times the cross-sectional area of the red region (18%). All fibers isolated from the white region of the iliobibularis had a single, well defined endplate of "en plaque" type but fibers isolated from the red region had an average of 16± endplates each, distributed along the entire length of the fiber at intervals of 985μm. Fibers with this type of end plates were considered to be tonic fibers, and their endplates were of "en grappe" type.

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The mitochondrial volume of fibers isolated from the red region (8 ± 0.36) was three times that of the fibers isolated from the white region (2.7 ± 0.12), and the capillary density of the red region was three times greater than those of the white region (2.2 ± 0.51 and 0.75 ± 0.12 capillaries per fiber, respectively). We suggest that the fibers of the white iliofibularis are best suited for fast but short term activity, and that the fibers of the deep red region would be recruited only during low speed locomotion and postural support.

Introduction

The locomotory capacity of lizards has been a focus of considerable attention from physiologists, morphologists, and ecologists (Marsh and Bennett, 1985; Bennett et al., 1984; Putnam et al., 1980). The histochemical and ultrastructural properties of skeletal muscle from Stelio stelio brachydactyla have not been studied in the context of locomotion and environmental adaptations as has been done for several species of lizards (Abu-Ghalyun et al., 1993; Bennett, 1980).

There are three fundamental fiber types identified within lizards upon histochemical demonstration of alkaline myofibrillar ATPase (m-ATPase), succinic dehydrogenase (SDH) and \(\infty\) — glycerophosphate dehydrogenase (\(\infty\) — GPDH). These fibers can be categorized as being fast-twitch-glycolytic (FG), fast-twitch-oxidative glycolytic (FOG), or tonic (Putnam et al., 1980). Succinic dehydrogenase reflects the oxidative metabolic capacity (Gleeson, 1980), and m-ATPase demonstrated at alkaline pH indicates the speed of muscle contraction (Barany, 1967). Therefore, the application of histochemical analysis allows one to infer locomotory efficiency which is ecologically important to the lifestyle of the lizard.

The ultrastructural analysis has demonstrated morphological adaptations at the tissue and cellular levels. Earlier studies demonstrated that postural muscles have more slow myofibers, whereas propulsive or locomotory muscles have many fast myofibers (Elice and Hulbert, 1981; Gleeson et al., 1980; Ovalle, 1978; Smith and Ovalle, 1973). According to Hess (1970), slow and fast muscle fibers differ in their morphology and nerve supply. The tubular system (T-tubules and sarcoplasmic reticulum) were shown to account for differences in contraction speed, and the nerve endings are either of "en plaque" or "en grappe" type. The "en plaque" nerve endings occur on fast muscle fibers while the "en grappe" nerve endings occur on muscle fibers of slow type. Quantitative comparisons were made of mitochondria, capillaries, and cross-sectional areas in relation to muscle region and species (Rosser
et al. 1994). A comparison of these variables between the species shows that for the iliofibularis there are significant differences in all measured variables in some lizards (Gleeson et al. 1984; Putnam et al. 1980; Bennett et al. 1984).

We therefore, undertook this study to fiber type the iliofibularis of Stellio stellio brachydactyla and examine its ultrastructure to correlate the behavioural performance with the structural capacities of the lizard. Materials and methods Animals

Adult lizards (Stellio stellio brachydactyla) of both sexes were collected from the eastern desert of Jordan and kept in glass aquaria under natural conditions for 2 weeks. The lizards used for experimentation were supplied with water at libidum, and a diet of insects daily. The preferred body temperature was maintained by allowing lizards to bask in a photothermal gradient maintained by using heat lamp over the aquarium.

Muscle Histochemistry

The iliofibularis muscle were removed from the hindlimbs of freshly decapitated lizards. The two muscles of each lizard were divided into two groups. One was used for histochemical study of muscle fiber types, and the other for endplate staining (Gleeson, 1980; Naik, 1963).

Muscle samples were immediately mounted in Tissue- Tek II O. C. T. Compound (Miles Laboratories, Inc., Naperville, IL 60566) on a cryostat chuck and frozen in melting isopentane cooled in liquid nitrogen (-160°C). Serial sections (10μm thick) were cut on a cryostat microtome (Briht’s Instrument Co. Ltd., Cambridgeshire, UK) at -20°C and were mounted on glass coverslips. Sections were stained for myofibrillar ATPase (m-ATPase) activity at pH 9.4, succinic dehydrogenase (SDH), and  \( \infty - \) glyceroephosphatde dehydrogenase (\( \infty - \) GPDH) as described by Gleeson et al. (1980). The temperature of the incubation medium was adjusted to the body temperature (35°C) (Hertz and Nevo, 1981). In this study, the m-ATPase stain was modified from Guth and Samaha (1970) and entailed incubation for 25 minutes. Dark fibers which have high m-ATPase activity were interpreted as fast fibers, while light fibers which have low m-ATPase activity were presumed to be tonic fibers. Fibers were classified into three types; dark, moderate, and light fibers based on the intensity of staining (Gleeson, 1983).

The stain for SDH was modified from Nachlas et al. (1975) and the procedure of Wattenberg and Leong was used for the demonstration of \( \infty - \) glyceroephosphatde (\( \infty - \) GPDH) (Wattenberg and leong, 1960). Serial sections were incubated in each stain for 1.5 h at 35°C. The cross-sectional area and the diameters of each fiber type
were calculated as described by Putnam et al. (1983). Fiber type counts were made on photographs of 10 randomly selected microscopic fields.

**Nerve endings**

Small bundles of fibers (20-100 fibers) were dissected free from the inner red and outer white regions of the iliofibularis and stained for regions of high acetylcholinesterase activity by a modified Naik procedure (Naik, 1963). They were fixed, outstretched at 4°C for 2 h in 10% neutral formalin buffered with 0.2 M acetate, pH 5.2, washed in distilled water, and incubated for 10-12 h in a freshly prepared solution of acetylthiocholine iodide. After staining, the teased preparations were washed and stained with 1% ammonium polysulphide for 5 min and cleared in glycerol for 3 h. Fibers from each region were isolated under glycerol and mounted on glass slides for viewing and photographing under low power with a light microscope.

**Muscle fixation for electron microscopy**

The iliofibularis of freshly decapitated lizards were pinned at in situ length on a dissecting dish containing fixation medium at 4°C to prevent the muscle fibers from shortening during fixation (Page, 1968). The medium contained 2.5% gluteraldehyde in cacodylate buffer (0.1 M, pH 7.4) at room temperature (22°C) for 2 h. Samples postfixed for 1 h in 1% osmium tetroxide, washed in water, dehydrated through a series of alcohols of increasing concentration, embedded in Epon and Araldite medium (Mollenhaur, 1964).

**Measurements of capillary density**

1 μm thick sections were cut from both red and white regions of the iliofibularis. Transverse and longitudinal sections were stained with 1% toluidine blue and examined by a Leitz semiautomatic image analyzer using the technique of Mathieu et al. (1983).

**Measurements of mitochondrial volumes**

Thin sections, 0.1 μm in thickness, were cut, stained with uranyl acetate and lead citrate, viewed with a Zeiss 10CR electron microscope (Hayat, 1975). Electron micrographs (1200-1600x) from both regions were magnified 4 times and analyzed for cellular organelles as described by Weibel (1979).

**Statistical Analysis**

All data are reported as means ± S.E.M. Significance of difference (p< 0.05) among paired data sets was computed with a one-tailed Student's t-test (Winer, 1962).
Results

Fiber types

Using the histochemical techniques, three major muscle fiber types in the iliofibularis muscle of Stellio stellio brachydactyla were identified (Fig. 1). Because the histochemical properties of these fibers are similar to those of muscle fibers from limbs of other lizards (Gleeson et al. 1980), we have simply referred to these fibers as fast-twitch-glycolytic (FG), Fast-twitch-oxidative glycolytic (FOG) and tonic fibers. The most abundant fiber types were FG fibers which were present in the white region and demonstrated the following pattern of color intensity: alkaline m-ATPase, dark; ∞ - GPDH, moderate; SDH, light. FOG fibers had been observed in the red region with the following histochemical characteristics: alkaline m-ATPase, dark; SDH, dark, and ∞ - GPDH, moderate. Tonic fibers were located in the central red region and exhibited the following pattern: alkaline m-ATPase, light; SDH, dark, and ∞ - GPDH, moderate (Table 1).

The diameters of tonic fibers were the smallest in all muscles of this species (81 ± 72μm), while FG fibers had the largest diameter (210 ± 80μm). However, the diameters of the FOG fibers were found to be intermediate (135 ± 26μm) (Table 2). There was regional variation in fiber distributions within the two regions of the iliofibularis. The outer (white) region contained fast glycolytic fibers while the inner (red) region contained mixed fibers of tonic and FOG types. The cross-sectional area of the white outer and inner regions of the iliofibularis were 72 ± 0.53% and 18 ± 0.56%, respectively (Table 3).

Nerve endings

The innervation patterns of muscle fiber have exhibited a morphological histochemistry for acetylcholinesterase (ACHE) activity. The subneural apparatus was stained as a black sulphide precipitate, which denotes zones of high ACHE activity. All fibers of the white region had a single motor end plate of "en plaque" type (Fig. 2). In the red region of the iliofibularis, two types of innervation were found; "en plaque" and "en grappe". Some teased preparations were singly innervated, while others from the same region possessed 16 ± 31 endplates of "en grappe" type per fiber (Fig. 2). These endplates occurred at an average intervals of 985 ± 62μm (Table 2).
Ultrastructural characteristics

The results of the determinations of mitochondrial volumes and capillary densities for fibers isolated from the red and white regions of the iliofibularis muscle are summarized in table 3, which shows the characteristics of both regions at the ultrastructural level: high values in mitochondrial volume (8 ± 0.36%) and capillary density (715 ± 19 capillaries/mm²) in the red region compared with the low values of mitochondrial volume (2.7 ± 0.12%) and capillary density (92 ± 28 capillaries/mm²) in the white region. Figure 3 shows a greater mitochondrial volumes and uniform distribution in a cross-section within the deep iliofibularis when compared with a cross-section from the white iliofibularis (Fig. 4). Furthermore, in the red region we could observe lipid droplets and glycogen granules around the mitochondria in fast-twitch-oxidative glycolytic fibers (Fig. 5). Moreover, capillaries in the red region demonstrate functional characteristics as indicated in Figure 6. The clustering of red blood cells in capillaries of the red region increases oxygen transport capacity.

Discussion.

The present work has shown that within the iliofibularis muscle of Stellio stellio brachydactyla the white (superficial) and red (deep) regions consist of three subgroups of muscle fibers referred to as FG, FOG, and tonic. The histochemical data presented here on Stellio stellio brachydactyla muscle properties agree well with earlier data from other species of lizards (Gleeson et al. 1980, 1984; Mutungi, 1990). The red region is fast-twitch-oxidative glycolytic and tonic fibers while the white region is fast glycolytic fibers. The red deep portion of the iliofibularis has a greater capacity for oxidative metabolism as shown by their staining for m-ATPase and are generally smaller in diameter than that of the fast fibers of the outer belly. Several authors have reported that fiber diameter is related to oxidative capacity. This has been demonstrated in mammalian and avian fiber types (Rosser et al. 1992; Sieck et al. 1986; George and Berger, 1966; Snyder, 1990).

The present mean value of the cross-sectional area of the red iliofibularis (18%) is less than the reported value for this region reported at 22% in Agama pallida (Abu-Ghalyun, 1995), 39% in Iguana iguana (Gleeson and Harrison, 1986), 30% in Dipsosaurus dorsalis (Gleeson et al. 1980). Although Stellio stellio brachydactyla exhibit striking changes in ground color, they were dark brown in the early morning and late afternoon, but tan at other times of the day (Hertz and Nevo, 1981). These differences probably reflect speed of contraction required for normal patterns of
running and posture which point towards adaptation of locomotory muscles. However, physiological parameters such as maximum velocity of contraction, contraction time, and twitch/tetanic ratio of the lizard locomotory muscles in the eastern Jordan or central Negev are needed to test this hypothesis. Referring to the measurements based on ultrastructural analysis (Table 3), the value of mitochondrial volume in the red region (8%) is lower than previously reported for the same muscle (9.6%, Gleeson et al. 1984; 12%, Mutungi 1990; 10%, Abu-Ghalyun, 1995).

In contrast, capillary densities in the red region (715 ± 19 capillaries/mm²) fall within the range reported for the iliofibularis of Agama pallida (795 capillaries /mm²). However, the magnitude is lower than that of V. exanthematicus muscle (932 capillaries/mm²). Moreover, it has been demonstrated that lipid droplets increases with increased muscle oxidative capacity (Kayar et al. 1988; Londraville and Sidell, 1990). Since lipid droplets facilitates oxygen transport from capillaries to mitochondria within the muscle fiber, the aerobic capacity of the red region may be partly inferred from the presence of lipid droplets in oxidative fibers.

The present study also demonstrates the presence of multiple endplates on tonic fibers of red region. This is consistent with multiple innervation in non-mammalian skeletal muscle (Prosk and Ridge, 1974; Abu-Ghalyun, 1990). The cholinesterase technique revealed that the nerve endings seen on teased fibers from the iliofibularis are of two types. "En plaque" ending which is found on fast fibers and "en grappe" which is seen on tonic fibers. The "en plaque" endings on fast fibers are relatively short and variable in structure, while "en grappe" endings occur spaced irregularly from each other along a single muscle fiber and frequently form clusters. This is seen in teased muscle fibers prepared from locomotory muscles of frogs (Hess, 1970). The mean number of endplates per fiber in Stellio stellio brachydactyla is 16 ± 3. This value is similar to that reported for D. dorsalis (14.7 ± 2.9, Gleeson et al. 1984) and V. exanthematicus (17, Mutungi, 1990), but higher than the reported value for the lizard Tiliqua nigrolutea (7-9, Proske and Vaughan, 1968). The mean distance between endplates (985 ± 68 μm) in tonic fibers is similar to reported values for this fiber (1124 ± 179μm, Gleeson et al. 1984 and 990-1200 μm (Ridge, 1971). Although no quantitative studies were performed on the endplate diameters, the two types of innervation are consistent with the general pattern of fast and tonic fibers innervation.

In general, the distribution of fiber type proportions, fiber area, and capillary supply in the iliofibularis muscle of Stellio stellio brachydactyla species indicates
functional differentiation of the muscle. The deeper region of the muscle seems better suited for posture maintenance and the superficial region is best suited for rapid movement. The range of activities from posture to running represents a gradual change from aerobic to anaerobic metabolism as speed increases. This sequential recruitment of fibers from tonic to FG has been confirmed for the iliofibularis muscle of the savannah monitor lizard (Jayne et al. 1988). The two regions of the iliofibularis of Stellio stellio brachydactyla are somewhat analogous to compartmentalized muscles of mammals in which the gastrocnemius is composed mainly of fast twitch fibers surrounding a smaller and deeper soleus which consists of mainly slow fibers (Ariano et al. 1973; Armstrong and Laughlin, 1985; Eisenberg, 1985). Compartmentalization can occur within individual muscles, each neuromuscular compartment having its own innervation, distinctive fiber type complement and function (English and Ledbetter, 1982; Bennett and Rubin, 1979). The benefit of grouping similar fibers together may be to optimize their mechanical advantage during contraction or to facilitate a more precise motoneuron control (Peters, 1989).
Acknowledgments

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Literature cited


Legends to Figures

Figure 1. A, B, C: Cross-sections from the iliofibularis muscle of *Stellio stellio brachydactyla* stained for: (A) alkaline myofibrillar ATPase (m-ATPase); (B) succinic dehydrogenase (SDH), and (C) SYMBOL 181 \( \text{\symbol{181}} \) glycerophosphate dehydrogenase (SYMBOL 181 \( \text{\symbol{181}} \) )-GPDH), respectively. Fiber marked G is FG fiber; O, FOG fiber; T, a tonic fiber. Magnification: X42.

Figure 2. Teased muscle fibers stained for acetylcholinesterase activity to localize endplates from the iliofibularis of *Stellio stellio brachydactyla*. A: single fiber isolated from the outer (white) region to demonstrate endplate (e) of the fast fiber and its motor neuron. B: single tonic fiber isolated from the inner (red) region to demonstrate multiple innervations (arrows). Magnifications: A: X42; B: X21.

Figure 3. Electron micrograph of a cross-section of the iliofibularis muscle from the red region showing greater mitochondrial density and uniform distribution of mitochondria (mt) within the red fibers. Magnification X4000.

Figure 4. Electron micrograph of a cross-section of the iliofibularis muscle from the white region. Note differences in the content of mitochondria compared with red region (Fig. 3). Magnification X7250.

Figure 5. Electron micrograph of a cross-section from the red iliofibularis of *Stellio stellio brachydactyla*. Note glycogen granules (G) and lipid droplets (l) around the mitochondria (mt). Magnification 7875X.

Figure 6. Electron micrograph of a capillary from the red iliofibularis fibers cut in transverse section. Note presence of red blood cells (RBCs) inside the capillary implying high oxygen content in the red iliofibularis. Magnification X2500.
List of Tables

Table 1. Histochemical characteristics of fiber types present in the iliofibularis muscle of *Stellio stellio brachydactyla*.

<table>
<thead>
<tr>
<th>Fiber type</th>
<th>Histochemical reaction intensity</th>
<th>comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>FG</td>
<td>m-ATPaxd dark; SDH light; GPDH moderate</td>
<td>Predominate in white iliofibularis</td>
</tr>
<tr>
<td>FOG</td>
<td>m-ATPaxd dark; SDH dark; GPDH moderate</td>
<td>Found in red iliofibularis</td>
</tr>
<tr>
<td>Tonic</td>
<td>m-ATPaxd light; SDH dark; GPDH moderate</td>
<td>Found in red iliofibularis</td>
</tr>
</tbody>
</table>

Table 2. Diameters and innervations of three histochemically distinct types of skeletal muscle fibers from the iliofibularis of *Stellio stellio brachydactyla*.

<table>
<thead>
<tr>
<th>Fiber type</th>
<th>Fiber diameter (μm)</th>
<th>Endplates/ fiber</th>
<th>Distance between endplates (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FG</td>
<td>210 ± 15</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>FOG</td>
<td>135 ± 26</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Tonic</td>
<td>81 ± 72</td>
<td>16 ± 31</td>
<td>985 ± 62</td>
</tr>
</tbody>
</table>

Values are means ±S.E.M., N = 10.

Table 3. Characteristics of red and white iliofibularis of *Stellio stellio brachydactyla*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Red</th>
<th>White</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional area (%)</td>
<td>18 ± 0.56</td>
<td>72 ± 0.53</td>
</tr>
<tr>
<td>Capillaries per mm²</td>
<td>715 ± 19</td>
<td>92 ± 28</td>
</tr>
<tr>
<td>Mitochondrial volume (%)</td>
<td>8 ± 0.36</td>
<td>2.7 ± 0.12</td>
</tr>
<tr>
<td>Myofibrillar volume (%)</td>
<td>85 ± 2.5</td>
<td>90 ± 1.6</td>
</tr>
<tr>
<td>Capillaries per fiber</td>
<td>2.2 ± 0.51</td>
<td>0.75 ± 0.12</td>
</tr>
</tbody>
</table>

Values are means ±S.E.M., N = 10, P < 0.05.