A Histochemical and Physiological Analysis of Performance in the Plantaris Longus Muscle of the Frog (*Rana Pipiens*) and the Toad (*Bufo Valliceps*)

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Abstract. Frogs typically move with powerful leaps for short periods of time, while toads display slower, lower levels of locomotion that can be sustained almost indefinitely. The objective of this experiment was to determine whether these differences in locomotory behavior of frogs and toads are reflected in the physiological properties of the plantaris longus (a.k.a. gastrocnemius) muscle, a primary muscle involved in movement. Frozen cross sections of frog (*Rana pipiens*) and toad (*Bufo valliceps*) skeletal muscles were stained for NADH dehydrogenase to investigate the primary pathway of ATP production in each fiber: anaerobic glycolysis or oxidative phosphorylation. Muscle fibers were also stained for myosin ATPase to determine whole muscle percent composition of slow twitch versus fast twitch fibers. Isolated whole muscle contractile performance was investigated by administering trains of stimuli and measuring rate of contraction fatigue at various points. Histological stains of the frog and toad gastrocnemius showed the frog gastrocnemius is composed of a significantly larger percentage area of fast twitch glycolytic fibers, while the toad gastrocnemius has a significantly larger percentage area composed of slow twitch oxidative fibers, in addition to a band of purely slow oxidative fibers comprising approximately 15–20% of the whole muscle. Fatigue tests showed that the frog gastrocnemius is capable of generating more force in the short term, while the toad gastrocnemius is able to sustain low levels of force over extended periods.

Introduction

Understanding the physiological basis for contractile properties of skeletal muscle is important in our understanding of the considerable diversity in behavioral responses and metabolic sources of energy that exists among amphibians during activity. Previous research has suggested that this range of diversity is well exemplified by *Bufo* (toads) and *Rana* (frogs) (Bennett 1974; Duellman and Trueb 1986).

The frog responds to threat or stimulation with rapid escape behavior involving powerful leaps. However, stamina is low and exhaustion ensues in 2–5 minutes (Bennett 1974). In contrast, the toad seems incapable of exhibiting behavior beyond the scope of a moderate walk and can sustain low levels of activity almost indefinitely. Predation is avoided by the adoption of an inedible posture (the toad puffs itself up) or reliance on poisonous skin secretions rather than rapid escape behavior (Bennett 1974; Mendiola et al. 1991).

The objective of this research was to determine whether such dif-

ferent patterns of locomotory behavior are evident in physiological properties of the frog and toad plantaris longus (a.k.a. gastrocnemius) muscle. Physiologists have known for years that vertebrate skeletal muscles are composed of different varieties of muscle fibers. These fibers vary in their source of energy (anaerobic glycolysis versus oxidative phosphorylation), their speed of contraction (fast versus slow twitch), and rate of fatigue (fatigue prone versus fatigue resistant) (Eckert et al. 1988).

The gastrocnemius is a large, thick-bellied muscle that extends the foot. It originates by a slender dorsal tendon from the distal border of the aponeurosis covering the knee, and also by a short, cylindrical tendon formed by the union of two branches of the tendinous arc along the medial surface of the knee. The gastrocnemius inserts distally by a thick, flat tendon that spreads out on the plantar surface of the foot to form the aponeurosis plantaris, from which numerous tarsal and foot muscles originate. Contraction results in a straightening of the ankle joint, and hence it is a primary muscle used in locomotion (Duellman and Trueb 1986).

Frozen cross sections of the gastrocnemius muscle in *Rana pipiens* and *Bufo valliceps* were stained for NADH dehydrogenase to quantitatively determine the primary pathway of ATP production in each muscle fiber: anaerobic glycolysis or oxidative phosphorylation. Myosin ATPase stains were used to determine percent composition of slow twitch versus fast twitch muscle fibers. Whole muscle contractile performance was assessed by administering trains of stimuli until zero tension was reached and then measuring fatigue rates. Results from the tests suggest that the frog gastrocnemius is better adapted to short bursts of high force responses because it contains a majority of large diameter fast twitch glycolytic fibers. The toad gastrocnemius is more suited to sustaining lower levels of tension over longer periods of time because it contains a much higher proportion of small diameter slow twitch oxidative muscle fibers.

Methods

Twelve adult frog specimens and thirteen adult toad specimens were utilized in this experiment. Histological stains were done by first mounting the isolated gastrocnemius muscle on a cryostat chuck with dental wax and covering it with O.C.T. frozen tissue embedding medium. The muscle was then frozen by brief (10–12 s) immersion in isopentane cooled with liquid nitrogen. Cross-sectional slices were made midway along the muscle in a cryostat 16 μm thick at −30°C and immediately transferred to slides. The tissue was air-dried from 15–60 minutes prior to incubation. A fraction of these slides was stained for myosin ATPase at 4°C for 30 minutes (Padykula and Herman 1955). The remaining slides were placed in

NADH-dehydrogenase incubation medium at room temperature in the dark for one to one and a half hours (Nachlas et al. 1958). Original staining procedures were slightly modified by Ogonowski and Lang (1979). A value for the percent of total muscle area that each fiber type composed was measured using computer imaging techniques in four randomly chosen sample areas for different tissue samples per muscle.

Physiological tension/fatigue studies involved attaching the isolated muscle to a force transducer and determining stimulation voltage and whole muscle length which produced the maximum tension response upon single 10 ms pulse stimulation. The muscle was then fatigued to zero tension by stimulating with trains consisting of five pulses (10 ms duration pulses @200 Hz) delivered at 0.5 Hz. Rate of fatigue (measured as % decrease in tension per second) was then determined at tension values corresponding to 75%, 50%, and 25% of maximum muscle tension.

Data were analyzed using paired and independent T-tests (Systat).

Results

Histochemical stains for the frog (Fig. 1) and the toad (Fig. 2) show that glycolytic fibers stain lighter than oxidative fibers for NADH-dehydrogenase due to the presence of fewer mitochondria. Fast twitch fibers stain darker for myosin-ATPase activity than slow twitch fibers due to a greater density of the faster ATPase enzyme. The stains reveal that the gastrocnemius of *Rana pipiens* is composed throughout of a mix of fiber types; the gastrocnemius of *Bufo valliceps* also exhibits a mix of fiber types in approximately 85% of its area, but also displays a pure band of slow oxidative fibers composing 16 ± 3.1% of the total cross-sectional area.

A quantitative analysis of these stains reveals that glycolytic fibers compose 78 ± 6.7% of the total area in the frog gastrocnemius and oxidative fibers compose 22 ± 6.7%. The toad gastrocnemius contains 48 ± 2.7% glycolytic fibers and 52 ± 2.7% oxidative fibers (Fig. 3). Frog fast twitch fibers compose 78 ± 5.0% of total muscle area and slow twitch fibers compose 22 ± 5.0%. The toad gastrocnemius contains 47 ± 3.4% fast twitch fibers and 52 ± 3.4% slow twitch fibers (Fig. 3). Note that in the frog gastrocnemius, fast glycolytic fibers compose the majority of total muscle area. In toad gastrocnemius, fast glycolytic and slow oxidative fibers compose approximately equal amounts of area.

Sample whole muscle tension traces (Fig. 4) of the frog and toad reveal that the frog gastrocnemius fatigues at a fairly constant rate until this rate increases when approximately 65% of maximum tension is reached (Fig. 5). The toad, however, fatigues fairly rapidly at first, and then maintains low levels of tension with slow fatigue rate (Fig. 5).

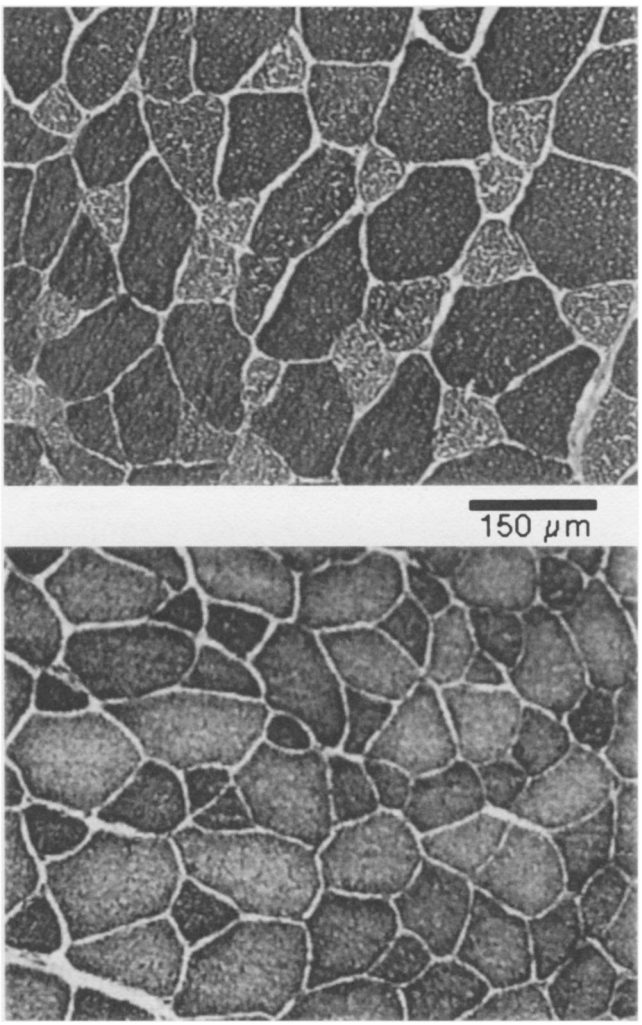

Fig. 1. Histological stains in the frog (*Rana pipiens*) for myosin ATPase activity (top) and NADH dehydrogenase (bottom).

Discussion

We have shown that the differences in locomotory behavior observed in the frog and toad are clearly reflected in the physiological properties of the gastrocnemius, a principle muscle of movement. The toad gastrocnemius has a significantly larger area composed of slow twitch oxidative fibers, which allows it to maintain lower levels of tension more efficiently than the frog gastrocnemius. However,

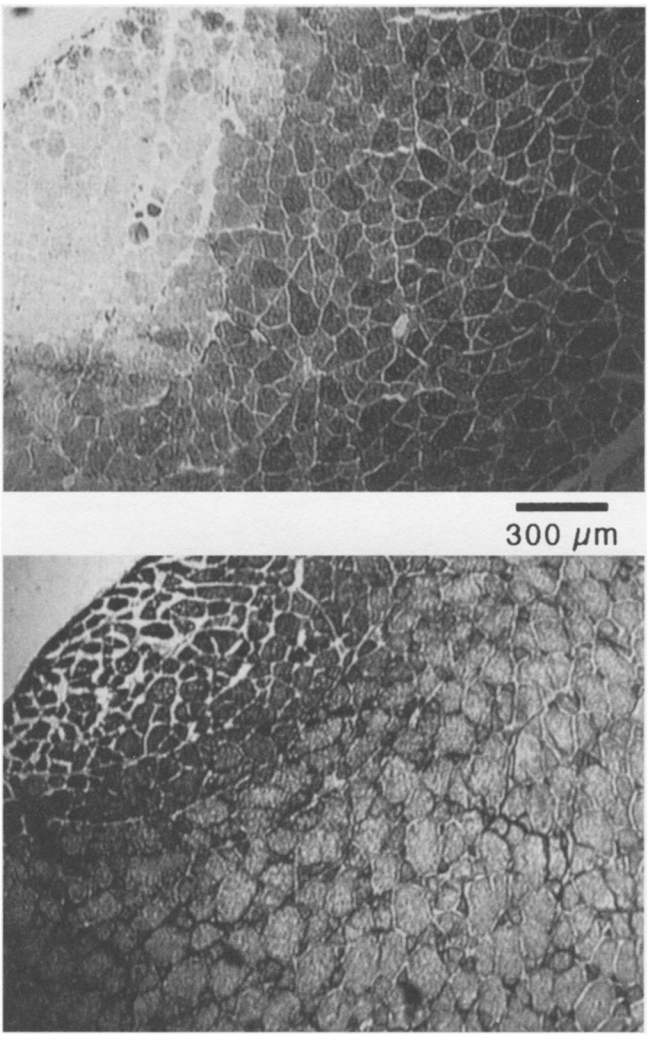

Fig. 2. Histological stains in the toad (*Bufo valliceps*) for myosin ATPase activity (top) and NADH dehydrogenase (bottom).

its lack of glycolytic fibers render it incapable of maintaining higher levels of tension for more than a few seconds. The frog gastrocnemius has a significantly larger area composed of fast twitch glycolytic fibers, can maintain higher tension longer, but fatigues fairly rapidly at lower tension levels.

Fast twitch glycolytic fibers are more capable of quick, powerful contractions than slow oxidative fibers due to their larger area, more

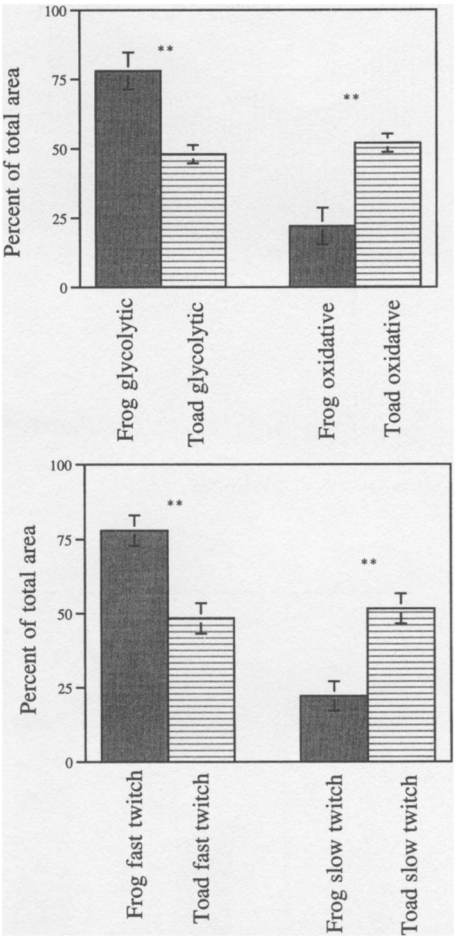

Fig. 3. A comparison of glycolytic and oxidative fibers, and fast and slow twitch fibers, as percent of total area, in the frog and toad gastrocnemius (** $p < .01$) Values represent means ± standard deviation.

developed sarcoplasmic reticulum, and larger amounts of glycolytic enzymes for rapid release of energy through anaerobic glycolysis. The ATPase site of the myosin head utilizes ATP at a faster rate in fast twitch fibers, leading to quicker speed of contraction. Slow twitch oxidative fibers, however, are capable of sustaining low levels of tension for longer periods of time, and typically possess a higher quantity of mitochondria for use in aerobic phosphorylation (Eckert et al. 1988).

Glycolytic fibers are also more prone to fatigue than oxidative

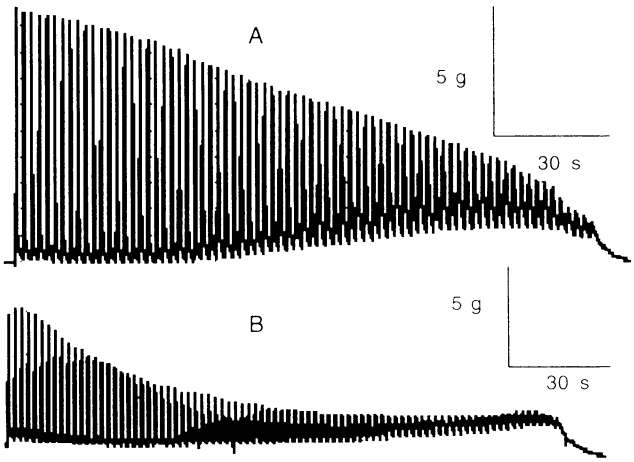

Fig. 4. Sample whole muscle tension traces recorded from the isolated gastrocnemius of the frog (A) and toad (B).

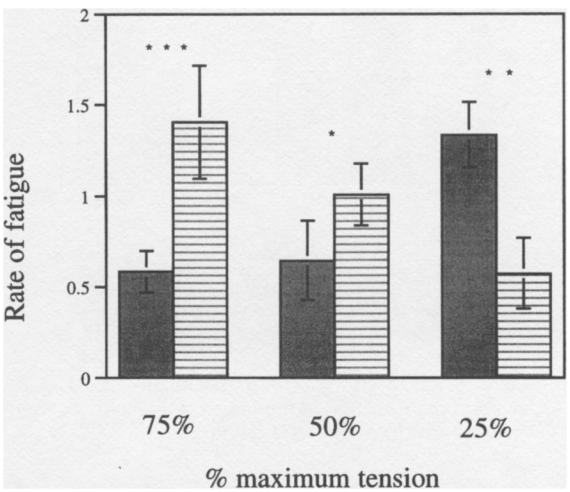

Fig. 5. A comparison of fatigue rate (% change in tension per second) at tension values that are 75%, 50%, and 25% of max tension. Solid bars represent the frog gastrocnemius. Striped bars represent the toad gastrocnemius (*p < .05, **p < .01, ***p < .001) Values represent means ± standard deviation.

fibers due to the production of lactic acid, a byproduct of anaerobic glycolysis. The lactic acid decreases the pH in the muscle cells, and thus alters the effectiveness of enzymes and proteins within the cell leading to reduced storage and release of calcium by the SR, reduced

actin/myosin affinity, and reduced troponin/calcium affinity (Eckert et al. 1988). Oxidative fibers, on the other hand, yield considerably more ATP from each nutrient processed, and do not result in lactic acid accumulation. This, coupled with the slow rate at which slow oxidative fibers use ATP and the large number of mitochondria, enable oxidative fibers to maintain tension efficiently for longer periods of time with little fatigue and depletion of energy stores (Eckert et al. 1988).

The histological stains showed that the frog gastrocnemius has significantly more area composed of fast twitch glycolytic fibers than the toad gastrocnemius (Figs. 1–3). This pattern is reflected in the results of the physiological experiments, which showed an initial fatigue rate in the frog muscle that was fairly constant, with a more rapid fatigue rate at less $\leqslant 25\%$ of maximum muscle tension. The toad gastrocnemius, however, fatigued at a more rapid rate early on, but because it was composed of a larger area of oxidative fibers than the frog, it was able to maintain lower levels of tension longer with a significantly slower rate of fatigue (Fig. 5).

Similar differences in histochemical profiles were reported by Sperry (1981) for *Bufo americanus* and *Rana pipiens.* In addition, Mendiola et al. (1991) concluded that the gastrocnemius of the *Rana perezi* contains a significantly larger area of fast glycolytic fibers and a smaller area of slow twitch fibers than the gastrocnemius of *Bufo calamita.* Finally, Bennett (1974) concluded, by measuring activity of glycolytic enzymes, that the toad possesses a maximum lactate production capacity that is 15–25% of the frog, and that differences in the activity patterns of *Rana pipiens* and *Bufo boreas* are not purely behavioral, but are a reflection of the physiological differences within the muscles of these animals. However, Putnam and Bennett (1983) reported no different histochemical profiles in locomotor muscles of *Rana pipiens and Bufo boreas* despite these enzymatic activity differences.

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