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STRUCTURAL CORRELATES OF SPEED AND ENDURANCE IN SKELETAL MUSCLE: THE RATTLESNAKE TAILSHAKER MUSCLE

PAUL J. SCHAEFFER[1], KEVIN E. CONLEY[2] AND STAN L. LINDSTEDT[1,*]

[1]*Physiology and Functional Morphology Group, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640, USA* and [2]*Department of Radiology, University of Washington Medical Center, Seattle, WA 98195, USA*

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Summary

The western diamondback rattlesnake *Crotalus atrox* can rattle its tail continuously for hours at frequencies approaching 90 Hz. We examined the basis of these fast sustainable contractions using electromyography, data on oxygen uptake and the quantitative ultrastructure of the tailshaker muscle complex. The tailshaker muscle has no apparent unique structures; rather, the relative proportions of the structures common to all skeletal muscles appear to be present (1) to minimize activation, contraction and relaxation times *via* an extremely high volume density of sarcoplasmic reticulum (26 %) as well as, (2) to maximize ATP resysnthesis *via* a high volume density of mitochondria (26 %). The high rate of ATP supply is reflected in the *in vivo* muscle mass-specific oxygen uptake of this group of muscles which, at 585 ml O_2 kg^{-1} min^{-1} during rattling at 30 °C body temperature, exceeds that reported for other ectotherm and many endotherm muscles. Since the change in oxygen uptake paralleled that of the rattling frequency over the range of measured body temperatures, there was a nearly constant O_2 cost per muscle contraction (0.139±0.016 μl O_2 g^{-1}). Electromyographic analysis suggests that each of the six muscles that make up the shaker complex may be a single motor unit. Finally, the maximum rate of mitochondrial oxygen uptake is similar to that of various mammals, a hummingbird, a lizard, an anuran amphibian and of isolated mitochondria (at 10 000–40 000 molecules O_2 s^{-1} μm^2 of cristae surface area, when normalized to 30 °C), suggesting a shared principle of design of the inner mitochondrial membrane among the vertebrates.

Key words: western diamondback rattlesnake, muscle structure, mitochondrial oxygen consumption, *Crotalus atrox*.

Introduction

Extremes of performance often provide insights into physiological mechanisms unlikely to surface in organisms of ‘average’ performance. These insights are largely the consequence of an increased ratio of signal to noise and have been argued to be a contribution unique to comparative physiology (Schmidt-Nielsen, 1967; Yates, 1979).

Without question, the tailshaker muscles of the rattlesnake exemplify an extreme of skeletal muscle function. Like most venomous animals, rattlesnakes are very conspicuous, rattling a loud, unmistakable warning apparently intended primarily for large herbivores (Klauber, 1982). This behavior has two features that make it unexpected among the reptiles. First, these animals can rattle their tails at very high frequencies, approaching 90 Hz (Chadwick and Rahn, 1954), presenting a difficult task of coordinating excitation–contraction coupling with millisecond timing in a muscle group that may exceed a mass of 10 g. Further, this frequency can be maintained continuously for minutes to hours (Martin and Bagby, 1973), suggesting an aerobic capacity uncommon among the reptiles and rare among all vertebrates. Because of its unusual coupling of high frequency and endurance, we feel this muscle group may be an ideal model system for the study of several properties common to all vertebrate skeletal muscle, namely excitation–contraction coupling, as well as those mechanisms quantitatively linking the supply of ATP to its demand.

A number of previous studies have described the key features of this muscle group, providing a foundation for our work. Zimmerman and Pope (1948) first examined the tailshaker muscle, describing the muscular anatomy of the tail as a complex of six muscles whose fibers are oriented at about 45 ° to the body axis. A classic study of Chadwick and Rahn (1954) measured the contraction frequency of rattlesnake tail muscles using mercury strobe lamps and reported a maximal rattling frequency approaching 90 Hz, later confirmed by

*Author for correspondence.

Martin and Bagby (1972, 1973). Schultz *et al.* (1980) and Clark and Schultz (1980) described the almost exclusively fast twitch fiber types present in the myofibers of two species of rattlesnakes.

In this paper, we present results addressing several unresolved questions about the muscles themselves. (1) Are all six tailshaker muscles active with each twitch during rattling? (2) How are the muscular contractions coordinated to producing the rattling? (3) What is the energetic cost of rattling and how does it vary with temperature? (4) What are the quantitative ultrastructural features of these muscles which are responsible for their unusual attributes? (5) How does mitochondrial oxygen uptake of the rattlesnake tailshaker muscle compare with that of other vertebrate muscles? Specifically, we examined the electromyographic activity of the tail complex during rattling, measured the rate of oxygen uptake during rattling and quantified some of the ultrastructural components of the muscles to probe the structural basis of the metabolic and contractile properties of this unusual muscle.

Materials and methods

Animals

Eight western diamondback rattlesnakes *Crotalus atrox* Baird and Girard (mean body mass 420 g, range 278–613 g) were captured in central Arizona, USA, between March 1991 and August 1992 (Arizona Game and Fish permit no. 285). Rattlesnakes of both sexes were utilized without consideration of differences. They were kept in animal quarters during the experimental portion of the study, at a constant temperature of 25 °C and with an approximately 12 h:12 h L:D cycle. At the end of the experimental period, each of the animals was killed by a lethal overdose of sodium pentobarbital, followed by decapitation and the removal of muscle tissues. The entire tailshaker muscle complex was carefully dissected out and weighed.

Determination of oxygen consumption

Whole-animal rates of oxygen consumption ($\dot{V}_{O_2}$) were determined using an open-flow system. The animals were placed into a cylindrical, double-walled chamber (3.0 l) constructed of clear acrylic. Chamber temperature was regulated by circulating water beneath and through the outer water jacket of the test chamber. Resting body temperatures were monitored with a 30 gauge cloacal thermocouple probe sheathed in a plastic tube, and $\dot{V}_{O_2}$ determination began after body temperature had equilibrated with chamber temperature.

Outside air entered through a small incurrent port in the lid and exited *via* a length of tubing opening at the bottom of the opposite side, ensuring flow past the snake. Excurrent air was directed through a column of Drierite, to remove water vapor, and then through a column of Ascarite to remove CO_2. The sample then passed through a Matheson mass-flow probe and finally into an Ametek O_2 sensor. The output from the O_2 analyzer was amplified and recorded on a Grass polygraph.

Resting $\dot{V}_{O_2}$, defined as the lowest constant rate of O_2 uptake over a period of at least 20 min, was measured while the snake remained inactive in the chamber, which was covered with material. Immediately following this, the covering was removed and the snake was aroused to rattle. Usually, a person moving in its field of view provided a sufficient stimulus to provoke the snake to rattle, which it did while keeping its body coiled and stationary. Rattling $\dot{V}_{O_2}$ was identified as a plateau of oxygen consumption ($\geqslant$5 min) during this period. Immediately after the rattling plateau had been reached, the animal was removed and the tail was pierced with a 28 gauge sterile needle to allow insertion of a 36 gauge thermocouple directly into the muscle to measure muscle temperature. This temperature was used in determining tail mass-specific rattling $\dot{V}_{O_2}$.

The O_2 system was calibrated by bleeding N_2 into the chamber (Fedak *et al.* 1981) at a known flow rate using a 50 ml min^{-1} calibrated Matheson flow probe. The N_2 flow rate was adjusted to yield a chart recorder deflection (fractional content of O_2) approximately equal to the deflection during rattling. All $\dot{V}_{O_2}$ values are converted to standard temperature and pressure (STP).

Electromyography

We recorded electromyograms (EMGs) from each of the six tail muscles of two snakes using bipolar, Teflon-coated, stainless-steel (0.076 mm) electrodes (bared tip of 1–1.5 mm) inserted into each muscle at body temperatures between 10 and 37 °C. The EMG electrodes were connected to an AM Systems model 1700 differential a.c. amplifier, set with a low bandpass of 1 Hz and a high bandpass of 20 kHz, and recorded on an A. R. Vetter eight-channel FM tape recorder. Rattling EMGs were transferred from the tape recordings to a 486 (66 MHz) computer *via* Peak Motion Measurement software (version 5.2.1) and analyzed with Data Pac II (version 4.0) software. The recordings were printed directly from the computer using a laser printer. Snakes were killed after the EMG recording, and the electrode placement was verified.

Quantitative ultrastructure

For the morphological study, both tail and body wall musculature were collected from each animal immediately following death. Tail muscle mass was determined, and the muscles of the tail complex were divided into the dorsal, lateral and ventral pairs and analyzed separately. The body wall musculature was sampled from the mid-section of the body, from muscles that overlie the ribs, near to the dorsal midline. All muscle samples were fixed in gluteraldehyde and processed for quantitative ultrastructural analysis following the techniques of Hoppeler *et al.* (1984).

The cellular structures quantified, as described previously by Weibel (1979) (at a final magnification of $\times$26 000), were myofibrils, mitochondria (both subsarcolemmal and intrafibrillar), sarcoplasmic reticulum (including T-tubule systems), lipid bodies, nuclei and ‘other’. Calculations of final volume densities and statistical analyses were accomplished

using a software program STEPONE (provided by F. Wainschstein, L. M. Cruz-Orive and H. Hoppeler). The surface area of the inner mitochondrial membrane was determined on ultrathin sections at a final magnification of ×200 000, following the technique of Schwerzmann *et al.* (1989).

Results

Muscle electromyograms

During rattling, each of the six muscles of the shaker complex was active with each tail movement. The muscle seems to be behaving as an oscillator, with the muscles on one side active out of phase with those on the opposite side of the tail. The EMG patterns at all temperatures appear as a series of single spikes rather than bursts of electrical activity (Fig. 1). At 10, 25 and 35 °C we measured muscle contraction frequencies of 23, 60 and 85 Hz, which correspond to contraction cycle times of 43, 17 and 12 ms, respectively. These rattling frequencies are nearly identical to those measured 40 years ago by Chadwick and Rahn (1954) in the prairie rattlesnake (*C. viridus*) using stroboscopic lights (Fig. 2).

Whole-animal $\dot{V}_{O_2}$

Resting O_2 uptake in these animals at 30 °C ($1.1\pm0.095\,ml\,O_2\,kg^{-1}\,min^{-1}$; mean ± S.E.M., N=8) is similar to that reported for other snakes of similar size (Ruben, 1976; Bennett and Dawson, 1976; Andrews and Pough, 1985; Beaupre, 1993) and lizards (Bennett and Dawson, 1976; Gleeson, 1981; Andrews and Pough, 1985; Bickler and Anderson, 1986). At each temperature, rattling results in a several-fold increase in $\dot{V}_{O_2}$. In Fig. 2, a regression line showing the rattling frequency of *C. viridus* (Chadwick and Rahn, 1954) is given together with our EMG and oxygen uptake results as a function of temperature. When plotted as $\log_{10}$ *versus* temperature, the slope of each line gives the Q_{10} value for that function. There appears to be a break in the resulting lines at about 20 °C, with a Q_{10} of 2.2–2.6 below 20 °C and approximately 1.6 above 20 °C; the lowest body temperature at which these animals are commonly active is about 20 °C (Beck, 1991). As the two lines (rattling frequency and rattling $\dot{V}_{O_2}$ *versus* temperature) are nearly parallel across the entire measured temperature spectrum, the mass-specific energy cost per contraction is nearly constant (P=0.48, ANOVA) at $0.139\pm0.016\,\mu l\,O_2\,g^{-1}$ (mean ± S.E.M., N=8) or $0.0028\,J\,g^{-1}$ contraction^{-1}.

Tail-specific $\dot{V}_{O_2}$

There was minimal detectable postural or activity difference between rest and rattling except in the rattling muscles, so we

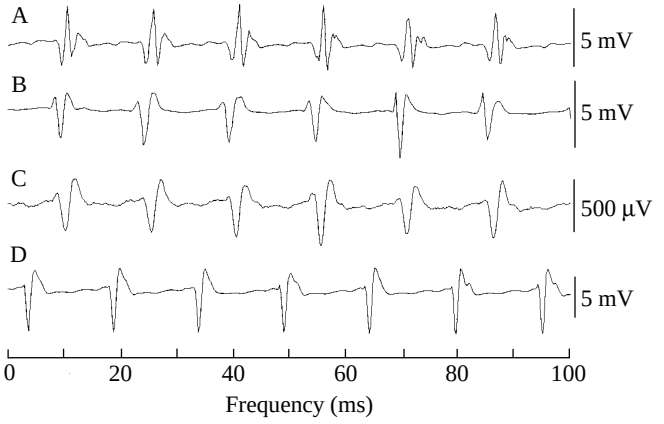

Fig. 1. Sample electromyograms (EMGs) of four of the six muscles that make up the tailshaker complex: (A) left dorsal, (B) left medial, (C) left ventral and (D) right ventral muscles. These EMGs were obtained during rattling at 25 °C and are not rectified; hence, the polarity of the spike is meaningless. Each contraction appears almost as a single 'spike', rather than a more typical burst of electrical activity, suggesting that each muscle is composed of one or very few motor units. Note that the signal for the right side (D) is out of phase with the signals for the left side muscles, which are synchronous.

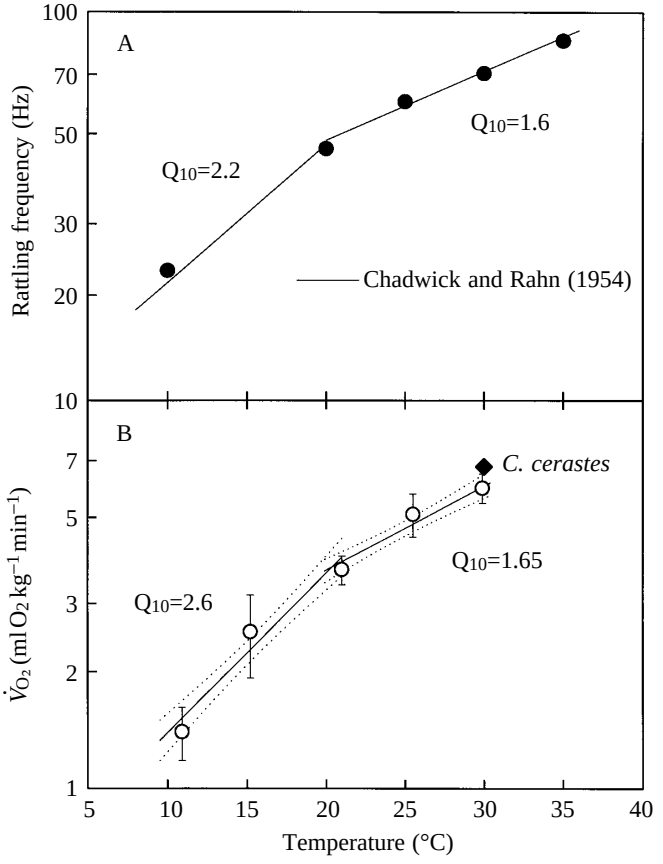

Fig. 2. Rattling frequency (A) and rattling $\dot{V}_{O_2}$ (B) are nearly parallel functions of body temperature in the western diamondback rattlesnake. Rattling frequency (measured from EMGs; this study) is plotted as filled circles. The stroboscopically measured tail movements of *Crotalus viridus* (Chadwick and Rahn, 1954) are shown as a line. Oxygen uptake data (open circles) at each of five temperatures are presented as the mean ± 95 % C.I., and the lines are linear regressions. The maximum oxygen uptake in the sidewinder rattlesnake (*Crotalus cerastes*) during treadmill locomotion is also given for comparison (filled diamond; Secor *et al.* 1992).

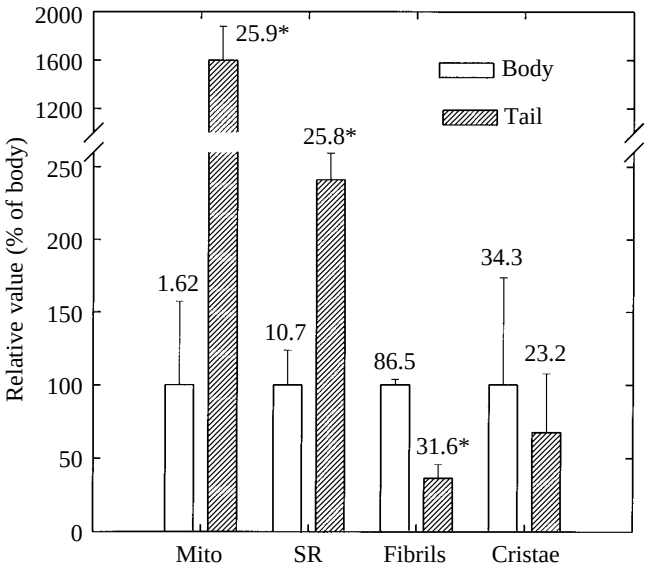

Fig. 3. Comparing the muscle ultrastructure of the tail with that of the body reveals several structural differences. Mean values for the volume densities of mitochondria (Mito), sarcoplasmic reticulum (and associated structures e.g. T-tubules, SR) and myofibrils (Fibrils) are given (+S.E.M., N=8; *tail value significantly different from body value, P<0.05). Absolute mean values are given at the top of each column, while *tail values relative to the body values* are given by the column height (i.e. in all instances body values constitute 100 %). The final pair of columns compares the surface area density of the inner mitochondrial membranes (Cristae) of representative (randomly chosen) mitochondria from both body and tail. The greater error represents in part the difficult nature of these measurements.

attributed the increase in oxygen uptake rate (resting *versus* rattling) exclusively to the active rattling muscles. Thus, we determine the $\dot{V}_{O_2}$ of rattling by subtracting the resting $\dot{V}_{O_2}$ from the rattling $\dot{V}_{O_2}$. Having extracted and weighed the tail muscles, the muscle mass-specific $\dot{V}_{O_2}$ of the tail was calculated. Tail muscle mass-specific O_2 uptake at 30 °C (muscle temperature) was 585±18 ml O_2 kg^{-1} min^{-1} (mean ± S.E.M., N=8).

Ultrastructural anatomy

We found no identifiable differences in ultrastructure among the six muscles that make up the tail complex. In the tail, the volume density of both mitochondria and sarcoplasmic reticulum (SR) are unusually high for any muscle tissue, especially reptilian (Fig. 3). For example, mitochondrial volume density in skeletal muscles of the Cuban iguana is about 3 % (Conley *et al.* 1989). As a consequence, the volume density of the contractile elements in the tailshaker muscle is unusually low. Although not quantified, a large portion of the cell space recorded as 'other' seemed to be dominated by glycogen (Fig. 4A), as was noted previously by Schultz *et al.* (1980). The body wall muscles show a more typical reptilian pattern, with very low mitochondrial densities and high contractile fiber densities (Figs 3, 4B). Both the muscle fibers and their myofibrils that constitute the body wall muscles are much larger in cross section than those of the tail. However, even in the body wall, the proportion of SR is high relative to other reptilian muscles (Fig. 3), probably a necessity for rapid prey striking movement. The inner membrane surface areas of the mitochondria in the tail and body wall muscles are similar (Fig. 3, Mann–Whitney rank sum test: t=488; P=0.097).

Mitochondrial respiration

As the inner membranes of the mitochondria are the ultimate sink for oxygen, we calculated the maximum rate of mitochondrial O_2 uptake of the tail muscles while rattling. Having determined the tail muscle mass-specific $\dot{V}_{O_2}$, the volume density of mitochondria in the tail and the surface area of the inner mitochondrial membranes, we multiplied the tail muscle mass-specific $\dot{V}_{O_2}$ by the percentage of mitochondria per mass of muscle, the area of inner mitochondrial membrane per volume of mitochondria and the number of molecules of O_2 per milliliter to calculate a passage of approximately 40 000 molecules O_2 m^{-2} s^{-1} at 30 °C (Fig. 5).

Discussion

The rattling frequency of 85 Hz at 35 °C is double the frequency of hummingbird flight muscle (40 Hz) operating at 40–43 °C (Hagiwara *et al.* 1968; Wells, 1990, 1993). Some insect muscles approach or exceed this value (Sotavalta, 1947; Elder, 1971; Josephson, 1973), as do the noise-making muscles of the swimbladders of some fish (Fawcett and Revel, 1961; Winn and Marshall, 1963), the remoter muscles of the lobster (Rosenbluth, 1969) and the cricothyroid muscle of the bat (Revel, 1962; Reger, 1978). However, unlike these muscles, the rattlesnake tailshaker muscles (1) operate aerobically, making noise continuously for periods of minutes to hours and (2) collectively may exceed 10 g in large snakes, requiring rapid, simultaneous transmission of motor neuron action potentials to a large muscle mass to synchronize the muscle contractions with millisecond timing.

The aerobic demand of rattling at 30 °C results in a rattling $\dot{V}_{O_2}$ that is equivalent in magnitude to the maximum oxygen uptake ($\dot{V}_{O_2max}$) measured during locomotion in what is likely to be the most actively moving of all crotalids, the sidewinder (Secor *et al.* 1992, and see Fig. 2), although unlike $\dot{V}_{O_2max}$, this level of oxygen uptake is sustainable. Tail muscle mass-specific $\dot{V}_{O_2}$ at 30 °C (muscle temperature), was 585±18 ml O_2 kg^{-1} min^{-1}, equal to or exceeding the maximum muscle mass-specific $\dot{V}_{O_2}$ reported for all but the most aerobic vertebrates. For example, this value approaches the maximum muscle mass-specific $\dot{V}_{O_2}$, calculated at 42 °C (muscle temperature), for the pronghorn antelope (666 ml O_2 kg^{-1} min^{-1}, Lindstedt *et al.* 1991), although it is significantly below that for hummingbird flight muscle (4362 ml O_2 kg^{-1} min^{-1}, Wells, 1990). To achieve high oxygen uptake and supply the ATP necessary for sustained performance, the volume density of mitochondria (26 %) may exceed that of any other reptilian muscle and equal that of some of the most aerobic mammalian muscles. Other muscles

capable of sustained, high-frequency repetitive contractions have similar or even higher mitochondrial densities, such as 30–35 % for hummingbird flight muscles (Wells, 1990; Zerbinatti *et al.* 1992) and 40 % for the stridulating muscles of the katydid *Neoconocephalus robustus* (Elder, 1971).

Despite the high aerobic capacity of these muscles, the actual cost per contraction is among the lowest for the vertebrates and invertebrates ($0.139\pm0.016\,\mu l\,O_2\,g^{-1}$) and is nearly constant across a broad range of temperatures. This suggests that the process of making noise in these animals does not vary in either efficiency or in the volume of muscle involved, only in the frequency of operation. The tailshaker muscles of the western diamondback rattlesnake seem to contain very few motor units, which contributes to the constant cost per contraction.

The EMG patterns at each temperature (see Fig. 1) appear as a series of single spikes rather than bursts of electrical activity, which suggests an unusual activation system. A single spike corresponding to each muscle contraction is consistent with a muscle composed of a single or very few motor units. Because the production of noise is not 'graded', as is force production during locomotion, for example, there may be very few motor units in all noise-making muscles. The muscles either make noise, which requires a certain cross-sectional area of myofibrils, or they are silent. At all rattling frequencies, the same muscle mass is required to produce sound; function in

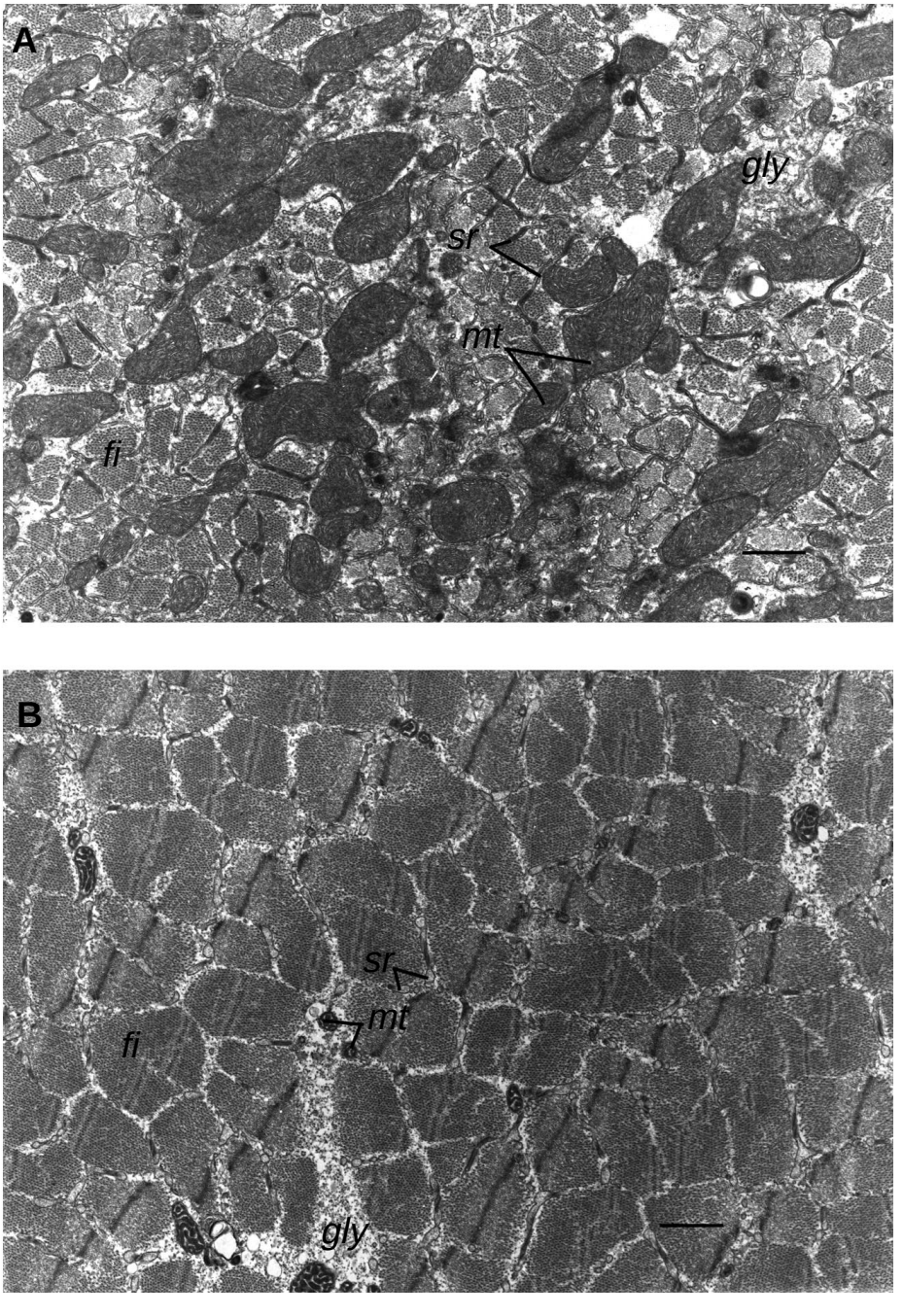

Fig. 4. Electron micrographs of the (A) tail and (B) body skeletal muscle cross sections of the western diamondback rattlesnake. Labeled structures include mitochondria (*mt*), sarcoplasmic reticulum (*sr*), myofibrils (*fi*) and glycogen (*gly*). Note the significantly larger mean myofibrillar area in the body skeletal muscle relative to the tail muscle which serves to minimize the diffusion distance from the sarcoplasmic reticulum to the crossbridges. Scale bars, 1 μm.

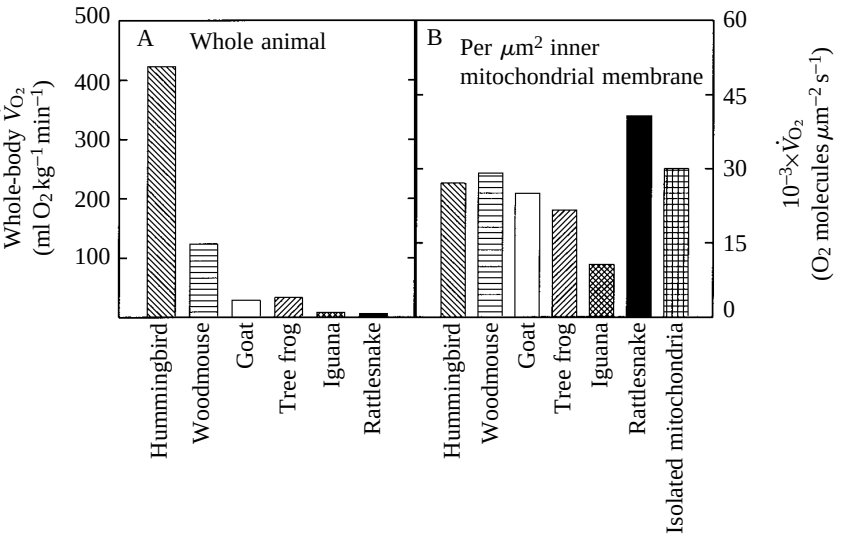

Fig. 5. Despite a mass-specific range of whole-body oxygen uptake of nearly two orders of magnitude (A), the maximum rate of oxygen uptake per unit of inner mitochondrial membrane area (B) is nearly constant when all data are normalized to a common temperature of 30 ˚C (using a Q_{10} of 2.2). The value given here for tree frogs is an estimate (based on data from Marsh and Taigen, 1987) assuming that the inner mitochondrial membrane density in these animals is equal to that of iguanas and rattlesnakes (25 $m^2\,cm^{-3}$). The low value for the iguanas may be explained in part by the apparently compromised ventilation that accompanies running in iguanas (Carrier, 1990). Hence, the value given here (from Conley *et al.* 1989) may be an underestimate. Remaining values have been taken from: woodmouse, Hoppeler *et al.* (1984); goat, Lindstedt and Thomas (1994); isolated cat mitochondria, Schwerzmann *et al.* (1989); hummingbird, Wells (1993) and Zerbinatti *et al.* (1992).

this muscle is essentially 'all-or-none'. Schultz *et al.* (1980), from horseradish peroxidase (HRP) labeling experiments using California red diamond and Wisconsin timber rattlesnakes, found the numbers of labeled neurons in their muscles to be exceptionally small. They assumed that many neurons must have been unlabeled, although they injected a sufficient quantity of HRP. We suggest that unlabeled neurons were not present, rather that each of the six muscles within the tailshaker complex may consist of a single motor unit. This interpretation is consistent with the EMG patterns and the constant amount of O_2 consumed per twitch with varying temperatures. However, if each muscle in the tail complex does consist of a single motor unit, this would be the only known vertebrate muscle of this type.

Fig. 1 also shows that the muscles on the right and left sides of the snake are out of phase, suggesting that the restoring force is provided by opposing muscle contractions. However, a weak but unmistakable rattling can be induced by simultaneously electrically stimulating the entire nerve bundle of motor neurons innervating the tail at the point where it leaves the vertebral column. Rapid (0.5 ms) high-voltage (70 V) stimulation at 50 Hz (the normal rattling frequency at 20 ˚C) of all the nerves results in a weak rattling noise.

During rattling, the oxygen directed to this one small muscle group is easily quantified, making it ideally suited to examine the oxygen uptake by skeletal muscle mitochondria. Among mammals, the maximum *in vivo* mitochondrial respiration rate is approximately constant at about 4–5 ml O_2 ml^{-1} mitochondria min^{-1}, despite enormous differences in aerobic capacity and 'life style' i.e. activity levels (Hoppeler and Lindstedt, 1985; Lindstedt *et al.* 1985, 1988). Because the density of inner mitochondrial membranes is nearly constant among mammals (Hoppeler and Lindstedt, 1985), there is a consistent (maximum) membrane-specific rate of oxygen uptake of 70 000 molecules O_2 m^{-2} s^{-1} at 40 ˚C (muscle temperature) in all mammals (Lindstedt and Thomas, 1994).

This relationship has been extended across other taxa, suggesting the presence of a design principle common to the skeletal muscle of all vertebrates, namely that ATP synthesis (oxygen utilization) may be a fixed function of the density of inner mitochondrial membrane (Rome and Lindstedt, 1996). However, inner mitochondrial membrane surface area is apparently not constant among all vertebrates. Our values for the rattlesnake (Fig. 3) are similar to values previously reported for the Cuban iguana (25 $m^2\,cm^{-3}$, Conley *et al.* 1989), but lower than the values reported for mammals (35 $m^2\,cm^{-3}$, Hoppeler and Lindstedt, 1985). Among birds, membrane density in pigeons (*Columba livia*) is similar to that in mammals (29 $m^2\,cm^{-3}$, James and Meek, 1979), but that of the hummingbirds is about double those of other tetrapods (58 $m^2\,cm^{-3}$, Zerbinatti *et al.* 1992). Given this structural variation in the mitochondrial oxygen sink, how much functional variation exists in mitocohondrial $\dot{V}_{O_2}$ among the vertebrates? We examined this question by comparing rates of mitochondrial oxygen uptake in birds (hummingbird flight muscle), mammals (woodmice and goats), amphibians (tree frogs) and reptiles (rattlesnake and iguana) (Fig. 5). Because the variation in muscle temperature in these studies (from 28 to 43 ˚C) will contribute significantly to variation in mitochondrial $\dot{V}_{O_2}$, we normalized values from these studies to a common operating temperature of 30 ˚C by applying a Q_{10} of 2.2 (Brooks *et al.* 1971). Among this diverse group of vertebrates, the mitochondrial $\dot{V}_{O_2}$ per unit inner membrane surface area differs fourfold, presumably within the range of error of our calculations. Despite whole-body rates of oxygen uptake varying by nearly two orders of magnitude, there is apparently a common maximum rate of oxygen uptake per unit area of inner mitochondrial membrane in animals as diverse as hummingbirds, woodmice, goats, tree frogs, iguana and rattlesnakes as well as in isolated mitochondria (Fig. 5). The degree to which the variations in rates of oxygen consumption from vertebrates of diverse phylogeny and ecology coincide argues very strongly for a common design in mitochondrial function and aerobic cellular organization (see Conley, 1994).

While mitochondrial volume density is high, perhaps the most obvious structural characteristic of these muscles is the exceptionally high volume density of SR. There are two apparent consequences of high SR density: (1) there are multiple sites for Ca^{2+} uptake, which is likely to ensure rapid muscle relaxation and (2) the distance between the SR and the crossbridges is minimized, ensuring simultaneous activation of the whole muscle. Thus, the combination of a very high density of SR coupled with small myofibrils is ideally suited for rapid activation and relaxation, probably criteria of all noise-making muscles. By cycling Ca^{2+} rapidly, the muscle can be activated nearly instantaneously, while the high density of SR also ensures that it relaxes rapidly to prepare for the next twitch.

In conclusion, rattling in rattlesnakes is accomplished by the high-frequency contraction of three pairs of muscles. Each muscle contracts with each tail movement cycle but the muscles on the left and right sides of the animal are out of phase; hence, the contraction of the three muscles on one side acts to restore the tail position for contraction of the muscles on the other side. While the oxygen uptake of this group of muscles is high, the cost per contraction is low and constant, irrespective of temperature and rattling frequency. Although the muscles have no unique structures, the proportions of structures present seem to be suited for rapid activation and relaxation as well as for a high rate of ATP production, but less so for high force production. The maximum synthetic capacity of the mitochondria, when expressed per unit area of inner mitochondrial membrane, is identical to that of other vertebrate skeletal muscle mitochondria. The one unique feature of these muscles may be that each of the six shaker muscles seems to be composed of a single motor unit; however, we suspect this may be a property common to all noise-making muscles.

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