THERMAL DEPENDENCE OF CONTRACTILE PROPERTIES OF RED AND WHITE FIBRES ISOLATED FROM THE ILIOFIBULARIS MUSCLE OF THE DESERT IGUANA (DIPSOSAURUS DORSALIS)

BY IAN A. JOHNSTON AND TODD T. GLEESON*

Department of Physiology, University of St Andrews, St Andrews, Fife KY16 9TS, Scotland, U.K.

Accepted 2 April 1984

SUMMARY

Single fibres were isolated from the 'red' and 'white' portions of the iliofibularis muscle from the desert iguana (Dipsosaurus dorsalis). Muscle fibres were chemically skinned with the non-ionic detergent Brij 58.

Maximal Ca$^{2+}$-activated tensions at 40°C were 32.3 ± 1.5 N cm$^{-2}$ for red and 33.0 ± 2.1 N cm$^{-2}$ for white muscle fibres (mean ± s.e. mean). Unloaded contraction velocities ($V_{\text{max}}$) were determined by the 'slack-test' method. Average values for $V_{\text{max}}$ at 40°C were 7.1 ± 0.4 and 16.1 ± 1.1 muscle length s$^{-1}$ ($L_0$ s$^{-1}$) for red and white fibres respectively. The red portion of the iliofibularis contained fibres with $V_{\text{max}}$ values between 1.1 and 12.9 $L_0$ s$^{-1}$.

Around 18% of the red fibre population had maximum contraction velocities less than 4.0 $L_0$ s$^{-1}$. These slower fibres were principally located in the region closest to the femur in situ. They probably correspond to the multiply-innervated 'tonic type' fibres that can be identified histochemically.

$Q_{10}$ values for $V_{\text{max}}$ over the range 5–15°C were higher for red (4.2) than white (2.3) muscle fibres. Above 25–30°C $Q_{10}$ values for $V_{\text{max}}$ were in the range 1.3–1.9 for both fibre types. Maximum Ca$^{2+}$-activated tensions were largely independent of temperature between 20 and 45°C. Dipsosaurus is maximally active in the field at 35–42°C, which corresponds to a zone of relative thermal independence of muscle contractile properties and locomotory performance.

INTRODUCION

The desert iguana is a herbivorous lizard native to the sandy desert regions of the southwestern United States and northern Mexico. During the daytime, when active in the field, it maintains body temperature within the range 40–42°C (Norris, 1953; DeWitt, 1967), warmer than the temperature in the burrow which remains at a constant 15–20°C during the spring (Norris, 1953; Porter, Mitchell, Bechman & DeWitt, 1973). Lower temperatures may be experienced in the winter (≤10°C), but the animals remain largely inactive (Norris, 1953).

* Usual address: Department of Environmental, Population and Organismic Biology, University of Colorado, Boulder, Colorado 80309, U.S.A.

Key words: Reptiles, contractile properties, skeletal muscle, temperature, Dipsosaurus dorsalis.
Reptiles have low stamina but are able to achieve high power outputs during short bursts of activity (Bennett, 1982). In general, effort greater than a slow walk is dependent on a well-developed capacity for anaerobic glycolysis (Bennett & Licht, 1972). A variety of studies have shown that aerobic energy production at temperatures below preferred body temperatures is much more thermally dependent than anaerobic glycolysis (Bennett, 1982). For example, at temperatures above 25°C maximal rates of lactate production are almost temperature independent for several lizard species \( (Q_{10} = 1.1-1.3) \) (Bennett & Licht, 1972). In contrast, experiments with *Dipsosaurus* on motor-driven treadmills have shown that oxygen transporting systems can only maintain sustainable walking speeds of 0.1 km h\(^{-1}\) at 20°C increasing to 0.3 km h\(^{-1}\) at 25°C and 0.8 km h\(^{-1}\) at 40°C \( (Q_{10} = 1.9) \) (John-Alder & Bennett, 1981). Thus, as body temperature falls, there is a proportionally greater decrease in the aerobic than anaerobic contributions to the total cost of the locomotion (Bennett, 1982).

Lizard limb muscles contain a mixture of red and white fibre types. The latter are focally innervated and correspond to the fast-twitch glycolytic fibres of other vertebrates (Proske & Vaughan, 1968; Gleeson, Putnam & Bennett, 1980). The status of reptilian red fibres is much less clear. Two kinds of red fibre may be distinguished on the basis of innervation and histochemical staining reactions for myofibrillar ATPase and succinic dehydrogenase activities (Gleeson *et al.* 1980; Gleeson, Nicol & Johnston, 1984). For the iliofibularis muscle from the desert iguana, capillary and mitochondrial volume densities are 629 mm\(^{-2}\) and 7.6%, respectively, for red muscle, and 73 mm\(^{-2}\) and 3.8% for white muscle (Gleeson, Nicol & Johnston, 1984). Morphological and contractile properties of white fibres suggest adaptation for anaerobically-supported burst activity, while red fibres are adapted for aerobically-supported activities such as postural support and slow movement.

It is clear that the different thermal properties of both energy producing systems and muscle contraction will impose constraints on both stamina and burst activity for animals such as *Dipsosaurus*, which experience daily changes in body temperature. The present study investigates the thermal dependence of contraction in red and white fibres isolated from the iliofibularis muscle of *Dipsosaurus* and attempts to relate the results to the effects of temperature on locomotory performance.

**MATERIALS AND METHODS**

Adult male desert iguanas (*Dipsosaurus dorsalis*, 45–58 g) were collected near Palm Springs, California during May 1983 (California Scientific Collecting Permit No. 0330). Lizards were transported to the U.K. (Department of the Environment Import Licence No. R4470) and maintained in the laboratory in glass aquaria. They were provided with a photothermal gradient on a photoperiodic regime of 16 h light: 8 h dark. Lizards had access to fresh water and were fed a diet of lettuce, carrots and dogfood three times a week.

Ilioibularis muscles were removed from the hindlimbs of freshly decapitated animals. Bundles of 50–100 fibres were dissected from the 'red' and 'white' portions of each muscle (Gleeson *et al.* 1980). Subsequent dissection was carried out in a 2 mm deep glass trough containing silicon oil (British Drug Houses MS 550). A small amount of relaxing solution (see below) was injected into the oil around the fibres.
Lizard muscle fibres

Single fibres were isolated using a binocular microscope and transferred to the apparatus using jewellers' forceps. The thin coating of silicon oil helps prevent dehydration during the 15–30 s required to transfer and mount the fibres.

Maximum contraction velocity was determined using the 'slack test' method (Hill, 1970). The apparatus and experimental protocol have been described in detail elsewhere (Johnston & Sidell, 1984). The skinned fibre chamber consists of a series of three water-jacketed baths which can be moved in <2 s to change the solution bathing the fibre. Fibres were mounted between two small glass hooks using Plexiglas acetone glue (Altringham & Johnston, 1982). One glass hook was rigidly attached to the silicon beam of an AE 801 strain gauge element (A.M.E. Horton, Norway). This was mounted on a one-way micromanipulator which allowed muscle length to be altered. The other hook was attached to a stout balsa beam glued to the central element of a loud-speaker movement from which most of the paper cone had been removed. The position of the beam was monitored using a photodiode-LED array and controlled by a servo-circuit utilizing velocity feedback (Johnston & Sidell, 1984). Temperature was controlled by circulating an antifreeze-water mixture through the chamber from a Grants Instruments (Cambridge) Flow heater-cooler.

Basic relaxing solution contained (mmol l⁻¹): 20 imidazole-HCl, 110 KCl, 3 MgCl₂, 5 EGTA [ethyleneglycolbis-(β-aminoethylether) N,N⁴-tetraacetic acid], 10 phosphocreatine, 2·5 ATP; pH 7·2 at 20°C. In all experiments, the final pH of solutions was adjusted to 7·2 at 20°C and allowed to vary freely with the temperature. Imidazole was chosen as the buffer since it has been shown to have a ΔpH/ΔT°C curve similar to that for both tissue and blood of a variety of reptiles (Reeves, 1977) including Dipsosaurus (Bickler, 1981). Activating solutions were made by addition of 4·0–5·0 mmol l⁻¹ CaCl₂ (1 M Volumetric solution, BDH Chemicals, Poole, England) to relaxing solution. Free calcium and other ionic concentrations were calculated using an iterative computer programme written in BBC Basic based on that described by Fabiato & Fabiato (1979). Correction was made for changes in ionic composition with both changes in pH and temperature. Calcium concentrations required to activate fibres maximally (0·8–3 μmol l⁻¹) were determined empirically for each temperature studied in a series of preliminary experiments. Free concentrations (μmol l⁻¹) of Mg and MgATP were in the range 0·50–0·54 and 2·21–2·35 respectively. Fibre segments 1·3–3 mm in length were mounted and skinned for 10 min in a 1 % solution of Brij 58 (polyoxyethylene 20 cetyl ether) dissolved in relaxing solution. They were subsequently transferred to a second bath containing relaxing solution, and sarcomere length was determined by laser diffraction. Initial sarcomere length was set to 2·3 μm. Fibres were subsequently transferred to activating solution and tension development monitored on a strip chart recorder (Fig. 1A). Following attainment of steady state tension, a series of quick releases (≤1 ms) were given of sufficient magnitude to abolish tension. The time taken to redevelop tension (ΔT) following each release was recorded using a Tetronix 5113 storage oscilloscope (Fig. 1B). Between each release (ΔL), fibres were re-extended to their original length (>15 ms). A typical record of muscle length and tension during a slack test experiment is shown in Fig. 1A,B. Usually slack or unloaded contraction velocity was determined from an average of four or five different length steps of increasing magnitude. Fibre diameter and length were measured in situ using a high power microscope (×100).
Fig. 1. (A) Typical record showing a maximal isometric activation of a single red fibre (44 μm diameter) from *Dipsosaurus* iliofibularis muscle. Arrows indicate transference of fibre from relaxing to activating solution (A) and vice versa (R). Vertical lines correspond to isotonic releases in B. (B) A record illustrating the method of determining maximum contraction velocity by the slack-test method. Increasing tension (P) is shown in the up direction and decreasing length (L) in the down direction. Fibre length was 2450 μm. The average time taken to redevelop tension (ΔT) following a step decrease in length (ΔL) was 3 ms. Increments between length steps were 50 μm. Unloaded contraction speed was equivalent to 16667 μm s⁻¹ or 6.7 muscle lengths (L₀ s⁻¹).

Experiments on the contractile properties of single red and white fibres were performed at 40 °C. Red fibres were dissected from the red portion of the iliofibularis. Small bundles of two to three red fibres and single white fibres were used in experiments on the effects of temperature on contraction velocity. A proportion of red...
Table 1. Contractile properties of single skinned fibres isolated from the ‘red’ and ‘white’ portions of iliofibularis muscles (40°C)

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>No. fibres</th>
<th>Maximum Ca²⁺-activated force (P_n, N cm⁻²) (Mean ± s.e.)</th>
<th>Maximum contraction velocity (V_∞) (muscle length s⁻¹) (Mean ± s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>37</td>
<td>32.5 ± 1.5</td>
<td>7.1 ± 0.4</td>
</tr>
<tr>
<td>White</td>
<td>16</td>
<td>33.0 ± 2.1</td>
<td>16.1 ± 1.1</td>
</tr>
</tbody>
</table>

fibres, from the region of the muscle closest to the femur in situ, had much lower contraction velocities than the average (see below). This region of the muscle was avoided for experiments on the effect of temperature.

RESULTS

Contractile properties of red and white muscles

Average diameters of red and white muscle fibres were 30–40 μm and 90 μm, respectively. Maximal Ca²⁺-activated tensions at 40°C were similar for red and white fibres (Table 1). The values obtained were within the range reported for mammalian muscle fibres at comparable temperatures but somewhat higher than for frog and fish fibres at their normal body temperatures (Hellman & Podolsky, 1969; Altringham & Johnston, 1982; Stephenson & Williams, 1981). At 40°C average values for unloaded contraction velocity were 2-3 times higher for white than for red muscles (Table 1). Contraction speeds for the ‘red’ portion of the iliofibularis muscle showed a continuous distribution from 1.5 to 12.9 muscle lengths s⁻¹ (Lo s⁻¹) (Fig. 2). A high proportion of fibres from the region closest to the femur in situ had contraction speeds less than 4.0 Lo s⁻¹. These fibres accounted for 18% of the total red fibre population.

Effects of temperature on contractile properties

Skinned muscle fibres isolated from the desert iguana were viable up to 45°C, showing no development of resting tension and complete relaxation following successive cycles of activation and relaxation. Maximal Ca²⁺-activated tension increased two-fold between 5 and 20°C and thereafter showed little change with increasing temperature (Fig. 3). The effects of temperature on slack velocity are shown in Fig. 4. Temperature coefficients were defined as:

\[ Q_{10} = \left( \frac{R_2}{R_1} \right)^{10/(T_2 - T_1)} \]

(where R₂ and R₁ are velocities at temperatures T₂ and T₁ respectively and T₂ > T₁) and were calculated on data from individual fibres. Q₁₀ values were in the range 1.5 to 2.5 over the range 15–35°C for both fibre types. Vₘₐₓ for muscle shortening below 15°C was more temperature dependent for red than for white fibres. For example, Vₘₐₓ (Lo s⁻¹) for fast glycolytic fibres increased from 1.5 ± 0.1 at 5°C to 3.5 ± 0.2 at 15°C (Q₁₀ = 2.33) compared with an increase from 0.53 ± 0.06 to 2.2 ± 0.3 (Q₁₀ = 4.15) for fast oxidative glycolytic fibres over the same temperature range. Above 40°C, Q₁₀ values declined somewhat, relative to 35–40°C (Table 2; Fig. 4).
DISCUSSION

The ‘red’ region of the iliofibularis muscle contained fibres with a wide spectrum of shortening speeds. Slower fibres occurred most frequently in the region closest to the femur *in situ*. It seems likely that these fibres correspond to a population whi

---

Fig. 2. Frequency distribution showing the range of maximum contraction speeds of 37 individual fibres isolated from the ‘red’ portion of the *Dipsosaurus* iliofibularis muscle.

Fig. 3. Effect of temperature on maximum Ca$^{2+}$-activated tension generation for single white (open circles) and bundles of two or three red fibres (closed circles) isolated from *Dipsosaurus* iliofibularis muscle.
can be distinguished histochemically on the basis of their alkaline labile myofibrillar ATPase activity (Gleeson et al. 1980) and multiple pattern of innervation (Gleeson et al. 1984). Fibres with an alkaline stable myofibrillar ATPase show high staining

Table 2. $Q_{10}$ for muscle contraction speed and locomotory activity for Dipsosaurus

<table>
<thead>
<tr>
<th>Temperature range (°C)</th>
<th>Muscle speed ($V_{max}$) ($L_0 s^{-1}$)</th>
<th>Burst speed (m min$^{-1}$)*</th>
<th>Distance running capacity (metres run in 6 min)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White fibres</td>
<td>Red fibres</td>
<td></td>
</tr>
<tr>
<td>5-15</td>
<td>2.3</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>15-25</td>
<td>2.1</td>
<td>2.5</td>
<td>4.2†</td>
</tr>
<tr>
<td>25-35</td>
<td>1.9</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>35-45†</td>
<td>1.3</td>
<td>1.5</td>
<td>1.2†</td>
</tr>
</tbody>
</table>

* Data from Bennet (1980).
† Calculated over the range 20-25°C. Animals performed poorly at temperatures below 20°C.
‡ $Q_{10}$ values on locomotory performance were determined over the range 40 to 43-44°C.
activities for aerobic and glycolytic enzyme markers and comprise the remaining 80% of the red portion of the iliofibularis (Gleeson et al. 1980). These fibres are focally innervated, as are the white fibres, which are characterized by a low staining intensity for mitochondrial enzymes (Gleeson et al. 1984). The red portion of the iliofibularis differs from the white with respect to its sensitivity to acetylcholine and its resistance to fatigue following repetitive stimulation (Gleeson et al. 1980). The three main fibre types in *Dipsosaurus* iliofibularis muscle probably correspond to the tonic, fast oxidative glycolytic and fast glycolytic fibre types of other vertebrate classes. Multiply innervated fibres in *Dipsosaurus* and other lizards (Proske & Vaughan, 1968; Finol & Ogura, 1977; Guthe, 1981) differ from those of amphibians with respect to their capacity for aerobic metabolism. For example, frog tonic fibres only show a weak staining reaction for mitochondrial enzymes (Smith & Ovalle, 1973; Spurway, 1980, 1983). In this respect, reptilian slow fibres resemble more closely the multiply innervated myotomal muscles of fish (Johnston, 1981). Red fibre types in *Dipsosaurus* are well adapted for sustained force generation. Tonic fibres may be responsible for postural support and slow movements, such as accompany changes in body attitude during courtship and territorial defence behaviour, whilst fast oxidative glycolytic fibres may be recruited during walking and at sustained running speeds.

Q\textsubscript{10} values for muscle shortening speed are similar for red and white muscles except at temperatures below 15°C (Table 2). This is below the temperature at which these animals are normally active in the field (Porter et al. 1973). Below 25°C, the Q\textsubscript{10} for muscle V\textsubscript{max} is substantially less than for either burst speed or distance running capacity (Table 2). The data suggest that contraction speed does not limit limb cycle times and/or performance over the lower range of temperatures at which *Dipsosaurus* are active. In contrast, the Q\textsubscript{10} values for muscle V\textsubscript{max} over the range 25-35°C parallel those obtained for locomotory performance (Table 2). The Q\textsubscript{10} values for anaerobic and aerobic metabolism at these body temperatures are 1.2 and 3.7 respectively (Bennett & Dawson, 1972). It is of interest that the Q\textsubscript{10}(25-35°C) for contraction velocity of red iliofibularis fibres is more closely matched to anaerobic than aerobic energy production. It seems likely that at fast walking speeds, the ATP demand of the fast oxidative glycolytic fibres is largely met by anaerobic pathways. Over the range 35-40°C, the thermal dependence of anaerobic and aerobic scopes for activity is more similar and matches that for muscle V\textsubscript{max} (Table 2; Bennett & Dawson, 1972). Above 40°C, running ability declines, suggesting that other factors, such as reduced metabolic scope may limit activity capacity (Bennett, 1980). Maximum isometric tensions (P\textsubscript{0}) show a degree of thermal independence over the entire range of temperatures at which *Dipsosaurus* is active in the field (Fig. 3). Similar results have been obtained for skinned fibre preparations isolated from fish adapted to different temperatures (Johnston & Brill, 1984) and for tetanic and twitch tension development for various lizard muscles (Morris, 1982; Putnam & Bennett, 1982). It should be noted that burst running speeds recorded in the field are much higher than under laboratory situations where motivation and stress may reduce performance (Belkin, 1961; Bennett, 1980). It is of interest, however, that Bennett (1982) found that laboratory behaviour was repeatable among groups of animals within a species, within a group during the experiments, and among individuals of a group.

Muscle contraction speeds for fast glycolytic fibres of *Dipsosaurus* are similar to...
Lizard muscle fibres

those of mammalian fast muscles at comparable temperatures (Close, 1972; Stephen-son & Williams, 1981). \( V_{\text{max}} \) of *Dipsosaurus* white fibres at 5°C is similar to that of the sartorius muscle of cold-temperate frogs (Julian, 1971) suggesting only a limited degree of temperature compensation in this parameter between species. There are, however, significant differences in the effects of temperature on maximum tension development and Ca-regulation of contraction between species (Johnston & Brill, 1984). For example, skinned fibres from cold-water fish fail to relax completely following activations in excess of 15°C, while fibres from the antarctic fish *Notothenia neglecta* contract in relaxing solutions (pCa 7.4) at temperatures above 20–22°C independently of free Ca concentrations (Johnston & Brill, 1984). \( \text{Ca}^{2+} \)-sensitivity of rabbit myofibrils is lost at temperatures slightly above upper lethal core temperatures (41°C; Fuchs, Hartshorne & Barns, 1975). In contrast, fibres from the desert iguana showed stable regulation of contraction over the whole range of temperatures from 5 to 45°C.

TG gratefully acknowledges support from N.S.F. Grant P.C.M. 8202432.

REFERENCES


