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CONTRACTION PARAMETERS, MYOSIN COMPOSITION AND METABOLIC ENZYMES OF THE SKELETAL MUSCLES OF THE ETRUSCAN SHREW *SUNCUS ETRUSCU*S AND OF THE COMMON EUROPEAN WHITE-TOOTHED SHREW *CROCIDURA RUSSULA* (INSECTIVORA: SORICIDAE)

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Summary

In the Etruscan shrew, the isometric twitch contraction times of extensor digitorum longus (EDL) and soleus muscles are shorter than in any other mammal, allowing these muscles to contract at outstandingly high contraction frequencies. This species has the highest mass-specific metabolic rate of all mammals and requires fast skeletal muscles not only for locomotion but also for effective heat production and for an extremely high ventilation rate. No differences could be detected in the fibre type pattern, the myosin heavy and light chain composition, or in the activity of the metabolic enzymes lactate dehydrogenase and citrate synthase of the two limb muscles, the EDL and the soleus, which in larger mammalian species exhibit distinct differences in contractile proteins and metabolic enzymes. All properties determined in EDL and soleus muscles of *Suncus etruscus*, as well as in the larger *Crocidura russula*, are typical for fast-oxidative fibres, and the same holds for several other skeletal muscles including the diaphragm muscle of *S. etruscus*. Nevertheless, the EDL and soleus muscles showed different mechanical properties in the two shrew species. Relaxation times and, in *C. russula*, time to peak force are shorter in the EDL than in the soleus muscle. This is in accordance with the time course of the Ca^{2+} transients in these muscles. Such a result could be due to different parvalbumin concentrations, to a different volume fraction of the sarcoplasmic reticulum in the two muscles or to different Ca^{2+}-ATPase activities. Alternatively, the lower content of cytosolic creatine kinase (CK) in the soleus compared with the EDL muscle could indicate that the observed difference in contraction times between these shrew muscles is due to the CK-controlled activity of their sarcoplasmic reticulum Ca^{2+}-ATPase.

Key words: shrew, *Suncus etruscus*, *Crocidura russula*, skeletal muscle, extensor digitorum longus, soleus, myosin heavy chain, myosin light chain, lactate dehydrogenase, citrate synthase, creatine kinase, myoglobin, parvalbumin, Ca^{2+} transient, time to peak of force, relaxation, myosin ATPase, fibre composition.

Introduction

The Etruscan shrew *Suncus etruscus* is one of the smallest mammals, with adult body masses of wild individuals of approximately 1.8 g. In this species, an ability to contract at extremely high speed and frequency is exhibited by the heart and skeletal muscles. The mass-specific metabolic rate of the Etruscan shrew exceeds that of all other mammals (Fons and Sicart, 1976; Weibel et al., 1971) and, to achieve sufficient oxygen transport in the body, it exhibits heart rates of up to 1500 min^{-1} and respiratory rates as high as 900 min^{-1} (Jürgens et al., 1996). From an allometric relationship, a stride frequency at the maximal sustained running speed of 780 min^{-1} is predicted for the Etruscan shrew (Heglund and Taylor, 1988). Moreover, shivering at high frequencies is an important mechanism for thermogenesis in this species during cold stress and rewarming from torpor. Fons et al. (1997) observed very high skeletal muscle contraction rates in *S. etruscus* during rewarming from torpor which, in combination with heat production by brown adipose tissue, generated warm-up rates of up to 1 °C min^{-1}. In the common European white-toothed shrew *Crocidura russula* (mean body mass 8.6 g), ventilatory rates of 500 min^{-1} (Nagel, 1991) have been measured, and the maximal stride frequency is estimated to be 620 min^{-1}. During cold tremor, electromyogram (EMG) frequencies of grouped discharges of up to 3500 min^{-1} have been recorded in this species (Kleinebeckel et al., 1994).

In the present study, we investigated the characteristics of twitch and tetanic contractions of skeletal muscles in *S. etruscus* and in the somewhat larger shrew species *C. russula*. Furthermore, we looked for the properties of the muscle fibres

rendering these high contraction frequencies possible. To detect whether there might be functional differences between different muscles within the shrew species, we selected the soleus and the extensor digitorum longus (EDL) muscles of the hindlimb for our measurements. In larger mammals, these muscles are typical slow- and fast-twitch muscles, respectively.

The mechanical properties of muscles are generally correlated with their myosin chain compositions. In larger mammals, the myosins of the fast-twitch EDL muscle are predominantly composed of type II myosin heavy chains (MHCs), whereas in the slow-twitch soleus muscle the myosins consist mainly of MHC type I. To determine the myosin composition of these muscles in the shrews, we determined the staining pattern of myosin ATPases of these muscles and, in addition, used immunohistochemistry to characterize the MHC type. Electrophoretic techniques were used to differentiate further between the subtypes of myosin heavy and light chains.

In addition to the myosin composition of the muscle, the contractile characteristics are also determined by the rate at which Ca^{2+} is released from the sarcoplasmic reticulum and subsequently resequestered. Thus, we not only measured the mechanical properties of EDL and soleus muscles of *S. etruscus*, but also recorded the time course of the change in sarcoplasmic Ca^{2+} concentration during the contraction–relaxation cycle of the two muscles using the Ca^{2+}-sensitive dye FURA2.

To characterize further the muscle properties, we also measured the activities of two metabolic enzymes: lactate dehydrogenase (LDH), which is associated with glycolytic metabolism and occurs in high concentrations in fast glycolytic fibres; and citrate synthase (CS), an enzyme associated with aerobic metabolism, which is observed in high concentrations in slow oxidative fibres. The LDH/CS activity ratio, which varies systematically with body size (Emmett and Hochachka, 1981), was calculated for the two shrews and compared allometrically with that of larger mammals.

During short-term high work loads, the maintenance of a constant cytoplasmic ATP level in the muscle requires a high activity of CK, which restores ATP levels from the phosphocreatine pool. Therefore, we additionally measured the content of creatine kinase (CK) in the EDL and soleus muscles of *S. etruscus*.

We compared the mechanical and biochemical characteristics of the muscles from the two shrew species with data from larger mammalian species to look for a scaling of muscle contraction time and muscle fibre composition with adult body mass of the species.

Some of the biochemical parameters were determined not only in the EDL and soleus muscles but also in other skeletal muscles, such as the diaphragm and gastrocnemius muscles of the shrews.

Materials and methods

Animals and muscles

Experiments were carried out using skeletal muscles from the shrew species *Suncus etruscus* (Savi) and *Crocidura russula* (Hermann). Adult animals were caught in Southern France in the area around Banyuls-sur-Mer during the summer and housed in a terrarium at room temperature. The shrews were fed with mealworms and crickets and had access to water *ad libitum*.

Before preparation of the muscles, the animals were killed by inhaling an overdose of halothane (Halothan; Hoechst). Several skeletal muscles, including the soleus, EDL, gastrocnemius and diaphragm, were dissected from the animal under a microscope using microsurgery instruments. During the dissection, the tissue was superfused with carbogen-equilibrated modified Krebs–Henseleit solution (120 mmol l^{-1} NaCl, 3.3 mmol l^{-1} KCl, 1.2 mmol l^{-1} $MgSO_4$, 1.2 mmol l^{-1} KH_2PO_4, 1.3 mmol l^{-1} $CaCl_2$ and 25 mmol l^{-1} $NaHCO_3$, pH 7.4) at room temperature (21–27 °C) to prevent desiccation and hypoxic damage. For biochemical studies, the dissected muscles were transferred to Eppendorf tubes, frozen by immersion in liquid nitrogen and subsequently stored in a deep freeze at −80 °C for future use.

Contraction measurements

The contractile characteristics of the soleus and EDL muscles were measured at room temperature. Since the room temperature varied between 21 and 27 °C over the period during which the experiments were carried out, we corrected all measured data to 25 °C assuming a Q_{10} of 2.5. This is the mean of the Q_{10} range determined for fast-twitch muscles of mice over a similar temperature range (Stein et al., 1982; Asmussen and Gaunitz, 1989). The value was applied as an approximation since Q_{10} values have not been reported for shrew muscles.

For the contraction measurements, one end of the muscle was fastened to the measuring chamber using surgical thread (20–30 μm diameter), and the other end was tied to a tiny platinum ring using the same kind of thread. The ring was used to connect the muscle to the hook of the force transducer. Because of the very small forces exerted by the shrew muscles (<3 mN), elongations of thread and knots during muscle contractions were negligible. The muscles were completely submerged in the measuring chamber fluid (carbogen-equilibrated Krebs–Henseleit solution) at room temperature. The bath solution was continuously pumped through the chamber, ensuring an oxygen partial pressure high enough for sufficient oxygen supply to the muscle. The muscles were pre-stretched by using a micrometer screw to a degree at which maximal force was generated following a supramaximal electrical stimulus of 1 ms duration. The electrical stimulation was applied *via* two platinum electrodes which were approximately 0.5 mm in diameter each and which were immersed in the bath solution opposite each other at either side of the muscle. The electrodes were placed at a distance of approximately 1 mm from the muscle surface.

Commercially available force transducers were not appropriate for measuring twitch contractions of the small shrew muscles because of insufficient sensitivity and/or low limiting frequencies. We therefore developed a force transducer

based on the semiconductor sensor device AE 802 (SensoNor a.s., Horten, Norway), which consists of a relatively stiff silicon strip (1 mm×5 mm, 0.1 mm thick), clamped on one side to a rod-shaped contact holder. The silicon strip on each side contains an ion-implanted resistor (basic resistance 1.2 kΩ). The two resistances change in opposite directions upon bending of the strip, which is used in a Wheatstone bridge configuration to transform the strain produced by the applied force into voltage. The sensitivity of the force transducer was increased by extending the free end of the silicon beam by another 5 mm with an L-shaped hook formed from a glass capillary of 0.4 mm outer diameter and glued to the silicon with a two-component glue (UHU plus endfest 300). This glass hook had the additional advantage of electrically insulating the transducer from the organ bath solution when its tip, holding the muscle, was submerged. Because of the stiffnesses of the silicon strip and the glass extension, the maximal movement of the transducer tip was only 3 µm at the maximally applied force of 3 mN. Since the muscles are approximately 2 mm long, the device is appropriate for recording isometric contractions. Calibration of the transducer revealed a linear force–voltage relationship (r^2=0.96) in the range 0–3 mN. No mechanical oscillations due to resonance phenomena occurred during the recordings of shrew muscle contractions with times to peak force of between 7 and 10 ms. Without modifications, the silicon strip has a natural frequency of 12 kHz and responds to frequencies up to 5 kHz. With our modifications, the frequency response of the force transducer is approximately linear up to at least 500 Hz.

The voltage of the Wheatstone bridge was amplified and recorded with a LINSEIS L1000 chart recorder. Its frequency response exhibits a magnitude decrease of 1 dB between 0 and 50 Hz at the maximal pen deflection used of 30 mm, and of 3 dB at 100 Hz for a pen deflection of 10 mm. The recordings were made at a paper speed of 500 mm s^{-1}, and the contraction parameters were measured directly from the paper of the chart recorder. Fig. 1 shows a digital scan of a typical original recording, the measured contraction parameters are explained in the legend to this figure.

Using the same experimental arrangement, we also investigated the properties of the EDL and soleus muscles during repetitive stimulations. Bursts of stimuli lasting 100–200 ms were delivered to the muscle. The stimulation frequency was increased in steps of 5 Hz between 0 and 50 Hz and in steps of 50 Hz between 50 and 300 Hz. We determined the stimulation frequency at which force summation occurred and at which a smooth tetanic contraction was reached. In addition, we measured the maximum rate of force development [d(F/F_{max})/dt] and the maximal tetanic force (F_{max}).

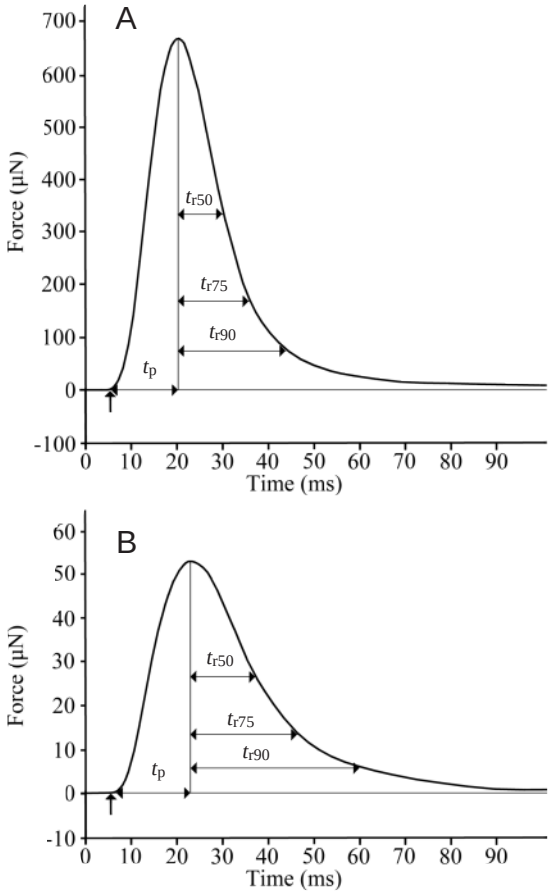

Fig. 1. Examples of the typical time course of the force during an isometric twitch contraction at 21 °C of (A) an extensor digitorum longus (EDL) and (B) a soleus muscles of *Suncus etruscus* stimulated simultaneously with a supramaximal electrical pulse of 1 ms duration. The curves are digital scans of original recordings. Time to peak (t_p) force is the time between stimulus onset (↑) and the attainment of maximal force; the 50, 75 and 90 % relaxation times (t_{r50}, t_{r75}, t_{r90}, respectively) are the times for the decay of the force from its maximum to 50, 25 and 10 %, respectively, of the maximum.

Ca^{2+} transients

Muscle fibre bundles containing 15–30 fibres were prepared from one EDL muscle and one soleus muscle and mounted in the measuring chamber described above. Intracellular Ca^{2+} transients of single twitches were measured fluorometrically using a microscope photometric apparatus and the fluorescent dye FURA2. The fluorescence intensity of this dye depends on the concentration of free Ca^{2+}. FURA2 was excited by light of wavelengths 340 and 380 nm. The emitted light was passed through a 500–530 nm bandpass filter, and the light intensity was measured using a photomultiplier. Measurements were made at several locations on each fibre bundle. The force was recorded using the transducer described above. The force and fluorescence signals were sampled at a rate of 1 kHz and stored digitally on a personal computer. Fig. 2 is an example of a plot of the force transient and the calculated ratio of the fluorescence intensities ($R_{340/380}$), which is a measure of the intracellular Ca^{2+} concentration. The FURA2 technique is described in detail by Wetzel and Gros (1998).

Electrophoresis

Electrophoresis of myosin heavy chain isoforms was carried out for 18 h at 4 °C according to the method of Kubis and Gros (1997) with the following minor modifications. The slab gels

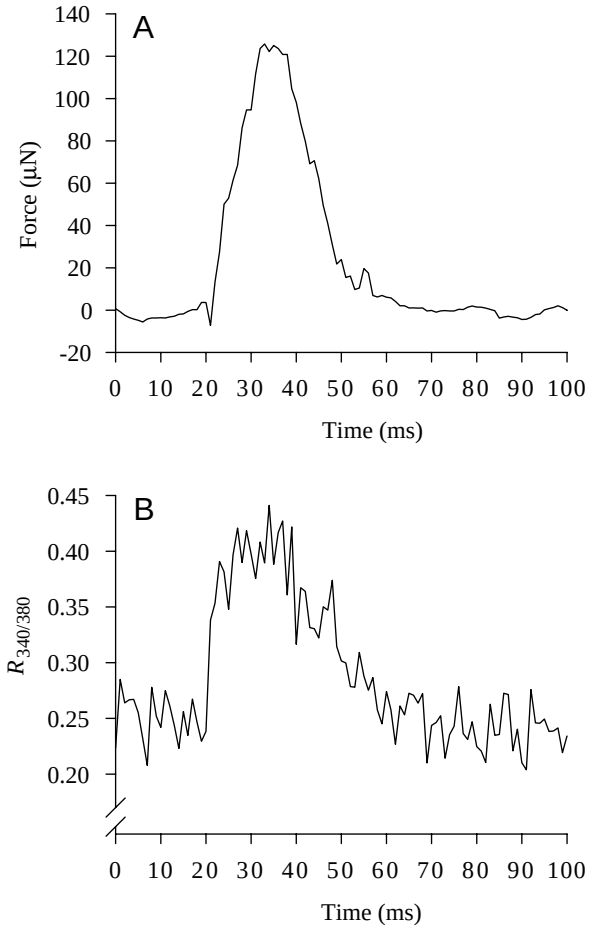

Fig. 2. Typical traces of the force signal (A) and the corresponding FURA2 signal (B) recorded during an isometric twitch contraction at 22 °C of an electrically stimulated (1 ms pulse) muscle fibre bundle of the extensor digitorum longus (EDL) muscle of *Suncus etruscus*. The signals were sampled at a rate of 1 kHz, stored and processed on a personal computer. $R_{340/380}$, the ratio of the FURA2 fluorescence intensities at the excitation wavelengths 340 and 380 nm, is a measure of the intracellular Ca^{2+} concentration. The curves are means of three consecutive recordings.

were 1 mm thick, 25 cm long and 16 cm wide. Running conditions were 14 mA and 450 V. Two-dimensional electrophoresis of myosin light chains was performed using the method of O'Farrell (1975). Proteins of the muscle homogenate supernatants were separated by SDS–PAGE using slabs with a 5 % stacking and a 15 % separating gel and visualized by silver staining using the method of Heukeshoven and Dernick (1985).

Histochemical fibre differentiation

Because the EDL and soleus muscles of the Etruscan shrew are extremely small (weighing less than 0.5 mg), they were inserted into a slit cut into liver tissue before freezing in liquid-nitrogen-cooled propane. This procedure facilitated handling and sectioning of the muscles in the cryomicrotome. Muscle fibre type identification was performed as follows. Acid-labile myosin ATPase (typical of type II fibres) was inhibited by preincubating the sections for 5 min in 0.2 mol l^{-1} barbital acetate buffer (pH 4.0), alkaline-labile myosin ATPase (typical of type I fibres) was inhibited by preincubating the sections for 5 min in 0.1 mol l^{-1} barbital buffer (pH 10.5) at 22 °C (Brooke and Kaiser, 1970). Subsequent staining for ATPase was performed using the standard calcium method (Padykula and Herman, 1955).

Immunohistochemistry

For immunohistochemical studies, complete muscles were dissected from the shrew's legs after determination of their *in situ* length using dividers at a moderate plantarflexion of the foot. Excised muscles were re-stretched and fixed at their *in situ* length in a mixture of methanol, acetone, acetic acid and water (35:35:5:25, v/v) at 22 °C for 3 h. The fixed muscles were dehydrated, embedded in Paraplast (Sigma, Germany) and cut in serial sections 5 μm thick. Monoclonal antibodies raised in mice and directed against fast-type myosin heavy chain (MHC) isoforms (340-3B5, anti-MHC IIa/IIb, antigen from rabbit tibialis anterior muscle) and a slow-type MHC (219-1D1, anti-MHC I, antigen from adult chicken heart) were used. These antibodies have been shown to bind to the corresponding MHC isoforms of rat, guinea pig, sheep, pig, coypu (*Myocastor coypus*) and man and not to cross-react with other muscle proteins (for specificity, see Bredman et al., 1992). To detect sites in the tissue where antibodies bind to the appropriate antigens, the peroxidase/antiperoxidase (PAP) technique was applied (Wessels et al., 1991). In brief, serial sections were incubated overnight with the primary antibodies at 22 °C followed by incubation with goat anti-mouse IgG (90 min), donkey anti-goat IgG (90 min) and goat PAP complex (90 min). The visualization was performed by incubation with 3,3′-diaminobenzidine. After dehydration, sections were mounted in Entellan (Merck, Germany).

A polyclonal antibody against parvalbumin raised in sheep (antigen from rabbit tibialis anterior muscle) was used. This antibody has been shown not to bind to any protein of muscle extracts other than parvalbumin (Leberer and Pette, 1986; Schmitt and Pette, 1991). A peroxidase-labelled horse anti-sheep antibody (60 min, 22 °C) was applied to serial sections after incubation with anti-parvalbumin IgG (60 min). Visualization was performed by incubation with 3,3′-diaminobenzidine. A polyclonal antibody against cytosolic muscle creatine kinase (CK) (rabbit anti-chicken MM-CK) was used. The cytosolic protein fractions were separated electrophoretically and immunoblotted on nitrocellulose membranes. Visualization was performed by coupling of peroxidase-labelled anti-rabbit IgG and incubation with 3,3′-diaminobenzidine.

Muscle homogenization

Muscle homogenates were prepared by disrupting frozen muscles with tungsten carbide balls (5 mm in diameter) in a rapidly shaking chamber. For this purpose, small and stable vessels appropriate for treatment of small sample volumes and for transfer into the ultracentrifuge were manufactured from Teflon. The disruption procedure was performed using a

Mikro-Dismembrator (Braun, Melsungen) in a room cooled to –20 °C. After removing the balls, the vessels containing the homogenate were centrifuged at 70 000 ***g*** in an ultracentrifuge (Kontron TGA-50). Supernatants and pellets were frozen and stored at –80 °C. Homogenates were prepared exclusively from freshly frozen muscles which had not been used previously for other experiments. To obtain a muscle mass large enough for our biochemical studies, the pooled EDL and soleus muscles of nine *S. etruscus* and 3–6 *C. russula* were used for homogenization.

Myosin preparation

Myosin was prepared by a procedure modified from Rushbrook and Stracher (1979). Muscle samples were homogenized in 50 mmol l^{-1} Tris/HCl, pH 8.5 at 0 °C. After centrifugation at 20 000 ***g*** for 20 min, pellets were extracted with 0.6 mol l^{-1} KCl, 1 mmol l^{-1} EGTA, 0.5 mmol l^{-1} dithiothreitol (DTT), 0.5 mmol l^{-1} phenylmethyl-sulphonylfluoride (PMSF), 10 mmol l^{-1} potassium phosphate, pH 6.8 at 0 °C. After a further centrifugation at 20 000 ***g*** (30 min), the supernatants were diluted to 60 mmol l^{-1} KCl with ice-cold water, and precipitated actomyosin was resuspended in 2 mol l^{-1} KCl, 0.2 mol l^{-1} potassium phosphate, pH 6.8. Suspensions were centrifuged at 20 000 ***g*** for 20 min. Supernatants were diluted into 0.6 mol l^{-1} KCl, and ATP, $MgCl_2$, EGTA and DTT were then added to give final concentrations of 5 mmol l^{-1} ATP, 5 mmol l^{-1} $MgCl_2$, 1 mmol l^{-1} EGTA and 1 mmol l^{-1} DTT. The solutions were centrifuged at 160 000 ***g*** for 2 h, and myosin in the supernatant was separated from the F-actin pellet. Myosin was dialysed against 0.6 mol l^{-1} KCl, 10 mmol l^{-1} Mops, 1 mmol l^{-1} EGTA, 1 mmol l^{-1} DTT and stored at –20 °C after addition of glycerol to 50 % (v/v). F-actin was redissolved in the same buffer and dialysed at 4 °C.

Measurement of myosin ATPase activity

Myosin filaments were reconstituted for 3 h at 4 °C by diluting the myosin preparations 1:20 with ice-cold water and adding $CaCl_2$ to a final concentration of 0.2 mmol l^{-1} and Triton X-100 to a final concentration of 0.05 %. After centrifugation at 2000 ***g*** for 10 min, the pellets were resuspended in 50 mmol l^{-1} imidazole, 6 mmol l^{-1} KCl, 4 mmol l^{-1} $MgCl_2$, pH 7.4. After addition of F-actin (20 µg of F-actin to 10 µg of myosin filaments), samples were diluted to a final volume of 500 µl with 50 mmol l^{-1} imidazole, 6 mmol l^{-1} KCl, 4 mmol l^{-1} $MgCl_2$, 1 mmol l^{-1} $CaCl_2$, pH 7.4, and the ATPase reaction was started by the addition of ATP to a final concentration of 4 mmol l^{-1} at 30 °C. The reaction was stopped with 500 µl of 0.6 mol l^{-1} perchloric acid after 30 min, and the supernatants were used for inorganic phosphate (P_i) measurement after centrifugation at 20 000 ***g*** for 10 min. Determination of [P_i] was carried out using the method of Kodama et al. (1986). Protein was determined by the procedure of Lowry et al. (1951).

Metabolic enzymes and myoglobin

Measurement of lactate dehydrogenase (LDH) activity was performed according to Bernstein and Everse (1975), and the activity of citrate synthase (CS) was determined using the method described by Bass et al. (1969). In both assays, the change in NADH concentration was followed at 340 nm and 30 °C.

The concentration of myoglobin was measured according to the method of Reynafarje (1963). Homogenates of pooled muscles were used for the measurements of enzyme activities and myoglobin concentration (see Table 4).

Results

Times to peak force, relaxation times and Ca^{2+} transients

Table 1 shows time to peak force (t_p) and relaxation times (t_{r50}, t_{r75} and t_{r90}) of EDL and soleus muscles from the two shrew species. In *S. etruscus*, both muscles show a rapid time to peak force, with no significant difference between the soleus and the EDL (Table 1), and in both muscles t_p is shorter than measured in any other mammalian species (Table 2). Relaxation times, which are also shorter than reported for muscles of larger mammals, are significantly longer in soleus than in EDL muscles (Table 1). The difference between the two muscles is a little more pronounced in *C. russula*, in which time to peak force is also significantly longer in the soleus muscle than in the EDL muscle. Times to peak force at 25 °C are 11 ms in the EDL muscle and 13 ms in the soleus muscle of *S. etruscus*, which gives times to peak force of 3.7 and 4.3 ms, respectively, at a body temperature of 37 °C applying at Q_{10} of 2.5. At body temperature, a twitch contraction cycle (t_p+t_{r90}) is completed within 13 ms in the EDL muscle and within 18 ms in the soleus muscle. In the fourfold larger shrew *C. russula*, the corresponding times are 15 ms and 24 ms for the EDL and soleus muscles, respectively.

For *S. etruscus*, such short contraction times are confirmed by contraction measurements performed on muscle fibre bundles prepared from EDL and soleus muscles (Table 3). In contrast to the whole muscles of *S. etruscus*, both relaxation time and time to peak force were found to be significantly longer in the fibre bundle from the soleus muscle than in the fibre bundle from the EDL muscle. The significant difference in t_p between fibre bundles of the EDL muscle and of the soleus muscle is probably due to the fact that the variation of this parameter within one muscle bundle, i.e. between different fibres of the bundle, is relatively small compared with the variation of this parameter between muscles of different individuals. Like the force relaxation transient, the corresponding Ca^{2+} transient is significantly longer in the soleus than in the EDL muscle fibre bundle.

In both muscles, the Ca^{2+} signal had smaller t_p and larger t_r values than the force signal, i.e. the shape of the force signal differs from that of the Ca^{2+} signal. The characteristic of longer relaxation times of Ca^{2+} transients compared with force transients has been observed previously in the EDL muscles of the rat and mouse. It is typical for fast muscle fibres characterized by a right-shifted steep pCa/force relationship, i.e. the force decreases rapidly with decreasing Ca^{2+}

Table 1. *Characteristics of isometric twitches of whole extensor digitorum longus and soleus muscles from two species of shrew at 25 °C*

Species	Body mass (g)	Muscle mass (mg)	t_p (ms)	t_{r50} (ms)	t_{r75} (ms)	t_{r90} (ms)
Suncus etruscus	2.2±0.3 (*N*=12)	EDL (*N*=6) 0.45±0.08	11.0±1.1	11.6±2.6	16.7±2.9	27.0±3.8
		Soleus (*N*=5) 0.37±0.04	12.7±2.3^{NS}	17.3±4.6*	27.7±6.5‡	42.2±10.8‡
Crocidura russula	8.6±1.4 (*N*=6)	EDL (*N*=6) 1.9±0.2	14.2±1.2	11.1±2.0	17.9±4.3	31.2±8.5
		Soleus (*N*=5) 2.3±0.4	17.9±0.9‡	22.5±3.5‡	36.4±4.9‡	55.3±7.8‡

Data represent mean ± S.D. for (*N*) animals or muscles, respectively.

t_p, time to peak force; t_{r50}, t_{r75}, t_{r90}, 50, 75, 90 % relaxation times, respectively.

Significance levels are calculated for the difference between extensor digitorum longus (EDL) and soleus muscles within the two species: NS, not significant; *P<0.05; ‡P<0.01 (unpaired t-test).

Table 2. *Comparison of times to peak twitch force of extensor digitorum longus and soleus muscles from a variety of different species*

		EDL		Soleus		
Species	Mean body mass (g)	t_p (ms)	Fast-twitch/ slow-twitch fibres (%)	t_p (ms)	Fast-twitch/ slow-twitch fibres (%)	Reference
Suncus etruscus	2.2	11	100/0	13	100/0	This study
Crocidura russula	8.6	14	100/0	18	100/0	This study
White mouse	35	14	100/0	41	54/46	Asmussen and Gaunitz (1989)
Young Wistar rat	120	20	95/5	48	30/70	Asmussen and Gaunitz (1989)
Adult Wistar rat	200	27[a]	97/3	80[a]	0/100	Geers and Gros (1990)
Guinea pig	350	20	94/6	94	0/100	Asmussen and Gaunitz (1989)

The fibre type composition of the muscles is also indicated. Twitches were isometric.

EDL, extensor digitorum longus; t_p, time to peak force.

[a]Value converted to hold for 25 °C using a Q_{10} value obtained from Asmussen and Gaunitz (1989).

concentration, and the sensitivity of the filaments towards Ca^{2+} is so low that the active force drops to zero before the Ca^{2+} concentration reaches its steady state. This phenomenon is discussed in detail by Wetzel and Gros (1998).

Twitch and tetanic forces, summation frequencies

The maximal twitch force observed in a whole-muscle preparation of a soleus muscle of *S. etruscus* amounted to 250 µN, and the corresponding value for the EDL muscle of *S. etruscus* was 900 µN. In *C. russula*, we measured maximal forces of 310 and 770 µN in these muscles. The maximal force generated by the muscle *in vitro* is mainly determined by the number of muscles fibres remaining intact during and after the preparation procedure. Because of the difficulty in preparing the extremely small leg muscles of the small shrews, this parameter varies greatly between different muscles.

During repetitive stimulation in *S. etruscus*, force summation began at stimulation frequencies of 20 Hz in the soleus and 25 Hz in the EDL muscle. The tetanic contraction was fused at 100 Hz in the soleus, but only at 200 Hz in the EDL muscle. Maximal tetanic force occurred in both muscles at 200 Hz. The highest recorded values of tetanic force were 720 µN in the soleus and 2600 µN in the EDL muscle. In *C. russula*, summation occurred at 15 Hz in the soleus and at 20 Hz in the EDL muscle, smooth tetanic contraction was reached at 50 Hz in the soleus and at 100 Hz in the EDL muscle, and maximal force was generated in both muscles at 100 Hz.

The two muscles also exhibited differences in the rate of force development during tetanic contractions. In soleus muscles stimulated at 200 Hz, the maximum rate of increase of relative force, $d(F/F_{max})/dt$, was 1.5 % ms^{-1} in both *S. etruscus* and *C. russula*, whereas in the EDL muscle a much faster increase, 3.3 % ms^{-1}, was observed in both species.

Muscle fibre types

Histochemical staining for myosin ATPases revealed the same fibre type pattern in the EDL and soleus muscles of *S.*

Table 3. *Quantities characterizing the force transient and the Ca^{2+} transient at 25 °C during an isometric twitch of a bundle of fibres from a single extensor digitorum longus and a single soleus muscle from the shrew species* Suncus etruscus

Signal	Muscle (*N*)	t_p (ms)	t_{r50} (ms)	t_{r75} (ms)
Force of muscle fibre bundle	EDL (9)	10.6±0.9‡	9.3±1.7‡	12.6±1.9‡
	Soleus (27)	13.2±1.5	17.6±2.3	30.0±3.7
Ca^{2+} transient	EDL (6)	5.3±2.4‡	16.1±4.9*	23.7±4.1‡
	Soleus (19)	9.5±3.2	21.8±9.2	37.4±13.3

Data represent mean ± S.D. for (*N*) measurements on a single bundle of muscle fibres.

t_p, time to peak force; transient t_{r50}, t_{r75}, 50, 75 % relaxation times.

Significance levels are calculated for the difference between the fibre bundles from the extensor digitorum longus (EDL) and the soleus muscle. *P<0.05; ‡P<0.01 (unpaired t-test).

etruscus after both acidic and alkaline preincubation. Only alkaline-resistant myosin ATPase could be detected, which is typical of type II fibres. All fibres of these shrew muscles showed the same degree and pattern of staining, in contrast to the fibres of the rat EDL muscle, which was stained for comparison. In the latter muscle, four different fibre types could be distinguished.

Myosin composition and ATPase activity

SDS–PAGE and two-dimensional electrophoresis of the pellets of the EDL and soleus muscles of *S. etruscus* revealed no difference in the pattern of myosin heavy chains (Fig. 3)

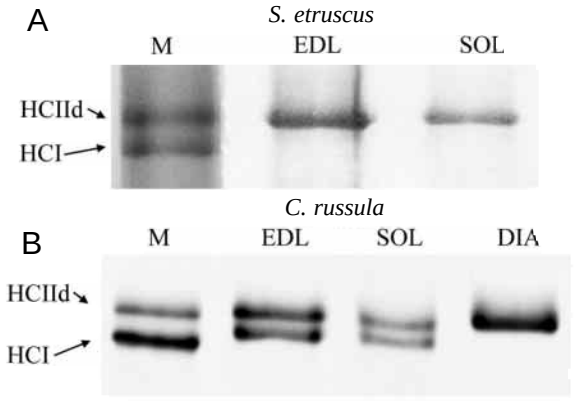

Fig. 3. Result of one-dimensional SDS–PAGE electrophoresis of myosin preparations from (A) the extensor digitorum longus (EDL) (17 pooled muscles) and the soleus (SOL) (16 pooled muscles) muscles of *Suncus etruscus* and (B) the EDL (three pooled muscles), the SOL (three pooled muscles) and the diaphragm (DIA) (one muscle) muscles of *Crocidura russula*. Marker proteins (M) are MHC I (HCI) and MHC IId (HCIId) from rabbit muscle.

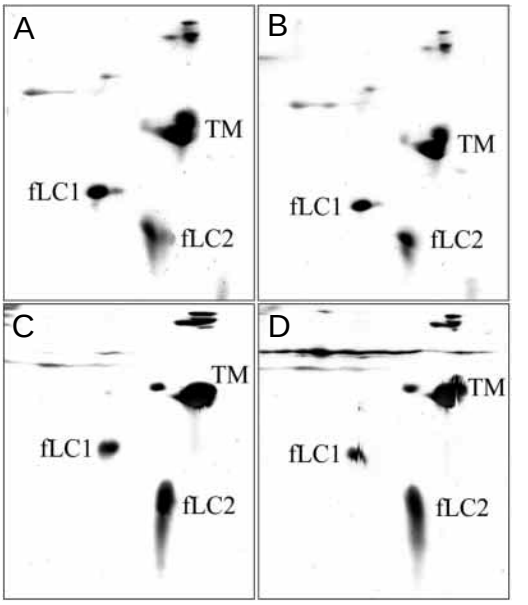

Fig. 4. Result of two-dimensional electrophoresis of myosin samples performed to identify myosin light chain (LC) isoforms. Myosin preparations were (A) from the extensor digitorum longus (EDL) and (B) from the soleus muscles of *Suncus etruscus*, and (C) from the EDL and (D) from the soleus muscles of *Crocidura russula*. LC isoforms can be identified by the positions of the respective spots relative to the spot for tropomyosin (TM). fLC1, fast myosin light chain type 1; fLC2, fast myosin light chain type 2.

and myosin light chains (Fig. 4A,B). An additional study of the diaphragm muscle led to an identical result. Only one type of MHC is found electrophoretically in all three muscles investigated. Comparison with rabbit MHCs indicates that the shrew MHC migrates like the rabbit fast type IId chain. In *C. russula*, two MHC isoforms are present in the soleus as well as the EDL muscles (Fig. 3) which, according to the pattern of rabbit isomyosins, are the fast IIb and IId heavy chains. There was no significant difference between the EDL and soleus muscles in heavy chain composition. We found mean values of 46 % MHC IId and 54 % MHC IIb in the soleus muscle and mean values of 42 % MHC IId and 58 % MHC IIb in the EDL muscle. The diaphragm muscle of *C. russula* contains 93 % MHC IId and 7 % MHC IIb.

In both species, MHC I is absent. This was confirmed immunohistochemically, since there was binding of monoclonal myosin type II antibodies but not of myosin type I antibodies in soleus as well as in EDL muscles of both species. The same holds for the diaphragm muscle of *C. russula*; the diaphragm of *S. etruscus* was not studied immunohistochemically. Two types of fast light chain (LC) have been found in *S. etruscus* and in *C. russula*. The myosin LC pattern is the same in the EDL and soleus muscles. The molecular masses of fLC1 and fLC2 were determined to be 24 and 21.5 kDa in the soleus, EDL and diaphragm muscles of both species (Fig. 4A–D).

Unfortunately, we were not able to measure the myosin

ATPase activity separately in the two muscles because the amount of tissue available was insufficient. In a mixture of diverse skeletal muscles from *S. etruscus*, a mean myosin ATPase activity of 35 μmol g^{-1} h^{-1} was found, which is almost the same as the corresponding value for *C. russula* of 36 μmol g^{-1} h^{-1}. These activities are slightly higher than the activity in rat tibialis anterior muscle (68 % MHC IIb, 24 % MHC IId), which was 32 μmol g^{-1} h^{-1}, but are considerably higher than the activity in rat soleus muscle (100 % MHC I), which was 5 μmol g^{-1} h^{-1}.

Cytoplasmic proteins

No reaction with polyclonal parvalbumin antibodies could be detected in either *S. etruscus* or *C. russula* in either the EDL or the soleus muscle. The cytosolic concentration of myoglobin amounts to a mean value of 150 μmol l^{-1} in the soleus and the EDL muscles of *S. etruscus*.

Electrophoresis of the muscle homogenate supernatant of the EDL and soleus muscles of *S. etruscus* exhibits similar protein patterns, but a pronounced difference is seen in a band formed by a protein that, according to marker proteins, has a molecular mass of approximately 42 kDa (Fig. 5). In the EDL muscle, this protein makes up 24.6 %, and in the soleus muscle 8.6 %, of the total cytosolic protein content. The identification of this protein was performed on the gastrocnemius muscle of *S. etruscus*, which also contained the 42 kDa protein, since no EDL and soleus muscles had been left for this investigation. Immunoblotting with anti-MM-CK antibodies revealed this protein to be creatine kinase (CK). In mammals, different genes code for three types of CK subunit, all of which have a molecular mass of 43 kDa (Ulrich, 1990).

Another significant difference between the two muscles is seen in the 62 kDa fraction, which makes up 6.5 % of the total cytosolic protein content in the EDL muscle and 18 % in the soleus muscle. This fraction may consist mainly of subunits of pyruvate kinase, which are present in relatively large concentrations in SDS-treated muscle homogenate supernatants and for which, in humans, a molecular mass of 61 kDa has been reported (Harkins et al., 1977). However, as we did not identify this protein unequivocally, the finding is not discussed further. A smaller difference was seen in the 18 kDa fraction, which is a little smaller in the soleus muscle (4.3 %) than in the EDL muscle (6.5 %). The mean myoglobin concentration of 150 μmol l^{-1} obtained using the method of Reynafarje (1963) in both EDL and soleus muscles corresponds to approximately 5 % of the total cytosolic protein content, suggesting that myoglobin (17 kDa) could be the major protein of this fraction. A significant difference in the myoglobin concentration of the EDL and soleus muscles, however, has not been confirmed using the Reynafarje method.

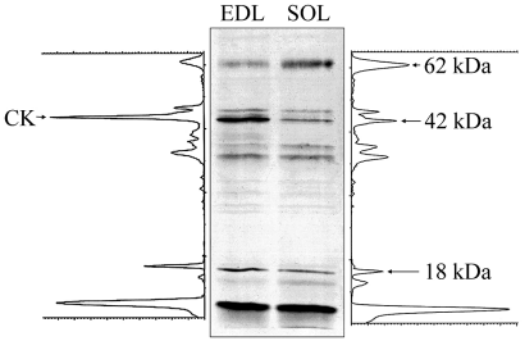

Fig. 5. Patterns of electrophoretically separated cytosolic proteins from the extensor digitorum (EDL) (17 pooled muscles) and soleus (SOL) (16 pooled muscles) muscles of *Suncus etruscus* and corresponding densitograms. CK, creatine kinase.

Metabolic enzymes

Measurement of LDH specific activities (LDH/c_{pr}, where c_{pr} is the cytoplasmic protein concentration; mmol min^{-1} mg^{-1}) revealed similar values for EDL and soleus muscles in *S. etruscus*. The same applies to CS, which showed almost the same specific activity (mmol min^{-1} mg^{-1}) as LDH (Table 4). In EDL and soleus muscles of *C. russula*, the specific activity of LDH is four times the specific activity of CS (Table 4). In both species, the specific activity of LDH, representing the capacity for glycolytic metabolism, is very low compared with the specific activity of LDH in muscles of bigger mammalian species, e.g. the rat. In contrast, the specific activity of CS, representing the aerobic capacity of the muscle, is rather high compared with CS specific activities found in the muscles of the rat (Table 4) and of other larger mammals. Fig. 6 shows an

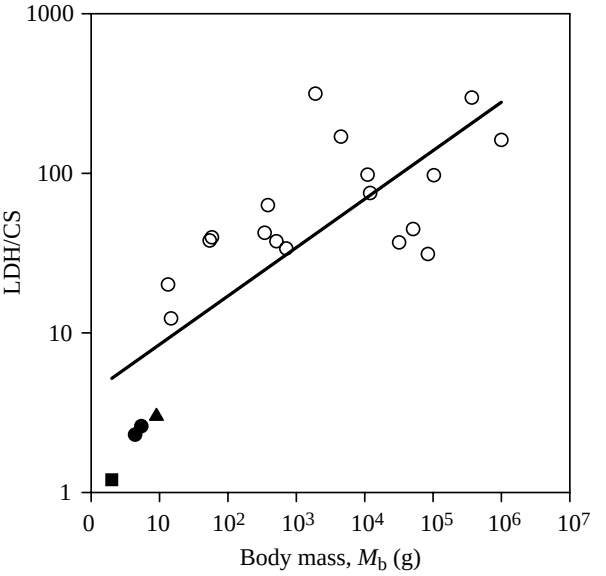

Fig. 6. Ratio of lactate dehydrogenase (LDH) to citrate synthase (CS) activity in gastrocnemius muscle of mammalian species of different body masses (M_b). The symbols represent the data for *Suncus etruscus* (■) and *Crocidura russula* (▲), measured here, and those for the shrew *Sorex vagrans* (M_b 4–5 g) (●) and several larger mammalian species (○), taken from Emmett and Hochachka (1981). The solid line is the result of a linear regression on a double logarithmic scale in which all data points were included [log(LDH/CS)=0.304logM_b+0.623; r^2=0.62].

Table 4. *Specific activities of lactate dehydrogenase and citrate synthase in the extensor digitorum longus, soleus and gastrocnemius muscles of* Suncus etruscus, Crocidura russula *and Wistar rat*

Species	Muscle (N)[a]	LDH/c_{pr} (mmol min^{-1} mg^{-1})	CS/c_{pr} (mmol min^{-1} mg^{-1})	LDH/CS
Suncus etruscus	EDL (17)	1.31	0.94	1.4
	Soleus (16)	1.14	0.84	1.4
	Gastrocnemius (4)	1.49	1.28	1.2
Crocidura russula	EDL (5)	2.87	0.7	4.1
	Soleus (4)	2.83	0.64	4.4
	Gastrocnemius (2)	3.94	1.39	2.8
Wistar rat	EDL (6)	12.2	0.17	72
	Soleus (6)	5.3	0.22	24
	Gastrocnemius	–	–	53[b]

c_{pr}, cytoplasmic protein concentration; CS, citrate synthase; LDH, lactate dehydrogenase.
c_{pr} is given in mg ml^{-1}. LDH and CS activities are given in mmol min^{-1} ml^{-1}.
[a]Number of pooled muscles in *Suncus etruscus* and *Crocidura russula*; number of muscles in Wistar rat.
[b]Taken from Emmett and Hochachka (1981).

interspecific comparison of the LDH/CS activity ratio in the gastrocnemius muscle, for which data for mammalian species covering a wide body size range are available in the literature (Emmett and Hochachka, 1981).

Discussion

Mechanical properties of the muscles

The small shrew species exhibit the shortest isometric times to peak (t_p) force (Table 2) and twitch contraction times (t_p+t_{r90}) of EDL and soleus muscles ever measured in mammalian muscles at 25 °C. On average, t_p+t_{r90} of the EDL and soleus muscles were 38 ms and 55 ms in *S. etruscus* and 45 ms and 73 ms in *C. russula* (at 25 °C), respectively, which is in fairly good agreement with the observation that summation of contractions begins at approximately 25 Hz in the EDL and 20 Hz in the soleus muscle of *S. etruscus* and at 20 Hz in the EDL and 15 Hz in the soleus muscle of *C. russula*. Taking into account that complete relaxation (including complete decay of the Ca^{2+} transient) may exceed t_p+t_{r90} by a maximum of 30 % and assuming a Q_{10} of 2.5, upper limits for twitch frequencies at 37 °C of approximately 3600 min^{-1} in the EDL and 2500 min^{-1} in the soleus muscle are estimated from our data for *S. etruscus*. The corresponding figures for *C. russula* are 3100 min^{-1} for the EDL and 1900 min^{-1} for the soleus muscle. Because of a similar fibre composition, similar frequencies are also likely to hold for other skeletal muscles of these shrews.

These frequencies are far above the respiratory rates of up to 900 min^{-1} measured in *S. etruscus* (Jürgens et al., 1996) and 500 min^{-1} measured in *C. russula* (Nagel, 1991). They are also much higher than the stride frequencies at maximum sustained running speed of 780 and 620 min^{-1} estimated from scaling studies for the two species (Heglund and Taylor, 1988). For respiration and for locomotion, lower maximal contraction frequencies than those calculated from twitch contraction times are expected since smooth movements of the corresponding muscles are only possible through asynchronous firing of their motor units. The firing rates of motor units are reflected in the electromyogram (EMG) of a muscle. In *C. russula*, the frequency of the grouped discharges observed in the EMG during shivering (Kleinebeckel et al., 1994) is only slightly higher than the maximal twitch frequency estimated for the EDL muscle. This indicates that the most rapid contractions are utilized for thermogenesis in the heterothermic crocidurine shrew.

Among the mammals investigated so far, *S. etruscus* exhibits the smallest difference between the twitch contraction times (t_p+t_{r90}) of the EDL and soleus muscles. Although small, this difference in mechanical muscle properties is significant, mainly as a result of significantly different relaxation times for the two muscles. In *C. russula*, this difference is a little more

pronounced. These results are in accordance with the results of tetanic contraction measurements. At a stimulation frequency of 200 Hz, the maximal rate of increase in the relative tetanic force is almost twice as high in the EDL muscle (3.3 % ms^{-1}) as in the soleus muscle (1.5 % ms^{-1}) in both species.

Muscle fibres and myosin composition

Although the EDL and soleus muscles of the small shrews exhibit different contractile characteristics (Fig. 1; Tables 1, 3), we could detect no significant differences in the fibre composition of these two muscles (Figs 3, 4). Histochemical staining for myosin ATPase led to a homogeneous type II fibre pattern that was identical in both muscles of *S. etruscus*. This finding agrees with results from other groups. For example, Savolainen and Vornanen (1995b) also detected only type II fibres in seven skeletal muscles, including the soleus muscle, of the shrew *Sorex araneus* (body mass 7–10 g) using the same type of ATPase staining that we used in this study. (These authors did not study the EDL muscle.) However, these authors observed two different fibre types after staining for succinate dehydrogenase and designated them as high- and low-oxidative forms. Since these two fibre subtypes appeared in all shrew muscles studied in approximately equal proportions, the different contractile properties of the EDL and soleus muscles in shrews cannot be explained by different fibre compositions of these muscles. Suzuki (1990) did not detect any type I fibres in 12 different limb muscles from the relatively large shrew species *Suncus murinus* (body mass 20–60 g). In this species, myofibre II subtypes have been found by measuring the activities of tetrazolium reductase and menadione-linked glycerol-3-phosphate dehydrogenase. The fibres were classified as IIA-W, IIA-S, IIAB and IIB. The soleus muscle was composed of 64 % IIA-W, 29 % IIA-S, 6 % IIAB and 1 % IIB fibres, the vastus lateralis muscle of 80 % IIB, 13 % IIAB and 7 % IIA-S fibres. The EDL muscle was not studied.

The finding of only type II myofibres in *S. etruscus* is confirmed by our results obtained from immunohistochemistry and from electrophoretic studies of the myosin heavy chain composition. Only MHC type II was detected immunohistochemically in *S. etruscus* and in *C. russula*. The electrophoretic methods we used revealed no significant differences in either myosin heavy or myosin light chain composition of the EDL and soleus muscles in either species (Figs 3, 4). Comparison with the electrophoretic pattern of rabbit MHCs indicated that, in *S. etruscus*, only MHC IId is present in these two muscles and that almost equal concentrations of MHC IId and MHC IIb occur in both muscles of *C. russula*. Thus, differences in myosin composition cannot explain the different mechanical properties of the EDL and soleus muscles in these shrews.

In their studies on *Sorex araneus*, Savolainen and Vornanen (1995a,b) differentiated two type II subtypes, which they identified as MHC IIb and MHC IId. They found 34 % (tibialis anterior muscle) to 97 % (masseter muscle) MHC IId in young individuals and 64 % (tibialis anterior muscle) to 99 % (masseter muscle) MHC IId in old individuals. The soleus muscle contained 84 % MHC IId in young and 98 % MHC IId in old animals; the corresponding values for the diaphragm muscle are 87 % and 97 %. The EDL muscle was not investigated.

That fast type II heavy chains must dominate in the entire skeletal musculature of *S. etruscus* and *C. russula* can also be deduced from the measurements of myosin ATPase activity. The activity found in pooled muscles of the shrews is in good agreement with the activity of myosin ATPase in the tibialis anterior muscle of the rat, a muscle that has been shown to exhibit over 90 % MHC II isoforms. In the rat, the myosin ATPase activity of the tibialis anterior muscle is seven times as high as that of the soleus muscle, which in this species contains only MHC I.

The uniformity of the myosin patterns found in the EDL and soleus muscles of the two shrew species is quite different from the situation in larger mammals, where there are muscles specialized for supporting the weight of the body and for performing contractions for locomotion. For example, in mice, rats and guinea pigs, the dynamically performing EDL muscle is composed of approximately 100 % fast-twitch fibres, as in the shrews. In contrast, in the soleus muscle, the percentage of slow-twitch fibres increases with increasing adult body mass of small mammalian species (Table 2): there are no slow-twitch fibres in the shrews, in adult mice, the soleus muscle contains approximately 50 % slow fibres, and in adult Wistar rats the soleus muscle is composed entirely of slow-twitch fibres. This leads to the conclusion that the lighter the animal the less important are slow-twitch-type fibres, which constitute muscles specialized for static contractions to maintain posture. Gravitational forces, which are proportional to mass, decrease with decreasing body size, and the locomotory behaviour of the smallest mammals reveals that moving is more important for them than standing.

In shrews, the presence of MHC I fibres seems not to be required to maintain posture. Irrespective of body mass, from the 60 g *S. murinus* to the 2 g *S. etruscus*, all shrew species studied so far, Crocidurinae and Soricinae, lack slow-twitch fibres. It seems that, in shrew species, instead of a body-mass-dependent ratio of MHC I to MHC II, the soleus muscle exhibits a body-mass-dependent ratio of different MHC II subtypes. In *S. murinus*, the soleus muscle is composed of 93 % IIA fibres (Suzuki, 1990), which predominantly contain the slow MHC IIa, the soleus muscle of *C. russula* contains the faster IId (46 %) and IIb (54 %) MHCs, in the soleus muscle of *S. araneus* 84–98 % of the MHCs are IId chains (Savolainen and Vornanen, 1995a), and a homogeneous MHC IId pattern is found in *S. etruscus*. The smaller the species, the more dominant is the MHC IId portion of a given muscle. Moreover, in the smallest shrew species, *S. etruscus*, there is even no difference in the MHC composition of different muscles (EDL, soleus and diaphragm). The uniformity of the muscles of *S. etruscus* and *C. russula* is confirmed by the fact that only one pair of myosin light chains has been found in the EDL, soleus and diaphragm muscles. A variation in the contractile

properties among type IID or type IIB muscle fibres, which could be caused by different fLC1/fLC3 ratios (Galler et al., 1994), can therefore be excluded.

The body-mass-dependent MHC II fibre subtype composition in shrews may be explained by the following properties. It has been shown in skinned fibre experiments using fibres of rat muscles (Galler et al., 1994) that type I fibres are very slow compared with type II fibres, but that the type II subtypes differ also in the increase in force following stretch activation and in their unloaded shortening velocity. The fastest force transients occurred in IIB fibres, the slowest in IIA fibres; the transients were intermediate in IID fibres. The same order was found for shortening velocities. The ATP turnover is therefore highest in IIB and lowest in IIA fibres; in this respect, the IID fibres resemble more closely the fast IIB than the slow IIA fibres. Fibre diameters decrease in the order IIB>IID>IIA>I. The diameters of IID fibres are much closer to the diameters of the thin IIA than to the thick IIB fibres. Therefore, type IID fibres are most appropriate when a combination of a high shortening velocity and a small fibre diameter is required in the rat. Provided that the fibre properties found in the rat also hold for shrews, type IID fibres seem to be the appropriate compromise between a high ATP turnover, which is required not only for physical performance but also for heat production to cope with the great heat loss of a small mammal, and a small fibre diameter which, together with a high capillary density, enables a high oxygen flux into the fibre, and hence ATP production, as a result of the short diffusion distance for oxygen. This may be the reason why, in the smallest mammalian species, which are characterized by the highest contraction frequencies and the highest mass-specific rates of oxygen consumption, all muscles studied are composed exclusively of type IID fibres or MHC IId.

Oxidative and glycolytic metabolism

Since we found a significant functional, but not a biochemical (at the myosin level), difference between the EDL and soleus muscles in both *S. etruscus* and *C. russula*, we also measured the activities of two metabolic enzymes important for the rate of production of ATP. We found that the specific activities of citrate synthase (a measure of the oxidative capacity of the muscles) and of lactate dehydrogenase (a measure of the glycolytic capacity of the muscles) were not significantly different between the two muscles and also did not differ greatly from the values in the gastrocnemius muscle (Table 4). Therefore, different proportion of these metabolic marker enzymes is also not an explanation for the differing mechanical properties of the muscles of small shrews.

The specific activity of CS is markedly higher in shrew muscles than in those of larger mammalian species. This observation is in accordance with the extremely high mitochondrial volume fraction in skeletal muscle of Etruscan shrews, 0.23 in leg muscles and 0.35 in the diaphragm (Hoppeler et al., 1981). The specific activity of LDH, in contrast, is markedly lower in muscles of the smallest shrew species than in muscles of larger mammalian species. Both the EDL and soleus muscles of *S. etruscus* exhibit an extremely low LDH/CS activity ratio of approximately 1, which is the lowest value found so far in a mammal (Fig. 6). In the gastrocnemius muscle, the LDH/CS activity ratios are 1 (*S. etruscus*) and 3 (*C. russula*), compared with 50 in the rat and 200 in the cow. These results indicate that, metabolically, the EDL, soleus and gastrocnemius muscles all have a high oxidative capacity in the two shrew species investigated, whereas the EDL muscle is mainly glycolytic and the soleus muscle is oxidative in larger mammalian species.

The finding that in the two shrew species both the EDL and soleus muscles consist only of oxidative fibres is supported by the observation that the myoglobin concentrations of these muscles are not significantly different. A mean concentration of $150\,\mu mol\,l^{-1}$ corresponds to the light red colour of the muscles. Red muscles of larger mammalian species generally show mean myoglobin concentrations of $300–400\,\mu mol\,l^{-1}$, whereas white muscles contain negligible amounts of myoglobin. It should be noted that no significant colour differences could be observed visually between different skeletal muscles of the shrews, indicating a similar myoglobin content in nearly all of them. The only exceptions were the masseter and temporalis muscles, which were darker red than all the other muscles. This result again underlines the uniformity of the musculature of the two shrew species.

In contrast to the muscles of larger mammals, fast shrew muscles seem to depend almost entirely on oxidative metabolism, i.e. their proper function depends on a steady supply of oxygen. Sufficient delivery of oxygen to the tissues of these small mammals is ensured by a very powerful respiratory and circulatory oxygen transport system (Jürgens et al., 1996). At the level of the muscle tissue, an adequate adaptation is achieved by the very high capillary densities ($2800–3200\,mm^{-2}$) and extremely small muscle fibre diameters (15–25 μm) (Pietschmann et al., 1982; Savolainen and Vornanen, 1995b), leading to very short diffusion distances for O_2. Thus, aerobic metabolism is ensured whatever the energy demand of the animal, so that glycolytic metabolism does not necessarily play a significant role. The glycolytic metabolic pathway may be underdeveloped also because, compared with the enormous mass-specific energy requirements of the organism (its rate of oxygen consumption can rise to $1000\,ml\,O_2\,kg^{-1}\,min^{-1}$; Jürgens et al., 1996), the amount of ATP that can be produced glycolytically during an oxygen debt would be almost negligible even at significantly higher levels of LDH.

Intracellular Ca^{2+} transients

The mechanical properties of a muscle are determined not only by the myosin composition and by the levels of metabolic enzymes in the muscle cells, but in addition by the rate at which Ca^{2+} is released from the sarcoplasmatic reticulum and subsequently resequestered. We therefore examined the sarcoplasmic Ca^{2+} transients of electrically stimulated muscle fibre bundles dissected from one EDL and one soleus muscle of *S. etruscus*.

In contrast to the absence of differences in MHC and myosin LC composition and in specific activity of the metabolic marker enzymes LDH and CS between the EDL and soleus muscles from *S. etruscus*, the Ca^{2+} transients shown by fibre bundles of the two muscles were significantly different in *S. etruscus* (Table 3). The recorded Ca^{2+} transients confirm the results of the contraction measurements. Corresponding to the shorter duration of the twitch contractions in the EDL muscle, the time to peak of the Ca^{2+} signal in the EDL muscle amounts to only 56 % and the t_{r75} decay time of the Ca^{2+} signal to only 63 % of that of the soleus muscle, indicating a considerably faster Ca^{2+} release from and re-uptake into the sarcoplasmic reticulum in the EDL muscle.

The shorter relaxation time of the EDL muscle could be caused by a higher concentration of a cytosolic Ca^{2+}-binding protein such as parvalbumin (Heizmann et al., 1982). Our immunohistochemical results provided no evidence for the presence of parvalbumin, but we cannot judge whether the polyclonal antibody against rabbit parvalbumin, which has been shown to cross-react with parvalbumin from rat, mouse, guinea pig, pig, sheep and coypu, did not cross-react with shrew parvalbumin or whether this protein is actually absent from the EDL and from the soleus muscle. Le Peuch et al. (1978) reported that parvalbumin is present in hindlimb muscles of *S. etruscus* at 30 μmol kg^{-1}, which corresponds to the moderate parvalbumin concentration reported for IID fibres of the rat (Leberer and Pette, 1986).

The difference in the Ca^{2+} kinetics between the two muscles could also be due to a larger volume fraction of the sarcoplasmic reticulum and, hence, more Ca^{2+} channels and Ca^{2+}-ATPase in fibres of the EDL than in fibres of the soleus muscles of *S. etruscus*, or to different specific activities of Ca^{2+}-ATPase isoforms; however, we did not determine these characteristics.

Our results might also be explained by an effect of creatine kinase activity on Ca^{2+}-ATPase activity. Cytosolic CK is present in remarkably high concentrations in both the EDL and soleus muscles of *S. etruscus*. It is shown here to occur in the EDL muscle at a concentration three times as high as in the soleus muscle of this species (Fig. 5). CK is reported to be attached not only to the mitochondria, the main site of ATP production, but also to several sites of ATP utilization in the muscle cell, such as myofibrils, sarcoplasmatic reticulum, sarcolemma and ribosomes (Bessman and Savabi, 1990; Minajeva et al., 1996). The presence of CK close to the Ca^{2+}-ATPase of the sarcoplasmatic reticulum has been shown to enhance Ca^{2+} uptake and release rates in muscles of mice (Steeghs et al., 1997). CK activity is functionally coupled to the activity of the Ca^{2+}-ATPase since it controls the local [ATP]/[ADP] ratio at the sarcoplasmic reticulum and thus determines the ATP concentration available for the Ca^{2+} pump and, therefore, the velocity of muscle relaxation (Wallimann and Hemmer, 1994; Minajeva et al., 1996).

It is possible that the difference in contraction times between the EDL muscle and the soleus muscle of the smallest mammal, which can be explained neither by a difference in the fibre composition of the muscle nor by differences in the specific activities of marker enzymes of the glycolytic and oxidative pathways, is at least in part due to the higher activity of CK and, therefore, to the higher activity of the sarcoplasmic Ca^{2+} pump in EDL than in soleus muscle.

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References

Asmussen, G. and Gaunitz, U. (1989). Temperature effects on isometric contractions of slow and fast twitch muscles of various rodents – dependence on fibre type composition: A comparative study. *Biomed. Biochim. Acta* **48**, S536–S541.

Bass, A., Brdiczka, D., Eyer, P., Hofer, S. and Pette, D. (1969). Metabolic differentiation of distinct muscle types at the level of enzymatic organization. *Eur. J. Biochem.* **10**, 198–206.

Bernstein, L. H. and Everse, J. (1975). Determination of the isoenzyme levels of lactate dehydrogenase. *Meth. Enzymol.* **41**, 47–52.

Bessman, S. P. and Savabi, F. (1990). The role of phosphocreatine energy shuttle in exercise and muscle hypertrophy. In *Biochemistry of Exercise VII* (ed. A. W. Taylor, P. D. Gollnick, H. J. Green, C. D. Ianuzzo, E. G. Noble, G. Métivier and J. R. Sutton). *Int. Ser. Sport Sci.* **21**, 167–178.

Bredman, J. J., Weijs, W. A., Korfage, H. A. M., Brugman, P. and Moorman, A. F. M. (1992). Myosin heavy chain expression in rabbit masseter muscle during postnatal development. *J. Anat.* **180**, 263–274.

Brooke, M. H. and Kaiser, K. K. (1970). Muscle fibre types: How many and what kind? *Arch. Neurol.* **23**, 369–379.

Emmett, B. and Hochachka, P. W. (1981). Scaling of oxidative and glycolytic enzymes in mammals. *Respir. Physiol.* **45**, 261–272.

Fons, R., Sender, S., Peters, T. and Jürgens, K. D. (1997). Rates of rewarming, heart and respiratory rates and their significance for oxygen transport during arousal from torpor in the smallest mammal, the Etruscan shrew *Suncus etruscus*. *J. Exp. Biol.* **200**, 1451–1458.

Fons, R. and Sicart, R. (1976). Contribution a la connaissance du métabolisme énergétique chez deux Crocidurinae: *Suncus etruscus* (Savi, 1822) et *Crocidura russula* (Hermann, 1780) (Insectivora, Soricidae). *Mammalia* **40**, 299–311.

Galler, S., Schmitt, T. L. and Pette, D. (1994). Stretch activation, unloaded shortening velocity and myosin heavy chain isoforms of rat skeletal muscle fibres. *J. Physiol., Lond.* **478**, 513–521.

Geers, C. and Gros, G. (1990). Effects of carbonic anhydrase inhibitors on contraction, intracellular pH and energy-rich phosphates of rat skeletal muscle. *J. Physiol., Lond.* **423**, 279–297.

Harkins, R. N., Black, J. A. and Rittenberg, M. B. (1977). Purification and characterization of human pyruvate kinase. *Can. J. Biochem.* **55**, 301–307.

Heglund, N. C. and Taylor, C. R. (1988). Speed, stride frequency and energy cost per stride: how do they change with body size and gait? *J. Exp. Biol.* **138**, 301–318.

Heizmann, C. W., Berchtold, M. W. and Rowlerson, A. M. (1982). Correlation of parvalbumin concentration with relaxation speed in mammalian muscles. *Proc. Natl. Acad. Sci. USA* **79**, 7243–7247.

Heukeshoven, J. and Dernick, R. (1985). Simplified method for silver staining of proteins in polyacrylamide gels and the mechanism of silver staining. *Electrophoresis* **6**, 103–112.

Hoppeler, H., Mathieu, O., Weibel, E. R., Krauer, R., Lindstedt, S. L. and Taylor, C. R. (1981). Design of the mammalian respiratory system. VIII. Capillaries in skeletal muscles. *Respir. Physiol.* **44**, 129–150.

Jürgens, K. D., Fons, R., Peters, T. and Sender, S. (1996). Heart and respiratory rates and their significance for convective oxygen transport rates in the smallest mammal, the Etruscan shrew *Suncus etruscus*. *J. Exp. Biol.* **199**, 2579–2584.

Kleinebeckel, D., Nagel, A., Vogel, P. and Klussmann, F. W. (1994). The frequencies of grouped discharges during cold tremor in shrews: an electromyographic study. In *Integrative and Cellular Aspects of Autonomic Functions: Temperature and Osmoregulation* (ed. K. Pleschka and R. Gerstberger), pp. 305–311. Paris: John Libbey Eurotext.

Kodama, T., Fukui, K. and Kometani, K. (1986). The initial phosphate burst in ATP hydrolysis by myosin and subfragment-1 as studied by a modified malachite green method for determination of inorganic phosphate. *J. Biochem.* **99**, 1465–1472.

Kubis, H. P. and Gros, G. (1997). A rapid electrophoretic method for separating rabbit skeletal muscle myosin heavy chains at high resolution. *Electrophoresis* **18**, 64–66.

Leberer, E. and Pette, D. (1986). Neural regulation of parvalbumin expression in mammalian skeletal muscle cells. *Biochem. J.* **235**, 67–73.

Le Peuch, C. J., Demaille, J. G. and Pechère, J. F. (1978). Radioelectrophoresis: A specific microassay for parvalbumins. Application to muscle biopsies from man and other vertebrates. *Biochim. Biophys. Acta* **537**, 153–159.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, O. P. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

Minajeva, A., Ventura-Clapier, R. and Veksler, V. (1996). Ca^{2+} uptake by cardiac sarcoplasmic reticulum ATPase *in situ* strongly depends on bound creatine kinase. *Pflügers Arch.* **432**, 904–912.

Nagel, A. (1991). Metabolic, respiratory and cardiac activity in the shrew *Crocidura russula*. *Respir. Physiol.* **85**, 139–149.

O'Farrell, P. (1975). High resolution two-dimensional electrophoresis. *J. Biol. Chem.* **202**, 739–747.

Padykula, H. A. and Herman, E. (1955). The specificity of the histochemical method for adenosine triphospate. *J. Histochem. Cytochem.* **3**, 170–195.

Pietschmann, M., Bartels, H. and Fons, R. (1982). Capillary supply of heart and skeletal muscle of small bats and non-flying mammals. *Respir. Physiol.* **50**, 267–282.

Reynafarje, B. (1963). Simplified method for the determination of myoglobin. *J. Lab. Clin. Med.* **61**, 138–145.

Rushbrook, J. I. and Stracher, A. (1979). Comparison of adult, embryonic and dystrophic myosin heavy chains from chicken muscle by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and peptide mapping. *Proc. Natl. Acad. Sci. USA* **76**, 4331–4334.

Savolainen, J. and Vornanen, M. (1995a). Myosin heavy chains in skeletal muscles of the common shrew (*Sorex araneus*): absence of a slow isoform and transitions of fast isoforms with ageing. *Acta Physiol. Scand.* **155**, 233–239.

Savolainen, J. and Vornanen, M. (1995b). Fibre types and myosin heavy chain composition in muscles of the common shrew (*Sorex araneus*). *J. Exp. Zool.* **271**, 27–35.

Schmitt, T. L. and Pette, D. (1991). Fibre type specific distribution of parvalbumin in rabbit skeletal muscle. *Histochemistry* **96**, 459–465.

Steeghs, K., Benders, A., Oerlemans, F., de Haan, A., Ruitenbeek, W. J. C., van Deursen, J., Perryman, B., Pette, D., Bruckwilder, M., Koudijs, J. J. P., Veerkamp, J. and Wieringa, B. (1997). Altered Ca^{2+} responses in muscles with combined mitochondrial and cytosolic creatine kinase deficiencies. *Cell* **89**, 93–103.

Stein, R. B., Gordon, T. and Shriver, J. (1982). Temperature dependence of mammalian muscle contractions and ATPase activities. *Biophys. J.* **40**, 97–107.

Suzuki, A. (1990). Composition of myofibre types in limb muscles of the house shrew (*Suncus murinus*): Lack of type I myofibres. *Anat. Record* **228**, 23–30.

Ulrich, K. (1990). *Vergleichende Biochemie der Wirbeltiere*. Stuttgart: Gustav Fischer Verlag.

Wallimann, T. and Hemmer, W. (1994). Creatine kinase in non-muscle tissue and cells. *Mol. Cell. Biochem.* **133/134**, 193–220.

Weibel, E. R., Burri, P. H. and Claassen, H. (1971). The gas exchange apparatus of the smallest mammal: *Suncus etruscus*. *Experientia* **27**, 724.

Wessels, A., Vermeulen, J. L. M., Viradh, S., Kalman, F., Lamers, W. H. and Moorman, A. F. M. (1991). Spatial distribution of 'tissue specific' antigens in the developing human heart and skeletal muscle. *Anat. Rec.* **229**, 355–368.

Wetzel, P. and Gros, G. (1998). Decay of Ca^{2+} and force transients in fast- and slow-twitch skeletal muscles from rat, mouse and Etruscan shrew. *J. Exp. Biol.* **201**, 375–384.