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Review

Etruscan shrew muscle: the consequences of being small

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Summary

The skeletal muscles of the smallest mammal, the Etruscan shrew *Suncus etruscus*, are functionally and structurally adapted to the requirements of an enormously high energy turnover. Isometric twitch contractions of the extensor digitorum longus (EDL) and soleus muscles are shorter than in any other mammal, allowing these muscles to contract at outstandingly high frequencies. The skeletal muscles of *S. etruscus* contract at up to 900 min^{-1} for respiration, up to 780 min^{-1} for running and up to 3500 min^{-1} for shivering. All skeletal muscles investigated lack slow-twitch type I fibres and consist only of fast-twitch type IID fibres. These fibres are optimally equipped with properties enabling a high rate of almost purely oxidative metabolism: they have a small diameter, their citrate synthase activity is higher and their lactate dehydrogenase activity is lower than in the muscles of any other mammal and they have a rapid shortening velocity. Differences in isometric twitch contraction times between different muscles are, at least in part, probably due to differences in cytosolic creatine kinase activities.

Key words: Etruscan shrew, *Suncus etruscus*, skeletal muscle, extensor digitorum longus, soleus, fibre composition, myosin heavy chain, myosin light chain, lactate dehydrogenase, citrate synthase, creatine kinase, myoglobin, Ca^{2+} transient, contraction, relaxation.

Introduction

The Etruscan shrew *Suncus etruscus* (Savi) and the bumblebee bat (*Craseonycteris thonglongyai*), both weighing on average less than 2 g, are the smallest extant mammals. A fossil record of an extinct shrew thought to weigh only 1.3 g was discovered recently (Bloch et al., 1998). The mean adult body mass of wild individuals of *S. etruscus* is 1.8 g. The species is found in Europe around the Mediterranean Sea and in Asia in a belt extending between 10 and 30°N.

Because of its large surface-to-volume ratio, the shrew's energy turnover is extraordinarily high. Under thermoneutral conditions (ambient temperature 35 °C), its rate of oxygen consumption is 100 ml kg^{-1} min^{-1}. At 22 °C, a rate of 270 ml kg^{-1} min^{-1} has been recorded (Fons and Sicart, 1976) and a maximal value of 1000 ml kg^{-1} min^{-1} (Weibel et al., 1971; Jürgens et al., 1996) has been estimated. These values are 25, 67 and 250 times higher than those of humans at rest. Although usually homeothermic, the species undergoes torpor cycles during times of food restriction and at low ambient temperatures.

Because muscles play a major role in the capture and chewing of food, in convective oxygen transport and in the generation of heat, they must be especially adapted to provide the enormous mass-specific rate of energy consumption of the body. In earlier studies investigating the properties of the blood and circulation, it was found that *S. etruscus* has a large relative heart muscle mass, 1.2 % of its body mass (Bartels et al., 1979), a value twice as high as expected from allometry, and a heart rate of up to 1511 beats min^{-1} (25 s^{-1}), which exceeds all values reported for other endotherms (Jürgens et al., 1996). The skeletal muscles of *S. etruscus* are also able to contract very rapidly: a respiratory rate of up to 900 min^{-1} (15 s^{-1}) was measured, a stride frequency of 780 min^{-1} (13 s^{-1}) is estimated from an allometric equation given by Heglund and Taylor (1988) and, during cold tremor, Kleinebeckel et al. (1994) observed electromyographic (EMG) frequencies of up to 3500 min^{-1} (58 s^{-1}). Although the relative amount of brown adipose tissue is higher in *S. etruscus* (on average 9.2 % of body mass) than in any other mammal, shivering has been shown to be important for rapid heat production and occurs during rewarming from torpor at body temperatures above 17 °C (Fons et al., 1997).

In a recent study, the contraction parameters, myosin composition and activity of the metabolic enzymes of the skeletal muscles of *S. etruscus* have been investigated and compared with corresponding properties of larger shrews and other larger mammals (Peters et al., 1999). In larger mammals, the soleus and the extensor digitorum longus (EDL) muscles are typical representatives of slow-twitch and fast-twitch muscles, so they were chosen to be functionally and structurally investigated in the shrew. In some cases, other

skeletal muscles, such as the diaphragm and gastrocnemius muscle, were also studied.

Materials and methods

Adult *Suncus etruscus* (Savi) 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*.

For the contraction measurements, the muscles were mounted in a measuring chamber and completely submerged in carbogen-equilibrated Krebs–Henseleit solution. To record the twitch contractions, a force transducer based on a semiconductor sensor device AE 802 (SensoNor a.s., Horten, Norway) was developed (Peters et al., 1999). Ca^{2+} transients of single twitches of muscle bundles containing 15–30 fibres were measured fluorometrically using a microscope photometric apparatus and the fluorescent dye Fura2. The Fura2 technique is described in detail by Wetzel and Gros (1998).

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 minor modifications. Two-dimensional electrophoresis of myosin light chains was performed using the method of O'Farrell (1975). Proteins of muscle homogenate supernatants were separated by SDS–PAGE using slabs with a 5 % stacking gel and a 15 % separating gel and visualized by silver staining. Muscle fibre type identification was performed after the method of Brooke and Kaiser (1970). Immunohistochemical studies with monoclonal and polyclonal antibodies are described in detail by Peters et al. (1999). Lactate dehydrogenase (LDH) activity was measured according to the method of Bernstein and Everse (1975), and citrate synthase (CS) activity was measured using the method of Bass et al. (1969). The specific activities of these enzymes are presented per milligram of cytoplasmic protein instead of per milligram wet mass of tissue because the tissue mass of the tiny muscles changes critically depending on the amount of water adhered to the muscle surface, either from the rinsing solution (fresh muscles) or from condensed water vapour (frozen muscles). The concentration of myoglobin was measured according to the method of Reynafarje (1963).

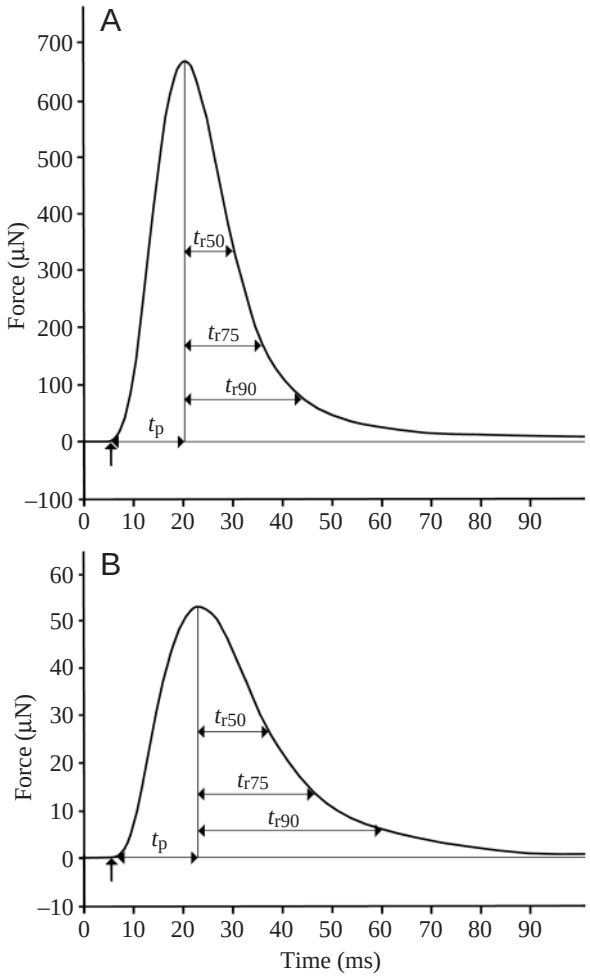

Fig. 1. Example of isometric twitch contractions at 21 °C of soleus (A) and extensor digitorum longus (B) muscle of *Suncus etruscus* stimulated simultaneously with a supramaximal electrical pulse of 1 ms duration. The curves are digital scans of original recordings. Time to peak force (t_p) is the time between the stimulus (upward-pointing arrow) and the occurrence of maximal force, and the 50, 75 and 90 % relaxation times (t_{r50}, t_{r75}, t_{r90}) are the times for the decay from maximal force to 50, 25 and 10 % of maximal force, respectively.

Results and Discussion

Contraction measurements

The time to peak force and relaxation time of twitch contractions of the soleus and EDL muscles (Fig. 1) are shorter in *S. etruscus* than in any other mammal. Applying a Q_{10} of 2.5 to the results obtained at room temperature (Table 1), it can be shown that, at 37 °C, a contraction cycle is completed within 13 ms in the EDL and within 18 ms in the soleus muscle. This is in accordance with the observation that contractions of motor units occurred at a frequency of 58 s^{-1} during shivering (Kleinebeckel et al., 1994). The maximal respiratory rate and the estimated maximal stride frequency are considerably lower than the frequency at which fusion of twitches occurs. This may be partly because smooth movements of a muscle require asynchronous firing of its motor units.

Time to peak force is not significantly shorter in the EDL than in the soleus muscle, whereas the times to 50 %, 75 % and 90 % relaxation are all significantly shorter in the EDL than in the soleus muscle (Table 1). This difference was confirmed by force measurements on fibre bundles from these muscles, which were performed together with recordings of the Ca^{2+} transients (Wetzel and Gros, 1998). Here, both time to peak force and times to 50 % and 75 % relaxation were significantly shorter in the EDL than in the soleus muscle, and the rise and

Table 1. *Characteristics of isometric twitch contractions of the extensor digitorum longus and the soleus muscles of* Suncus etruscus *(body mass 2.2±0.3 g,* N*=12) at 25 °C*

Muscle	Muscle mass (mg)	t_p (ms)	t_{r50} (ms)	t_{r75} (ms)	t_{r90} (ms)
Soleus (*N*=5)	0.37±0.04	12.7±2.3	17.3±4.6	27.7±6.5	42.2±10.8
EDL (*N*=6)	0.45±0.08	11.0±1.1	11.6±2.6*	16.7±2.9**	27.0±3.8**

Values are means ± S.D. for *N* muscles.

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

Significance levels are calculated for the difference between extensor digitorum longus (EDL) and soleus muscle: **P*<0.05, ***P*<0.01 (unpaired *t*-test).

Table 2. *Characteristics of force and Ca^{2+} transients of fibre bundles of the extensor digitorum longus and the soleus muscles of* Suncus etruscus *at 25 °C*

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

Values are means ± S.D. for *N* measurements per muscle bundle.

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

Significance levels are calculated for the difference between extensor digitorum longus (EDL) and soleus: **P*<0.05, ***P*<0.01 (unpaired *t*-test).

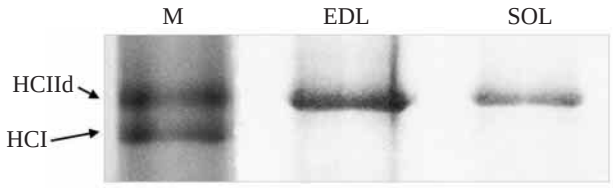

Fig. 2. Myosin heavy chain composition of extensor digitorum longus (EDL) (17 pooled muscles) and soleus (SOL) (16 pooled muscles) of *Suncus etruscus* demonstrated by electrophoresis. Lane M, myosin heavy chains HCI and HCIId of rabbit muscles.

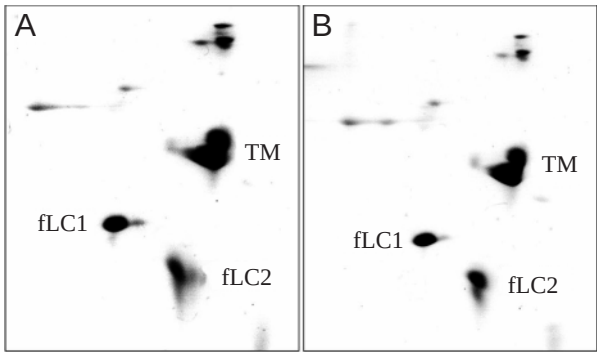

Fig. 3. Myosin light chain composition (fLC1 and fLC2) of extensor digitorum longus (A) and soleus (B) muscle of *Suncus etruscus* separated by two-dimensional electrophoresis. TM, tropomyosin.

decay of the cytosolic Ca^{2+} concentration were significantly faster in EDL muscle (Table 2).

To reveal the reasons for the functional difference between these two muscles, we investigated their fibre composition, their composition of myosin heavy and light chains and the activity of two enzymes important for ATP generation, lactate dehydrogenase and citrate synthase.

Myosin chain composition

Histochemical staining for myosin ATPases revealed identical fibre type patterns in the EDL and soleus muscle. All fibres showed the same degree and pattern of staining, indicating the presence of only alkaline-resistant myosin ATPase, which is typical of type II fibres. Immunochemistry using monoclonal antibodies against type I and type II myosin heavy chains also revealed only the presence of heavy chain type II in all leg muscles. In addition, SDS–PAGE and two-dimensional electrophoresis of EDL and soleus muscles showed no difference in the pattern of myosin heavy chains and myosin light chains. Only one type of heavy chain is found in both muscles, and the same holds for the diaphragm muscle of *S. etruscus*. This heavy chain type was identified as the isoform HCIId by comparison with myosin electrophoresis from rabbit muscles (Fig. 2). The same two types of fast light chains, fLC1 and fLC2, with molecular masses of 24 and 21.5 kDa, were detected in the EDL and soleus muscle (Fig. 3). Moreover, in a mixture of leg muscles from the Etruscan shrew, only these fast light chains were detected.

In contrast to larger mammals, no slow-twitch type I fibres

Table 3. *Time to peak force of isometric extensor digitorum longus and soleus twitches and the fibre composition of the two muscles in mammals of different size at 25 °C*

		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)
Wistar rat	200	27*	97/3	80*	0/100	Geers and Gros (1990)
Guinea pig	350	20	94/6	94	0/100	Asmussen and Gaunitz (1989)

EDL, extensor digitorum longus.
t_p, time to peak force; *converted from 20.5 to 25 °C using Q_{10} values obtained from Asmussen and Gaunitz (1989).

Table 4. *Fibre composition of the extensor digitorum longus and soleus muscles of some shrew species*

Shrew species	Body mass (g)	Fibre types, I/IIA/IIB/IID (%) EDL	Soleus
Suncus etruscus	2.2	0 / 0 / 0 / 100	0 / 0 / 0 / 100
*Sorex araneus**	7–10	–	0 / 0 / 2 / 98
Crocidura russula	8.4	0 / 0 / 58 / 42	0 / 0 / 54 / 46
Suncus murinus†	20–60	–	0 / 93 / 7 / 0

EDL, extensor digitorum longus.
*Data from Savolainen and Vornanen (1995); †data from Suzuki (1990).

Table 5. *Specific activities of lactate dehydrogenase and citrate synthase in the extensor digitorum longus, soleus and gastrocnemius muscles of* Suncus etruscus

Muscle	Specific activity LDH/c_{pr} (mmol min^{-1} mg^{-1})	Specific activity CS/c_{pr} (mmol min^{-1} mg^{-1})	LDH/CS activity ratio
EDL (N=17)	1.31	0.94	1.4
Soleus (N=16)	1.14	0.84	1.4
Gastrocnemius (N=4)	1.49	1.28	1.2

c_{pr}, cytoplasmic protein concentration given in mg ml^{-1}; N is the number of pooled muscles.
LDH, lactate dehydrogenase; CS, citrate synthase; EDL, extensor digitorum longus.

are present in the EDL and soleus muscle of *S. etruscus*. As shown in Table 3, the number of type I fibres in soleus muscles decreases and the percentage of fast-twitch fibres increases with decreasing body mass in a range of mammals. Shrew muscles, in general, seem to be composed of type II fibres only but, within this family, there is a correlation between body mass and HCII-subtype composition (Table 4). The number of type IIA fibres decreases and the number of IID fibres increases with decreasing body mass, leading to the unique situation that all the muscles of the smallest mammals are probably composed solely of type IID fibres. Nevertheless, as shown by the contraction measurements (Tables 1, 2), there are functional differences between different muscles.

Activity of metabolic enzymes

Can the functional difference between the EDL and soleus muscles of *S. etruscus* be attributed to different activities of the glycolytic and the oxidative metabolic pathway? Measurements of the activities of lactate dehydrogenase (LDH) and citrate synthase (CS) revealed no significant difference in activities between the two muscles (Table 5), suggesting that the functional difference between the EDL and soleus muscles cannot be explained by different activities of CS and LDH. The gastrocnemius muscle also did not differ from the EDL and soleus with respect to the activities of these enzymes. Moreover, the specific activity of CS turned out be higher in the muscles of *S. etruscus* than in those of other mammals, whereas the activity of LDH was much lower than in larger species. The ratio of LDH/CS activity, given in Fig. 4 as a function of body mass, is almost 1 in the smallest mammal, a value that is smaller than in any other mammal.

The measured enzyme activities indicate that the function of the type IID fibres depends almost entirely on oxidative ATP production; the activity of the glycolytic pathway appears to be negligible. The importance of oxidative metabolism is also underlined by the finding of a very high capillary density of up to 2800 mm^{-2} in the soleus muscle of the Etruscan shrew (Pietschmann et al., 1982) and a very high volume fraction of mitochondria (0.23 in leg muscles, 0.35 in diaphragm) (Hoppeler et al., 1981). Moreover, because of the foldings, the surface area of the inner mitochondrial membranes is considerably larger in the shrew than, for instance, in man (Bartels, 1980). All Etruscan shrew muscles studied to date are also uniform with respect to their myoglobin content, which is approximately 150 µmol l^{-1}.

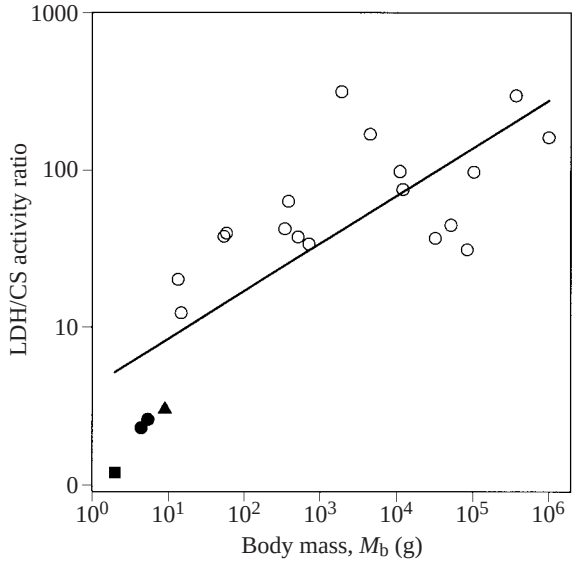

Fig. 4. Ratio of lactate dehydrogenase to citrate synthase (LDH/CS) activity in gastrocnemius muscle of mammals of different body masses (M_b). The symbols represent data for *Suncus etruscus* (■) and *Crocidura russula* (▲) (Peters et al., 1999) together with those for the shrew *Sorex vagrans* (body mass 4–5 g) (●) and several larger mammals (○), 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: $\log(\text{LDH/CS})=0.304\log M_b+0.623$; $r^2=0.62$).

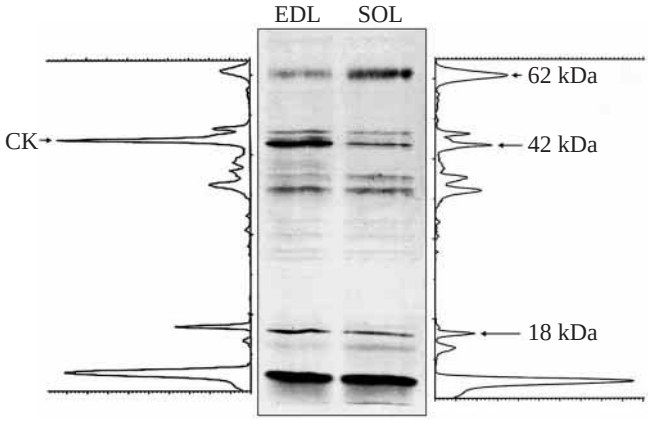

Fig. 5. Pattern of electrophoretically separated cytosolic proteins and corresponding densitograms of extensor digitorum longus (EDL) (17 pooled muscles) and soleus (SOL) (16 pooled muscles) of *Suncus etruscus*. CK, creatine kinase.

Specialization of Etruscan shrew muscles

From the results of the myosin studies and a consideration of energy metabolism, it can be concluded that oxidative fast-type IID fibres are most appropriate to meet the demands of the smallest mammal. The lighter the species, the less important are slow-twitch types of fibre, which constitute muscle specialized for static contractions to maintain posture. Gravitational forces, which are proportional to body mass, decrease with decreasing body size, and the locomotory behaviour of the smallest mammals reveals that moving is much more important for them than standing.

Type IID fibres combine a high shortening velocity which, according to studies in rats is highest in type IIB fibres, a little lower in type IID fibres and considerably lower in type IIA and I fibres, and a low fibre diameter, which is lowest in type I and IIA fibres, a little larger in type IID fibres and largest in type IIB fibres (Galler et al., 1994). Provided that the fibre properties found in the rat also hold for shrews, type IID fibres seem to be an appropriate compromise between a high ATP turnover, which is required not only for physical performance but also for heat production, and a small fibre diameter which, together with a high capillary density, enables a high oxygen flux into the fibre and, hence, a high rate of ATP production, as a result of the short diffusion distance for oxygen between the blood and the mitochondria.

The importance of the oxidative metabolic pathway is supported by the capacity of the shrew's type IID fibres to store oxygen bound to myoglobin. The myoglobin concentration is intermediate: type I and IIA fibres are known to have a myoglobin concentration higher than 150 μmol l^{-1}, and type IIB fibres are free of myoglobin in all mammals studied so far. The activity of metabolic enzymes leads to the conclusion that aerobic metabolism may meet any level of energy demand by the animal so that glycolytic metabolism is not necessarily required for ATP production. The glycolytic metabolic pathway may be underdeveloped 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 activity.

Reasons for the functional differences between the EDL and soleus muscles of Suncus etruscus

Despite the uniformity of Etruscan shrew muscles with regard to their myosin composition and their metabolic enzyme activities, differences in contraction times and the kinetics of Ca^{2+} transients have been observed. The rate at which Ca^{2+} is released from the sarcoplasmic reticulum and subsequently resequestered is significantly higher in the EDL than in the soleus muscle of *S. etruscus* (Table 2). A shorter relaxation time could be the result of a higher concentration of parvalbumin in the EDL, but immunohistochemical investigations have provided no evidence for the presence of parvalbumin in shrew muscles (Peters et al., 1999). A larger volume fraction of sarcoplasmic reticulum and, hence, greater numbers of Ca^{2+} channels and higher Ca^{2+}-ATPase activity in the EDL could also be responsible, but these have not been measured.

The results might be explained, at least in part, by an effect of creatine kinase (CK) activity on Ca^{2+}-ATPase activity. From electrophoretic studies of cytoplasmic proteins (Fig. 5) and

subsequent immunoblotting with anti-CK antibodies, it has been shown that cytosolic CK is present in remarkable amounts in shrew muscles and that its concentration in the EDL muscle is three times higher than in the soleus muscle. The presence of CK close to the Ca^{2+}-ATPase of the sarcoplasmic reticulum has been shown to enhance Ca^{2+} release and uptake rates in the muscles of mice (Steeghs et al., 1997). CK activity is functionally coupled to the activity of the Ca^{2+}-ATPase because CK controls the local [ATP]/[ADP] ratio at the sarcoplasmic reticulum and, thus, determines the ATP concentration available for the Ca^{2+}-ATPase and, therefore, the velocity of muscle relaxation (Minajeva et al., 1996).

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