

ATP utilization for calcium uptake and force production in skinned muscle fibres of *Xenopus laevis*

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1. A method has been developed to discriminate between the rate of ATP hydrolysis associated with calcium uptake into the sarcoplasmic reticulum (SR) and force development of the contractile apparatus in mechanically or saponin-skinned skeletal muscle fibres. The rate of ATP hydrolysis was determined in fibres of different types from the iliofibularis muscle of *Xenopus laevis* by enzymatic coupling of ATP re-synthesis to the oxidation of NADH.
2. The ATPase activity was determined before and after exposure of the preparations for 30 min to a solution containing 0.5% Triton X-100, which effectively abolishes the SR ATPase activity. The fibres were activated in a solution containing 5 mM caffeine to ensure that calcium uptake into the SR was maximal.
3. At saturating Ca^{2+} concentrations the actomyosin (AM) and SR ATPase activities in fast-twitch fibres, at 4.3 °C, amounted to 1.52 ± 0.07 and $0.58 \pm 0.10 \mu\text{mol s}^{-1} (\text{g dry wt})^{-1}$, respectively (means \pm s.e.m.; $n = 25$). The SR ATPase activity was 25% of the total ATPase activity. At submaximal calcium concentrations the AM ATPase activity varied in proportion to the isometric force.
4. The calcium sensitivity of the SR ATPase was larger than that of the AM ATPase and its dependence on $[\text{Ca}^{2+}]$ was less steep. The AM ATPase activity was half-maximal at a pCa of 6.11 (pCa = $-\log[\text{Ca}^{2+}]$) whereas the SR ATPase activity was half-maximal at a pCa of 6.62.
5. In Triton X-100-treated fibres, at different 2,3-butanedione monoxime (BDM) concentrations, the AM ATPase activity and isometric force varied proportionally. The SR ATPase activity determined by extrapolation of the total ATPase activity in mechanically skinned or saponin-treated fibres to zero force, was independent of the BDM concentration in the range studied (0–20 mM). The values obtained for the SR ATPase activity in this way were similar to those obtained with Triton X-100 treatment.
6. The AM ATPase activity in slow-twitch fibres amounted to $0.74 \pm 0.13 \mu\text{mol s}^{-1} (\text{g dry wt})^{-1}$, i.e. about a factor of two smaller than in fast-twitch fibres. The SR ATPase activity amounted to $0.47 \pm 0.07 \mu\text{mol s}^{-1} (\text{g dry wt})^{-1}$, i.e. rather similar to the value in fast-twitch fibres. The proportion of the total ATPase activity that was due to SR ATPase (40%) was larger than in fast-twitch fibres.
7. The temperature dependence of the AM and SR ATPase activities in fast-twitch fibres differed. In the temperature range 5–10 °C, the relative changes in AM and SR ATPase activities for a 10 °C temperature change (Q_{10}) were 3.9 ± 0.3 and 7.2 ± 1.5 , respectively. In the temperature range 10–20 °C, the Q_{10} values of the AM and SR ATPase activities were, respectively, 2.6 ± 0.4 and 3.1 ± 0.5 . As a result the SR ATPase activity at high temperature was a larger fraction of the total ATPase activity than at low temperature.

The most important energy-requiring processes in skeletal muscle are activation, which involves the release and uptake of calcium ions by the sarcoplasmic reticulum (SR), and contraction, i.e. the actomyosin (AM) interaction resulting in force development and muscle shortening. The energy required is liberated by hydrolysis of ATP. The energetic aspects of calcium cycling and the actomyosin interaction have been studied mainly in intact muscle by measuring the production of activation and maintenance heat. On the other hand, the structures and the fundamental mechanisms involved in calcium transport were mainly investigated *in vitro*, in isolated SR fragments or vesicles (for reviews see Homsher & Kean, 1978; Martonosi & Beeler, 1983; Inesi, 1985; Woledge, Curtin & Homsher, 1985).

These investigations have provided a general framework for our understanding of the energy cost of activation and contraction during tetanic contractions. However, much less insight is available concerning energy utilization during submaximal contractions. Calcium is an important regulator of contractile activity. The intracellular free calcium ion concentration is the result of several calcium fluxes, including both Ca^{2+} uptake and release, which are $[\text{Ca}^{2+}]$ dependent. From the energetic point of view the Ca^{2+} (inter)dependence of the AM and SR ATPase activities is very important, because it influences the overall muscle efficiency. Therefore, we wanted to study both processes simultaneously in the same preparations.

It has been shown that both the SR and the actomyosin interactions are functionally intact in mechanically skinned preparations, i.e. preparations from which the surface membrane has been removed. This is also the case in preparations in which the surface membrane has been permeabilized by incubation of the fibre for 30 min or less in a solution containing the glycoside saponin ($50 \mu\text{g ml}^{-1}$) (Endo & Iino, 1980). The non-ionic detergent Triton X-100 effectively abolishes the SR and to quantify the AM and SR ATPase activities in the same preparations, we therefore compared the ATPase activities before and after Triton X-100 treatment. Other methods which can be employed in skinned preparations for this purpose are based on selective pharmacological actions on the AM or the SR ATPase activities. BDM (2,3-butanedione monoxime) inhibits force production as well as the AM ATPase activity (Higuchi & Takemori, 1989). It has previously been used in low concentrations (2–5 mM) to study activation heat in cardiac muscle (e.g. Alpert, Blanchard & Mulieri, 1989). Several studies, however, demonstrated a dose-dependent effect of BDM on the shape of the Ca^{2+} transients, both in cardiac muscle (e.g. Gwathmey, Hajjar & Solaro, 1992) and in skeletal muscle (Horiuti, Higuchi, Umazume, Konishi, Okazaki & Kurihara, 1988), which might interfere with these determinations. BDM has the advantage over Triton X-100 treatment that its action is reversible, but because it might interfere with Ca^{2+} handling, we decided to employ both methods.

An important advantage of skinned fibres over intact fibres in studying the energy utilization during contraction is that the intracellular composition, for instance the calcium concentration, can be accurately controlled. Moreover, direct access to intracellular structures makes it possible to distinguish easily between different energy-requiring processes.

In *in vitro* studies, the temperature sensitivity of the SR Ca^{2+} pump has been investigated (e.g. Inesi, Millman & Electr, 1973). This temperature sensitivity is important for the resolution of the structural or enzymatic factors determining the SR ATPase activity and, in view of possible differences between *in vitro* and *in vivo* results, we considered it of interest to determine the temperature sensitivity of the SR ATPase activity in skinned fibres. This temperature sensitivity is important also for comparison of our skinned-fibre data, which were mainly obtained at low temperature, with the results from energetic studies on single *Xenopus* fibres performed at room temperature (e.g. Elzinga, Lännergren & Stienen, 1987; Lännergren, Elzinga & Stienen, 1993).

Previously, it has been found that the activation heat rate is a fairly constant fraction (30–50%) of the total energy expenditure over a sixtyfold variation in the rate of heat production for different muscles (Homsher & Kean, 1978). This implies that the energy costs of Ca^{2+} uptake and contraction co-vary. The variation in contractile proteins results from there being a large variety of actin and myosin isoforms. For the SR Ca^{2+} pump only two different isoforms are known, with very similar activities (cf. Martonosi & Beeler, 1983) but the Ca^{2+} ATPase content is variable. For instance, Leberer & Pette (1986) found that, in rabbit, Ca^{2+} ATPase content in fast-twitch skeletal muscle was 6–7 times higher than in slow-twitch muscles. To study these differences in fibres from *Xenopus* in more detail, we determined the AM and SR ATPase activities in slow-twitch and in fast-twitch fibres originating from the same muscle.

In summary, apart from a validation of the methods used to discriminate between the AM and SR ATPase activities in single skinned muscle fibres, this study focuses on the Ca^{2+} , temperature and fibre type dependences of the AM and SR ATPases. As these are the major sources of ATP hydrolysis, these results give information about the partitioning of most of the energy required during muscle contraction. This makes it possible to explain several of the differences in muscle efficiency observed experimentally.

METHODS

Preparation

Adult female *Xenopus laevis* (clawed frogs) were kept in tap water at room temperature and fed with meal worms or SDS Amphibia diet 3 (Special Diet Services, Witham, UK) every second day. The animals were killed by rapid decapitation, followed by pithing. Thin fibre bundles of about 1 mm in

diameter were dissected from the outer dorsal zone of the iliofibularis muscle. From the fibre bundles, thick fast-twitch fibres were selected. These fibres are of type 1 or type 2, as described by Lännergren & Smith (1966). Slow-twitch fibres with a granular appearance when viewed with a dissecting microscope under dark field illumination (type 3) were dissected from the tonus bundle. Fibre segments of about 6 mm length were isolated in cold dissecting solution. The composition of the dissecting solution was (mM): Na₂ATP, 7.3; MgCl₂, 10.6; ethylene glycol-bis(β -aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA), 20; creatine phosphate, 10; *N,N*-Bis[2-hydroxyethyl]-2-aminoethanesulphonic acid (BES), 100; pH 7.0 (adjusted with KOH); ionic strength 200 mM (adjusted with KCl). In isolating fibres, care was taken not to stretch them unduly. Each fibre segment was divided into two parts; one was used for histochemical fibre typing (as described below) and the other was skinned and used for the ATPase measurements.

In general the fibres were chemically skinned by incubating them in cold dissecting solution (~5 °C) to which 50 $\mu\text{g ml}^{-1}$ saponin was added for 30 min. This procedure permeabilizes the sarcolemma but leaves the sarcoplasmic reticulum functionally intact (Endo & Iino, 1980; Horiuti, 1986). In other cases, especially when the temperature sensitivities of force and ATP utilization were studied, fibres were skinned mechanically and split in bundles of myofibrils. Fibre diameters were measured in two perpendicular directions by means of a dissection microscope of $\times 50$ magnification. Cross-sectional area was calculated assuming an elliptical cross-section. Fibre length was measured at $\times 20$ magnification. After the measurements the preparation was dried in air and, after subsequent freeze-drying (4K Modulyo, Edwards, Crawley, UK), its dry weight was determined by means of an electrobalance (Model 29, Cahn Instruments, Cerritos, CA, USA).

The preparations were mounted in the experimental set-up (Stienen, Roosemalen, Wilson & Elzinga, 1990) by means of aluminium T-clips (Goldman & Simmons, 1984). The holes in these clips were passed over hooks, one of which connected to a carbon fibre extending a force transducer element (AE 801, SensoNor, Horten, Norway) and the other to a glass rod connected to a manipulator. Sarcomere length was measured in relaxing solution by means of He-Ne laser diffraction and was adjusted to 2.3 μm .

Fibre typing

The segment for fibre typing was taken out of the dissecting solution, put on a small piece of aluminium foil and frozen in liquid nitrogen. Subsequently it was freeze-dried and stored at -80 °C for a few weeks until a sufficient number of segments of different type had been obtained. These fibre segments were then transferred to a microwell plate and assayed for succinate dehydrogenase in a solution described by Pool, Diegenbach & Scholten (1979). This incubation medium consisted of (mM): sodium phosphate buffer (pH 7.6), 37; sodium succinate, 74; and Tetranitro Blue tetrazolium, 0.4. In this medium, slow-twitch type 3 fibres, which are rich in mitochondria, stained black quickly (< 2 min). Fast-twitch fibres (type 1 or 2) remained transparent or stained slightly during a longer period (~ 5 min).

ATPase activity and force measurements

The apparatus used to measure the ATPase activity has been described previously (Stienen *et al.* 1990). It consisted of

several temperature-controlled troughs in which the fibre could be immersed. During the actual measurement of ATPase activity, the preparation was kept in a small trough with quartz windows, of 30 μl volume. Hydrolysis of ATP inside the fibre was linked to the oxidation of NADH, measured photometrically by the absorption at 340 nm of near UV light from a 150 W xenon light source that passed beneath the fibre. The solution in the chamber was continuously stirred. The absorbance signal obtained was linearly related to the NADH concentration inside the measuring chamber and its slope was a measure of the ATPase activity of the preparation. Calibration of the absorption signal was carried out after each recording by adding a known amount of ADP to the solution via a stepper motor-controlled pipette. The rate of ATP hydrolysis was derived from the slope of the absorbance signal relative to the baseline found after exposure of the fibre to the solution. This minor change in baseline when the fibre was not present in the measuring chamber was the combined result of NADH bleaching by the UV light, contaminating ATPases present in the enzymes used (mainly in lactate dehydrogenase (LDH)), and slight evaporation of the solution during the measurements. The composition of the solutions used during the experiments is shown in Table 1.

Three different bathing solutions were used: a relaxing solution, a pre-activating solution with low EGTA concentration and an activating solution. The composition of the solutions was calculated with a computer program similar to that of Fabiato & Fabiato (1979), using the equilibrium constants given by Godt & Lindley (1982). The temperature sensitivity of the equilibrium constants was taken into account when making the solutions. Solutions with a lower concentration of calcium were obtained by appropriate mixing of the relaxing and activating solutions assuming an apparent stability constant for the Ca²⁺-EGTA complex at 4 °C of 10^{6.77}. The temperature in the measuring chamber was measured routinely during the experiments.

During the experiments, a segment was incubated in relaxing solution for 4 min, in pre-activating solution for 4 min, and in activating solution until a steady force level was attained, and from there it was transferred back again into relaxing solution. In the experiments in which the calcium or BDM concentration was varied, the ATPase activity was measured during control conditions, i.e. at saturating calcium concentrations without BDM. Thereafter, the measurement was repeated at different submaximally activating calcium or different BDM concentrations, followed by a maximally activating control measurement. Subsequently, the preparation was incubated for 30 min in dissecting solution containing 0.5% (v/v) Triton X-100 to disrupt the SR membrane at ~5 °C and the measurements were repeated. All measurements were carried out in the presence of 5 mM caffeine, 10 μM oligomycin B and 5 mM sodium azide. In some cases the specific inhibitor of the SR Ca²⁺ pump cyclopiazonic acid (CPA) was used at a concentration of 20 μM (cf. Kurebayashi & Ogawa, 1991).

The experiments were terminated when isometric force during the control measurements was < 80% of the force of the first activation. When the maximum isometric force found during the control measurement before and after Triton treatment differed by more than $\pm 10\%$, the measurements were discarded.

Force and ATPase activity were recorded with a pen recorder and after analog-to-digital conversion by a computer (M280,

Table 1. Composition of the solutions (mM)

Solution	[MgCl ₂]	[Na ₂ ATP]	[PEP]	[EGTA]	[HDTA]	[CaEGTA]	[KProp]
Standard							
Relaxing	7.35	5.58	5	20	—	—	88.6
Pre-activating	6.96	5.58	5	0.5	19.5	—	89.4
Activating	6.82	5.66	5	—	—	20	89.3
Temperature 5 °C							
Relaxing	8.01	6.06	10	20	—	—	46.4
Pre-activating	7.78	6.06	10	0.5	19.5	—	46.9
Activating	7.63	6.17	10	—	—	20	46.7
Temperature 20 °C							
Relaxing	8.31	5.79	10	20	—	—	34.7
Pre-activating	7.78	5.79	10	0.5	19.5	—	35.8
Activating	7.63	5.86	10	—	—	20	35.7

PEP, phosphoenolpyruvate; HDTA, hexamethylene diamine-tetraacetate. All solutions contained in addition 4 mg ml⁻¹ pyruvate kinase (500 U ml⁻¹, Sigma), 0.24 mg ml⁻¹ lactic dehydrogenase (870 U ml⁻¹, Sigma), 5 mM sodium azide, 10 μM oligomycin B, 0.8 mM NADH and 0.2 mM p¹,p⁵-di(adenosin-5')pentaphosphate. Potassium propionate (KProp) was added to adjust ionic strength to 200 mM. CaEGTA was made by dissolving equimolar amounts of CaCO₃ and EGTA. The free Mg²⁺ and MgATP concentrations were, respectively, 1 and 5 mM. The standard solutions contained 100 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid (Tes). The solutions used for the temperature experiments contained 100 mM *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid (Bes). The pH was adjusted with KOH to 7.1. The standard solutions were used in all experiments except those in which the temperature sensitivity was studied. The solutions used at 10 and 15 °C were obtained by mixing the corresponding 5 and 20 °C solutions in a 2:1 and a 1:2 proportion, respectively.

Olivetti) at a sampling rate of 10 Hz. The Ca²⁺ sensitivity of isometric force was approximated by means of the Hill curve:

$$\frac{F_{Ca}}{F_0} = \frac{[Ca^{2+}]^n}{([Ca^{2+}]_{50})^n + [Ca^{2+}]^n}$$

In this equation, F_0 indicates the maximum isometric force level at saturating Ca²⁺ concentration and n is an index of cooperativity of Ca²⁺ binding and cross-bridge attachment to the actin filament. $[Ca^{2+}]_{50}$ denotes the Ca²⁺ concentration at which force is half-maximal.

The temperature coefficient (Q_{10}) in a range of temperatures from T_1 to T_2 was calculated as follows:

$$Q_{10} = \left(\frac{\text{ATPase activity at } T_2}{\text{ATPase activity at } T_1} \right)^{10/(T_2 - T_1)}$$

In this way Q_{10} represents the change in ATPase activity with a 10 °C change in temperature. The Q_{10} for force development was calculated accordingly.

Data values are given as means ± s.e.m. of n experiments. Regression lines between force and ATPase activity obtained at different Ca²⁺ or BDM concentrations were calculated with force as independent variable. Differences between mean values were statistically tested by means of Student's t test at a 0.05 level of significance ($P < 0.05$).

RESULTS

Discrimination between AM and SR ATPase activities by Triton X-100 treatment

The method used to determine the AM and SR ATPase activities is illustrated in Fig. 1. In this figure ATPase activity and force development are shown for a saponin-treated fast-twitch fibre before and after Triton X-100 treatment. The experiments were carried out in the presence of 5 mM caffeine, which under our experimental conditions is very effective in inducing Ca²⁺ release from the SR (e.g. Endo, 1985; Stienen, van Graas & Elzinga, 1993).

Caffeine thus prevented the inhibition of the SR ATPase activity by Ca²⁺ inside the SR. It was verified that 5 mM caffeine was sufficient in a separate series of experiments in which the caffeine concentration was varied in the range from 2 to 20 mM. In the presence of caffeine, the total ATPase activity was slightly larger than in its absence, but it was independent of the caffeine concentration *per se*.

It can be seen in Fig. 1 that the ATPase activity was reduced substantially after Triton X-100 treatment while force development was only slightly reduced. The average

decrease in force during these experiments was 4%. Control experiments indicated that the ATPase activity left after Triton X-100 treatment reflects the ATPase activity of the actomyosin (cross-bridge) interaction: the AM ATPase activity. In these experiments it was found that the resting or basal ATPase activity measured in relaxing solution (pCa 9) was very small. The average basal activity (\pm s.e.m.) found in eight mechanically or saponin-skinned fibres amounted to $1 \pm 1\%$ of the maximal Ca^{2+} -activated activity. The basal ATPase activity of saponin or mechanically skinned fibres at pCa 9 was very similar to the basal activity found in Triton X-100-treated preparations and it was not affected by $20 \mu\text{M}$ cyclopiazonic acid (CPA). CPA, an inhibitor of the SR Ca^{2+} pump, also had no effect on the basal or the maximal Ca^{2+} -activated ATPase activity in Triton X-100-treated fibres. However, the effect of $20 \mu\text{M}$ CPA in fully activated mechanically or saponin-skinned preparations was substantial, indicating a pronounced activity of the SR Ca^{2+} pump in these fibres. In fact, as is illustrated below (Fig. 4), the ATPase activity left with $20 \mu\text{M}$ CPA represents almost exclusively the actomyosin ATPase. Therefore, before Triton X-100 treatment, the ATPase activity measured reflects the sum of AM and SR ATPase activities and the difference in ATPase activity before and after Triton X-100 treatment thus reflects the SR ATPase activity.

In fast-twitch fibres ($n = 25$), the ATPase activity was determined before and after Triton X-100 treatment. The average reduction in ATPase activity after Triton X-100

treatment, i.e. the SR ATPase activity, was $25.1 \pm 1.7\%$ of the total activity. The total ATPase activity before Triton X-100 treatment amounted to $2.04 \pm 0.10 \mu\text{mol s}^{-1}$ (g dry wt^{-1}). The average AM ATPase activity in fast-twitch fibres amounted to $1.52 \pm 0.07 \mu\text{mol s}^{-1}$ (g dry wt^{-1}) and the average SR ATPase activity was $0.58 \pm 0.10 \mu\text{mol s}^{-1}$ (g dry wt^{-1}). The corresponding average values expressed per litre cell volume were 0.15 and 0.06 mM s^{-1} , respectively. Assuming that the density of myosin heads which participate during contraction is 0.2 mM (cf. Glyn & Sleep, 1985), the AM ATPase activity corresponds to a rate of ATP turnover per myosin head of 0.8 s^{-1} . In intact *Xenopus* fibres the SR Ca^{2+} pump content is similar to that of fast mammalian muscle, i.e. about 0.04 mM (Leberer & Pette, 1986; Simonides & van Hardeveld, 1990). In skinned fibres, the volume is increased osmotically by about a factor of two. This reduces the concentration of Ca^{2+} pumps to about 0.02 mM . Assuming a stoichiometry of the Ca^{2+} pump in the SR of 2 Ca^{2+} per ATP, the SR ATPase rate corresponds to a catalytic activity of the SR Ca^{2+} pump of 6 s^{-1} .

Calcium dependence of the AM and SR ATPase activities

The calcium dependences of the AM and SR ATPase activities in saponin-treated fibres were determined by varying the free Ca^{2+} concentration during activation of the fibre. Figure 2 shows the relation between the isometric force and the total (AM + SR) ATPase activity obtained from these experiments.

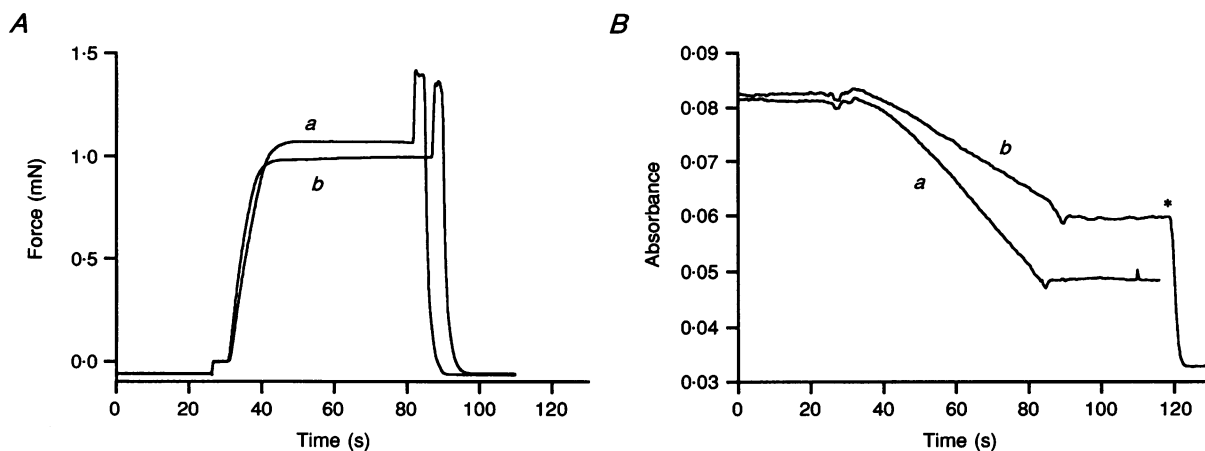


Figure 1. Force and ATPase activity in a fast fibre chemically skinned by means of saponin

Left-hand recordings, force development; right-hand recordings, NADH absorbance. Traces marked *a* were obtained *before* while traces marked *b* were recorded *after* abolishing the SR ATPase activity of the fibre with Triton X-100. The fibre was activated by transferring it from the pre-activating solution (pCa 4.5) into the activating solution (pCa 9), and relaxed at the end of the measurement by transferring it back into relaxing solution (pCa 9). At the asterisk a calibration of the absorbance signal was carried out which corresponded to 0.5 nmol of ATP hydrolysed. The zero level in the absorbance signal was arbitrarily chosen. Experimental conditions: fibre diameters, $220/190 \mu\text{m}$; fibre segment length, 2.15 mm ; temperature, $5.2 \text{ }^\circ\text{C}$.

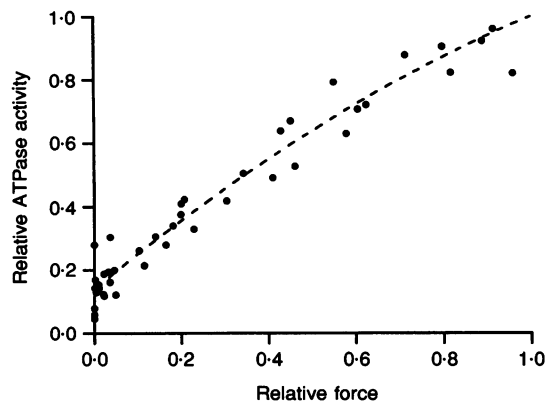


Figure 2. Relation between isometric force and total (AM + SR) ATPase activity of saponin-skinned fast fibres activated at different calcium concentrations

ATPase and force are normalized to the values obtained at saturating Ca^{2+} concentrations. Dashed line, second order polynomial fit: $y = ax^2 + bx + 1 - a - b$. The results at zero force were obtained at different Ca^{2+} concentrations below the threshold for force generation. These were excluded from the fit. Number of fibres, 16.

It can be seen that the relative ATPase activity in general is larger than the relative force. It can be seen also that the relation between ATPase activity and force is somewhat curved. In five fibres which were skinned by Triton X-100, force and ATPase activity at submaximal Ca^{2+} concentrations were determined and normalized to the maximum isometric values. Linear regression analysis in which force was treated as the independent variable yielded:

$$\begin{aligned} \text{Normalized ATPase activity} \\ = (0.04 \pm 0.03) + (0.93 \pm 0.05) \\ \times \text{normalized force } (r = 0.97). \end{aligned}$$

The intercept and slope of this regression line were not significantly different from 0 and 1, respectively. Thus in Triton-skinned fibres, force and ATPase activity at submaximal Ca^{2+} concentrations varied in proportion. This observation confirms that the ATPase activity left after

Triton X-100 treatment reflects the activity of the actomyosin interaction: the AM ATPase activity.

In Fig. 3A, the results shown in Fig. 2 are plotted as a function of the Ca^{2+} concentration. This figure clearly shows that the Ca^{2+} sensitivity of the total (AM + SR) ATPase activity differs from that of the isometric force. Since the Ca^{2+} sensitivities of the AM ATPase activity and force are identical, Fig. 3A implies that the SR ATPase activity is more sensitive to Ca^{2+} than the AM ATPase activity. An estimate of the Ca^{2+} sensitivity of the SR ATPase activity in fast-twitch fibres was obtained by assuming that at saturating Ca^{2+} concentrations the SR ATPase activity was 25.1% of the total ATPase activity (i.e. the average value found in the previous section) and that, at submaximal Ca^{2+} concentration, the AM ATPase activity was proportional to the isometric force. The SR ATPase activity, which remained after subtraction of the

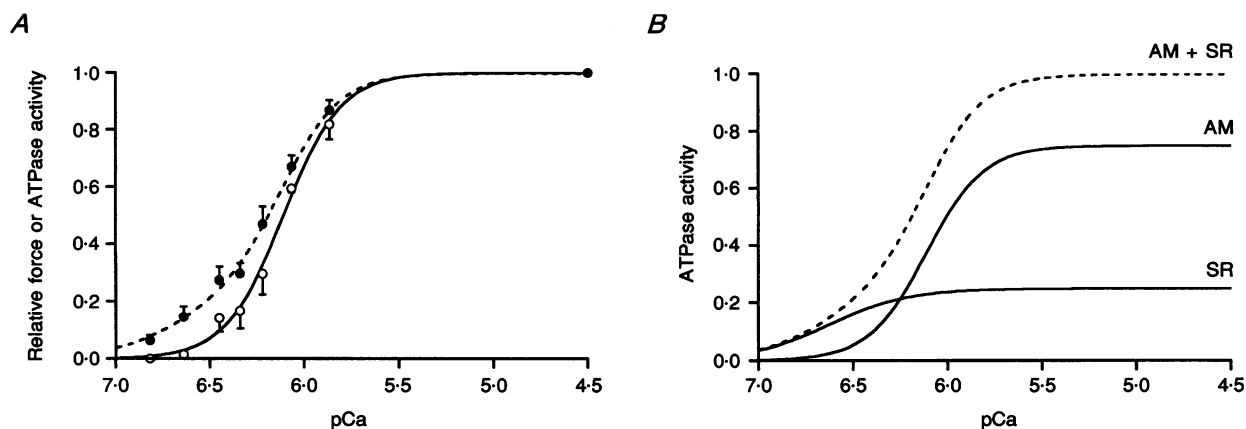


Figure 3. Calcium dependence of AM and SR ATPase activity

In A the results shown in Fig. 2 are plotted as a function of the calcium concentration. Continuous line, Hill curve fitted to isometric force; dashed line, sum of two Hill curves fitted to the isometric ATPase activity. This function was composed of two Hill functions, one for the AM ATPase activity obtained from the force-pCa relation, with a relative amplitude of 0.749, and the other for the SR ATPase activity, with a relative amplitude of $1 - 0.749$, i.e. 0.251. These amplitudes were obtained from the difference in ATPase activity before and after Triton X-100 treatment (see text). B, decomposition of the total (AM + SR) ATPase activity into the AM and SR components. The Hill parameters (means \pm standard error of the estimate) obtained for force and AM ATPase were $\text{pCa}_{50} = 6.11 \pm 0.01$ and $n = 2.87 \pm 0.17$. For the SR ATPase activity the fit yielded $\text{pCa}_{50} = 6.62 \pm 0.04$ and $n = 2.07 \pm 0.34$.

AM ATPase activity from the total ATPase activity, could be fitted by the sigmoidal (Hill) equation described in Methods, as was also the case for the isometric force. This is illustrated in Fig. 3*B*. The Hill equation fitted to isometric force and hence the AM ATPase activity yielded $pCa_{50} = 6.11 \pm 0.01$ and $n = 2.87 \pm 0.17$ (values are given as means \pm standard error of the estimate). For the SR ATPase activity the fit yielded $pCa_{50} = 6.62 \pm 0.04$ and $n = 2.07 \pm 0.34$. The ATPase activity of the SR therefore appeared to be more sensitive to Ca^{2+} and its dependence on the Ca^{2+} concentration less steep than that of the actomyosin interaction.

BDM dependence of the AM and SR ATPase activities

The effect of BDM on force and ATPase activity was investigated systematically in saponin-skinned fibres before and after Triton X-100 treatment up to a concentration of 10 mM. In a number of additional experiments on mechanically skinned fibres concentrations up to 40 mM were used. The effect of BDM on isometric

force and ATPase activity is illustrated in Fig. 4. It can be seen that BDM (20 mM, in this example) causes a considerable reduction in force and ATPase activity.

This figure also shows the effect of 20 μ M CPA, a specific inhibitor of the SR ATPase activity. The ATPase activity in the presence of 20 mM BDM is markedly depressed by CPA, indicating that a large fraction of the total ATPase activity which remains when force production is reduced is due to SR ATPase activity. As can be seen in Fig. 5*A*, active force was already reduced by 50% in the presence of 2 mM BDM. The effect of BDM on isometric force before and after Triton X-100 treatment was practically the same, but the depressing effect of BDM on ATPase activity (Fig. 5*B*) after Triton was larger than before. This indicates that a rather BDM-insensitive component of the total ATPase activity has been removed by Triton X-100. This can be seen more clearly in Fig. 6, in which the relative ATPase activity before and after Triton treatment shown in Fig. 5 is replotted as a function of the relative force. A linear relationship between force and ATPase activity was found before as well as after Triton X-100 treatment. Additional

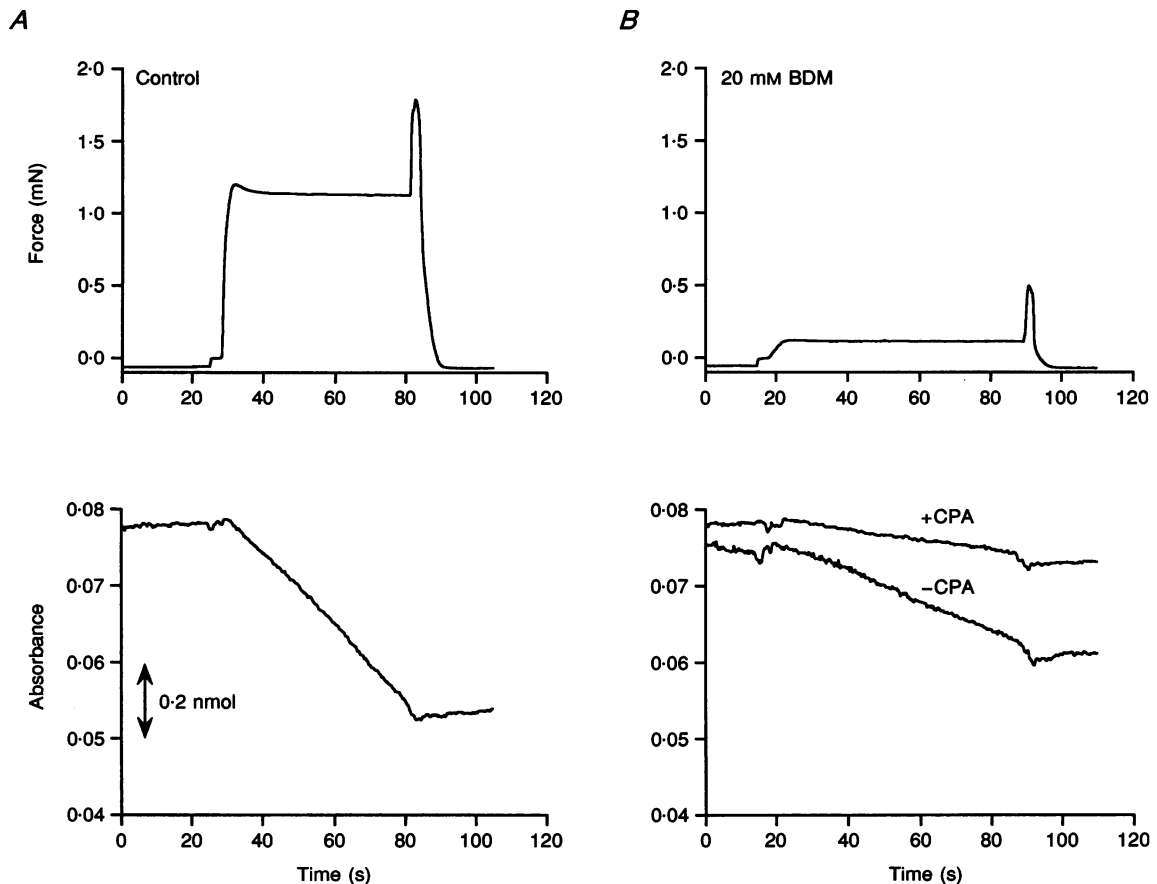


Figure 4. Effect of BDM on force and ATPase activity in a mechanically skinned fast fibre

Upper recordings show force, lower recordings NADH absorbance. *A*, control conditions; *B*, in the presence of 20 mM BDM. The absorbance traces marked + or -CPA were carried out in the presence and absence of 20 μ M CPA, respectively. CPA causes a marked reduction in the ATPase activity. The force recordings in the presence and absence of CPA were identical. Experimental conditions, fibre diameters, 180/140 μ m; fibre length, 2.35 mm; temperature, 4.5 $^{\circ}$ C.

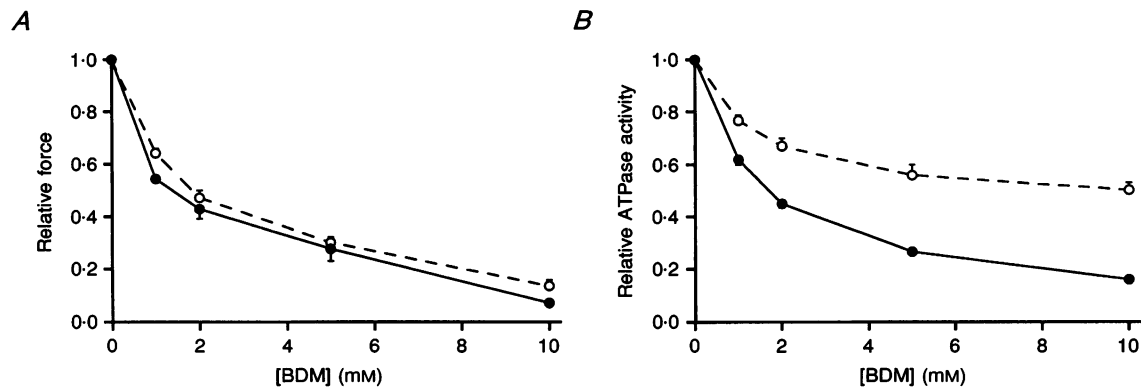


Figure 5. BDM dependence of isometric force (*A*) and ATPase activity (*B*) before and after treatment of saponin skinned fast-twitch fibres by Triton X-100

○, after saponin treatment; ●, after Triton X-100 treatment. The effect of BDM on isometric force was half-maximal at a concentration of 2 mM. The effect of BDM on relative ATPase activity was increased by the Triton X-100 treatment. The error bars denote s.e.m. values and are shown only when larger than the symbol size. Number of fibres, 10.

experiments performed on mechanically skinned fibres at 10, 20 and at 40 mM BDM indicated that the linearity of the force-ATPase relation before Triton treatment was preserved up to 20 mM BDM. After Triton treatment the relation was linear at least up to 40 mM BDM.

The intercept in the ATPase activity at zero force after Triton treatment was $5.8 \pm 1.6\%$ of the ATPase activity measured in the absence of BDM. This intercept was significantly different from zero and larger than the basal ATPase activity measured in relaxing solution. This indicates that a small residual ATPase activity remains after Triton X-100 treatment when force development is inhibited by BDM. This ATPase activity could be due to some membrane remnants and partly due to the basal, myosin ATPase activity. This residual activity is so small that it is very difficult to find out where it comes from. We consider it unlikely that it originates from remaining SR fragments since, as stated above, the ATPase activity at saturating calcium concentrations after Triton X-100 treatment was not affected in the presence of 20 μM CPA ($n = 2$). The linearity of the force-ATPase relationships both before and after Triton X-100 treatment indicates

that the SR ATPase activity is not influenced by BDM in the concentration range up to 20 mM.

The SR ATPase activity was determined in ten saponin-skinned and fifteen mechanically skinned preparations from the intercept in ATPase activity at zero force. It was found that the SR ATPase activity (mean \pm s.e.m.), expressed as a percentage of the total (AM + SR) ATPase activity, was $37.7 \pm 2.8\%$ in saponin skinned fibres and $26.9 \pm 1.4\%$ in mechanically skinned preparations. The average value for the SR ATPase activity obtained from all BDM experiments ($31.7 \pm 1.7\%$) was found to be significantly higher than the value obtained with Triton X-100 treatment (25.1%), due to the relatively high SR ATPase activity in the saponin-skinned fibres obtained with BDM. This difference, $\sim 6\%$, could be due to the residual ATPase activity which remains after Triton treatment. In the determinations of the SR ATPase activity by means of BDM, the extrapolation of the ATPase activity to zero force includes this residual activity, whereas in calculating the difference before and after Triton X-100 treatment the residual activity is included in the AM ATPase activity. However, the results were obtained on different batches of toads and,

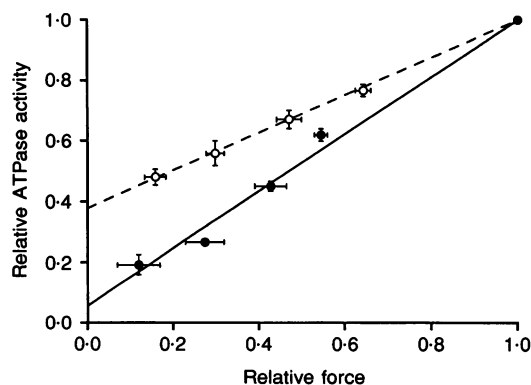


Figure 6. The relationship between isometric force and ATPase activity at different BDM concentrations before and after Triton X-100 treatment

The results of experiments at the same BDM concentrations were normalized to the values in the absence of BDM and then averaged. ○, before Triton X-100 treatment; ●, after Triton treatment. In the figure, BDM concentrations in increasing order (1, 2, 5 and 10 mM) correspond to ATPase activities in decreasing order. The error bars denote s.e.m. values. The lines were obtained by fitting a straight line: $y = a(x - 1) + 1$, to the individual data points.

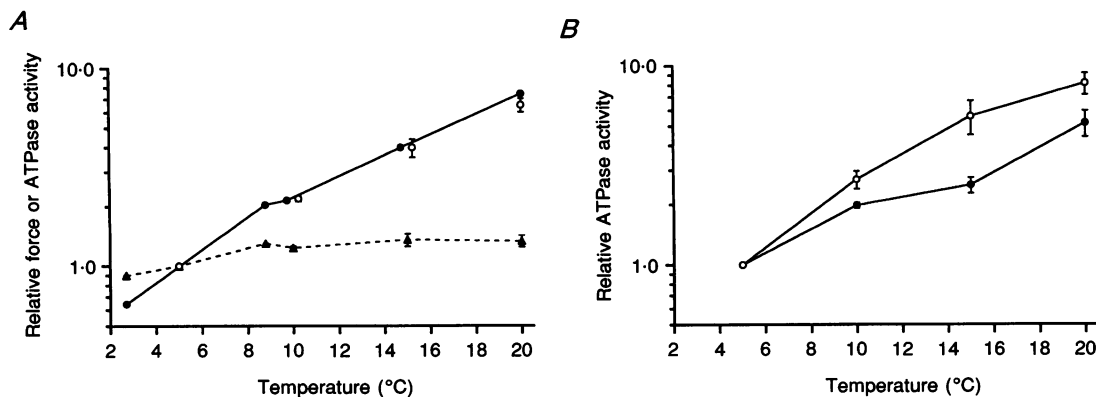


Figure 7. Temperature dependence of isometric force and ATPase activity

In *A* the results are shown before and after treatment of saponin-skinned fast-twitch fibres by Triton X-100. ▲, average force before and after Triton; ○, total (AM + SR) ATPase activity before Triton; ●, AM ATPase activity after Triton. *B*, the AM ATPase (●) and SR ATPase (○) activities obtained for mechanically skinned bundles of myofibrils by means of 20 mM BDM. Number of fibres, see Table 2.

in view of the variability in ATPase activity among fibres, batch differences may also be involved.

Temperature dependence of the AM and SR ATPase activities

To allow a correction for the differences in temperature at which the measurements were conducted, the temperature sensitivity of the ATPase activity after Triton X-100 treatment was systematically investigated at 2.7, 5.6 and 8.8 °C ($n = 11$). From these experiments the Q_{10} values of force and AM ATPase activity were calculated as indicated in Methods. The Q_{10} (mean \pm s.e.m.) value for force development amounted to 1.61 ± 0.06 and for the AM ATPase activity a value of 5.9 ± 0.4 was obtained. In addition, experiments were carried out ($n = 14$) in which the temperature sensitivity of force and ATPase activity was investigated before and after Triton X-100 treatment at about 5, 10, 15 and 20 °C. This allows us to make a comparison with heat measurements on tetanically stimulated intact muscle fibres from *Xenopus* (e.g. Elzinga *et al.* 1987), which were performed at 20 °C (see Discussion).

In most of the experiments mechanically skinned bundles of myofibrils with a minimum diameter of less than 130 μm were used to reduce P_i build-up and to prevent depletion of phosphoenolpyruvate inside the preparations. The results of these experiments are shown as a semilogarithmic plot in Fig. 7A. It can be seen that the isometric force is less temperature sensitive than the ATPase activity measured before as well as after Triton X-100 treatment. In general the temperature sensitivities decrease when the temperature is increased. An overview of the temperature coefficients is included in Table 2.

It can be seen that the temperature coefficients of the total ATPase activity before and after Triton X-100 treatment were fairly similar. The SR ATPase activity is a rather

small fraction of the total ATPase activity before Triton. Therefore, the temperature sensitivity of the SR ATPase activity could only be determined from these experiments with limited precision. To obtain an accurate estimate of the Q_{10} of the SR ATPase, we also studied the ATPase activity in mechanically split bundles of myofibrils in the absence and presence of 20 mM BDM. In the presence of 20 mM BDM, the SR ATPase activity is a larger fraction of the total activity. Extrapolation to zero force of the ATPase activity in the absence and presence of 20 mM BDM thus yields a more reliable determination of the temperature sensitivity of the SR ATPase activity. The fairly large value of 20 mM BDM was chosen because it was observed, in agreement with previous studies (Fryer, Neering & Stephenson, 1988; Higuchi & Takemori, 1989), that the effect of BDM on force development decreased at high temperatures. The reduction in force by 20 mM BDM was 85% at 5 °C and 50% at 20 °C. The SR ATPase activity obtained in these experiments from the intercepts at zero force was temperature dependent. At 5, 10, 15 and 20 °C it was, respectively, 28 ± 2 ($n = 14$), 33 ± 1 ($n = 4$), 46 ± 7 ($n = 3$) and 48 ± 4 ($n = 3$)% of the total ATPase activity measured in the absence BDM.

Although the diameters of the fibres were rather small, phosphate accumulation could influence the estimate of the SR ATPase activity. Phosphate depresses force more than the AM ATPase activity (Kawai, Güth, Winnikes, Haist & Rüegg, 1987) and it possibly also influences the SR ATPase activity. However, we did not observe any diameter dependence in the estimates of the SR ATPase activity, suggesting the phosphate accumulation does not affect our results.

The temperature dependences of the AM and SR ATPase activities derived from these experiments (on 14 fibres) are shown in Fig. 7B. The Q_{10} obtained for the SR ATPase activity decreased from 7.2 ± 1.5 in the range 5–10 °C to 3.1 ± 0.5 in the range from 10–20 °C. The Q_{10} for the AM

Table 2. Temperature coefficients of maximum force and ATPase activity

Temperature range (°C)	2.7–8.8	5–10	10–20
Force	1.61 ± 0.08 (11)	1.51 ± 0.08 (18)	1.08 ± 0.08 (18)
AM + SR ATPase activity (before Triton)		4.80 ± 0.03 (18)	2.97 ± 0.25 (18)
AM ATPase activity (after Triton)	5.92 ± 0.41 (11)	4.64 ± 0.28 (4)	3.46 ± 0.44 (4)
AM ATPase activity (BDM)		3.92 ± 0.25 (14)	2.62 ± 0.41 (14)
SR ATPase activity (BDM)		7.18 ± 1.50 (14)	3.09 ± 0.50 (14)

Q_{10} values (means ± S.E.M.) calculated from the changes in force and ATPase activity over the temperature range indicated. Number of fibres shown in parentheses. Number of data points used in the calculation of Q_{10} values in the BDM experiments: 14 (5 °C); 3 (10 °C); 3 (15 °C); 4 (20 °C).

ATPase decreased from 3.9 ± 0.3 (5–10 °C) to 2.6 ± 0.4 (10–20 °C). The results for the total (AM + SR) ATPase activity and of the AM ATPase activity in these experiments were similar to those obtained by Triton X-100 treatment. The residual ATPase activity, i.e. the intercept at zero force of the ATPase activity after Triton X-100 treatment at different BDM concentrations, may have an effect on the temperature dependence of the ATPase activities. However, since this residual activity is only a small fraction of the SR and the AM ATPase activities and because it will very probably be temperature sensitive as well, we think that it may cause an overestimation of the SR ATPase activity by a few percent, but that its effect on the Q_{10} values is negligible.

In two mechanically skinned fibre segments, the basal ATPase activity was determined by incubating the fibre in relaxing solution (pCa 9). This basal ATPase activity, presumably myosin ATPase activity, increased with temperature in absolute terms but at each temperature it was always less than 2% of the maximal Ca^{2+} -activated ATPase activity.

Type dependence of the AM and SR ATPase activities

A comparison was made between the AM and SR ATPase activities in fast-twitch (type 1 and 2) and slow-twitch fibres (type 3). These determinations were carried out by measuring the ATPase activity before and after Triton X-100 treatment. In slow-twitch fibres ($n = 8$) the ATPase activity was determined before and after Triton X-100 treatment. The

average reduction in ATPase activity after Triton X-100 treatment, i.e. the SR ATPase activity, was $40.1 \pm 3.9\%$ of the total activity. The total ATPase activity before Triton X-100 treatment amounted to $1.21 \pm 0.17 \mu\text{mol s}^{-1} (\text{g dry wt})^{-1}$. The AM ATPase activity in slow-twitch fibres amounted to $0.74 \pm 0.13 \mu\text{mol s}^{-1} (\text{g dry wt})^{-1}$ and the SR ATPase activity was $0.47 \pm 0.07 \mu\text{mol s}^{-1} (\text{g dry wt})^{-1}$. These results are illustrated in Fig. 8. It follows that the AM ATPase activity in fast- and slow-twitch fibres differed by about a factor of two but that the SR ATPase activities are very similar. Expressed per litre cell volume the AM and SR ATPase activities in slow-twitch fibres correspond to 0.07 and 0.05 mm s^{-1} , respectively. Using the same assumptions as for fast-twitch fibres, these values correspond to a rate of ATP turnover per myosin head of 0.37 s^{-1} and a catalytic activity of the SR Ca^{2+} pump of 5 s^{-1} .

The temperature at which the experiments were carried out ranged between 3.1 and 5.8 °C (mean, 4.3 °C) and, in an attempt to reduce the variability between fibres of the same group, we calculated for each fibre the AM and SR ATPase activity at 4.3 °C assuming that the temperature coefficients of the ATPase activity before and after Triton X-100 treatment were the same ($Q_{10} = 5.9$, cf. Table 2). The effect of this correction, however, was quite small. Even after correction for the difference in temperature, considerable variability within the groups of fibres remained. The AM ATPase activity in fast-twitch fibres varied between 1.01 and $2.32 \mu\text{mol s}^{-1} (\text{g dry wt})^{-1}$ and in slow-twitch fibres

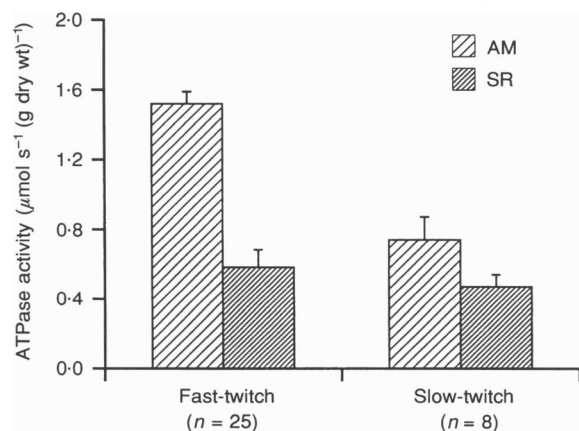


Figure 8. Type dependence of the AM and SR ATPase activities

between 0.36 and 1.65 $\mu\text{mol s}^{-1}$ (g dry wt) $^{-1}$. The SR ATPase activity in fast-twitch fibres varied between 0.21 and 0.92 $\mu\text{mol s}^{-1}$ (g dry wt) $^{-1}$ and in slow-twitch fibres between 0.21 and 0.80 $\mu\text{mol s}^{-1}$ (g dry wt) $^{-1}$. The variability in the AM ATPase activity in fast fibres could be due to the differences in myosin composition between type 1, type 2 and intermediate type 1/2 fibres (Lännergren & Hoh, 1984). However, the variability in SR ATPase activity in fast- and slow-twitch fibres and of the AM ATPase activity in type 3 fibres was rather unexpected. In most cases several fibres were studied on one day and, therefore, the time the fibres were incubated in relaxing solution after skinning, varied. This could have induced some variations. However, no systematic differences between early and late fibres of the same type were observed. Also segments of the same fibre incubated for different times in relaxing solution gave similar results. The differences in AM and SR ATPase activities between different segments from the same fibres were always less than 5% ($n = 3$). Also, we did not find any signs of differences in fibre 'quality' which would show up as a correlation between isometric force and AM or SR ATPase activity. Our results also indicated that the total ATPase activity did not correlate with the average fibre diameter or with the dry weight per unit length of the preparations, which is an accurate estimate of cross-sectional area of the fibre. This indicates that the ATPase measurements are not diffusion limited. Therefore, we think that some variability exists in the SR ATPase activity between fibres of the same type.

DISCUSSION

This study represents the first attempt to measure the maximal ATPase activity of the SR in skinned muscle fibres. This is of interest for two reasons. (1) The intracellular environment in the skinned fibres was similar to the composition of the cytosol with respect to the concentrations of free Mg^{2+} , ATP, ADP and P_i , and to the pH and ionic strength. Therefore, contrary to many of the results on SR fragments *in vitro*, the values obtained here are relevant under physiological circumstances. (2) Since the AM and SR ATPase activities and force development were studied in the same preparations under identical conditions, these results in addition provide an insight into the correspondence between activation and contractile processes at the cellular level.

Comparison with previous results

The rate of ATP turnover expressed per litre cell volume in fast fibres corresponded to 0.20 mM s^{-1} . In Triton X-100-skinned frog fibres at 4 °C, Stienen *et al.* (1990) obtained a value of 0.34 mM s^{-1} . They showed that this value was in good agreement with the estimates for the myofibrillar ATPase activity ($\sim 1.2 \text{ mM s}^{-1}$) based on measurements of the stable maintenance heat rate (Curtin, Howarth, Rall, Wilson & Woledge, 1986) in tetanically stimulated intact

frog fibres. In comparing the results from intact and skinned fibres, the increase in fibre volume by about a factor of two should be taken into account. Therefore, the results on skinned fibres are also in good agreement with previous results from biochemical determinations on whole muscle, i.e. 0.7 $\mu\text{mol (g muscle)}^{-1} \text{ s}^{-1}$ at 0 °C (Infante, Klaupikis & Davies, 1964). The rate of ATP turnover in skinned *Xenopus* fibres is less than in frog fibres. This is compatible with the difference in heat production during twitches between frog and toad observed by Smith (1972).

The absolute values for the total (AM + SR) ATPase activity at room temperature can be compared with previous measurements of the maintenance heat production in intact fibres (Elzinga *et al.* 1987). Based on the mean value of the total ATPase activity at 4.3 °C of 2.04 $\mu\text{mol s}^{-1}$ (g dry wt) $^{-1}$, a relative increase in ATPase activity at 20 °C by a factor of 7.6 (Fig. 7), and an enthalpy change for creatine phosphate (PCr) splitting of -34 kJ mol^{-1} (Woledge *et al.* 1985), this would correspond to a maintenance heat rate production of 0.53 W (g dry wt) $^{-1}$. This value is practically identical to the average of the values for type 1 and type 2 fibres obtained by Elzinga *et al.* (1987).

(Previously, higher values for the AM and SR ATPase activities in fast *Xenopus* fibres were observed in our laboratory (Lännergren *et al.* 1993). During the course of this study it was found that the difference was caused by an error in the temperature measurements: the actual temperature in the earlier experiments was about 8 °C instead of 4 °C.)

The SR ATPase activity expressed as a fraction of the total ATPase activity in fast-twitch fibres (0.25) is fairly similar to that found in biochemical and heat measurements on frog fibres at low temperature. In these experiments the rate of ATP splitting or the maintenance heat rate was studied at short and at long sarcomere lengths where active force production was small. For instance, Infante *et al.* (1964), Smith (1972), Homsher, Mommaerts, Ricchiuti & Wallner (1972) and Burchfield & Rall (1986) found fractions in the range from 0.25 to 0.4. Therefore, it can be concluded that the values for the energy cost of Ca^{2+} cycling obtained from intact preparations and skinned fibres are in good agreement.

The catalytic activity of the SR Ca^{2+} pump at 4.3 °C corresponded to 6 s^{-1} . Its temperature sensitivity (Fig. 7) is such that it would be about 60 s^{-1} at 20 °C. This is considerably larger than the initial rate of Ca^{2+} uptake found in mammalian SR vesicles of about 10 s^{-1} at 24–25 °C (Inesi & Scarpa, 1972). Since the estimates of the SR ATPase activity in skinned and intact fibres agree, this implies that the activity of the intact SR is about 10 times larger than that found in isolated vesicles. The reason for this discrepancy could be that (1) the Ca^{2+} pumps in vesicles are partly inactivated and/or (2) the SR content in functionally intact SR is larger than the estimate we used.

Calcium sensitivity of the AM and SR ATPase activities

The calcium sensitivity of the SR ATPase activity in fast-twitch fibres could be described by the Hill equation with apparent calcium affinity $pCa_{50} = 6.62 \pm 0.04$ and $n = 2.07 \pm 0.34$. The Hill equation which describes the calcium dependence of isometric force and AM ATPase activity had $pCa_{50} = 6.11 \pm 0.01$ and $n = 2.87 \pm 0.17$. The ATPase activity of the SR is therefore more sensitive to Ca^{2+} and its dependence on the Ca^{2+} concentration less steep than that of the actomyosin interaction. These calcium sensitivities of the AM and SR ATPase activities are such that the Ca^{2+} pump is already half-maximal around the threshold of force generation and near its maximum value at half-maximal force. This implies that the Ca^{2+} pump is also very potent during force relaxation. It also suggests that the energy required for activation during submaximal contractions, e.g. during unfused tetani, is a larger fraction of the total energy utilized than during tetanic contractions.

Evidence is presented in the literature suggesting that the hydrolysis of ATP associated with calcium uptake into the SR takes place in two consecutive steps: (1) the fast formation of an aspartylphosphate enzyme intermediate and (2) a combination of steps which lead to the transfer of Ca^{2+} across the SR membrane and the eventual hydrolysis of ATP (e.g. Martinosi & Beeler, 1983). To account for co-operative calcium binding, Inesi, Kurzmack, Coan & Lewis (1980) presented a scheme in which calcium binding occurs in two stages. The apparent binding constant K_{app} in rabbit hind leg muscle was $2.3 \times 10^6 M^{-1}$ ($pK_{app} = 6.36$) and the Hill coefficient was 1.82. In view of differences in species and in experimental conditions these values are in good agreement with our findings.

BDM dependence of the AM and SR ATPase activities

The results of this study indicated that the AM ATPase activity and isometric force varied in proportion at different BDM concentrations. The BDM dependence was such that already at 2 mM BDM the effects were half-maximal. These results are consistent with the findings of Higuchi & Takemori (1989), who showed that the isometric force and the ATPase activity of heavy meromyosin and myofibrils of rabbit psoas muscle are inhibited with a similar concentration dependence. From caged-ATP experiments Lenart, Tanner & Goldman (1989) concluded that BDM reduces the rate of cross-bridge reattachment. This view is supported by the results of Herrmann, Wray, Travers & Barman (1992) who showed that the predominant effect of BDM was a reduction in the number of cross-bridges in the strong actin binding state. It should be noted, however, that recent studies (Bagni, Cecchi, Colomo & Garzella, 1992; Zhao & Kawai, 1994) indicate that BDM may have additional effects. Our finding that isometric force and AM ATPase activity varied in proportion indicates that their

ratio, i.e. tension cost, is independent of the BDM concentration. In a simple two-state model of cross-bridge interaction, tension cost is proportional to the rate of cross-bridge detachment. Therefore, our results are also in agreement with a predominant effect of BDM on the apparent rate of cross-bridge attachment.

The effects of BDM on calcium handling by the sarcoplasmic reticulum reported in the literature are somewhat variable. In cardiac muscle it has been suggested that BDM at low concentrations (< 5 mM) mainly acts on the contractile apparatus but that at larger concentrations calcium flux through the sarcolemma as well as across the SR membrane are affected (Alpert *et al.* 1989). Based on studies on skinned muscle fibres and aequorin-injected intact fibres, Horiuti *et al.* (1988) concluded that BDM mainly acted on the contractile system but that it also enhanced the activity of the calcium-induced calcium release mechanism and that it depressed the calcium pump activity. Recently, Steele & Smith (1993) showed that at rather low Ca^{2+} concentrations, BDM promotes the net release of Ca^{2+} in saponin-skinned cardiac muscle. They propose that this could be due an inhibition of the Ca^{2+} -uptake pump. We observed a linear relation between force and ATPase activity in saponin-skinned preparations up to 20 mM BDM. This result indicates that the *maximum* activity of the calcium pump is not affected by up to 20 mM BDM. However, the calcium sensitivity of the pump may very well be reduced by BDM.

Temperature sensitivity of the AM and SR ATPase activities

To obtain a reliable estimate of the Q_{10} of the SR ATPase, we studied the ATPase activity in mechanically split bundles of myofibrils in the absence and presence of 20 mM BDM. The temperature sensitivities of the isometric force and the AM and SR ATPase activities decreased when temperature was increased from 2.7 to 20 °C. Near 5 °C the Q_{10} values of force and AM ATPase activity after Triton treatment amounted to 1.6 and 5.9, respectively. In the range 5–10 °C the Q_{10} of the SR ATPase was even 7.2. In the range 10–20 °C, the Q_{10} values were decreased to 1.1 (force), 2.6–3.5 (AM ATPase) and 3.1 (SR ATPase). The results of the total (AM + SR) ATPase activity and of the AM ATPase activity in the experiments in which BDM was used were similar to those obtained by Triton X-100 treatment. This provides additional evidence that the SR ATPase activity of saponin- and mechanically skinned fibres are identical.

At low temperature (5 °C), the Q_{10} values of isometric force and AM and SR ATPase activities are very similar to the values obtained in frog fibres (e.g. Burchfield & Rall, 1986, and references therein). At high temperature (20 °C), the Q_{10} values were less. The changes in temperature sensitivity seem to occur gradually. In SR vesicles from white skeletal muscle from rabbit hindleg, a transition in the temperature dependence of the ATPase activity was found in the range

5–48 °C, which was characterized by an abrupt change in the activation energies of the enzymatic reactions involved at 20 °C (Inesi *et al.* 1973). This was ascribed to a transition in membrane fluidity or to an intrinsic temperature dependence of the ATPase protein (Martonosi & Beeler, 1983). The absolute values and the changes in the Q_{10} values of the ATPase activity from 5.4 (5–20 °C) to 2.6 (20–37 °C) are similar to the values found in this study. This suggests that a similar transition point could be present in *Xenopus* albeit at a lower temperature (~10 °C). Since *Xenopus* is a poikilotherm, a lower transition temperature would be not surprising.

Type dependence of the AM and SR ATPase activities

The maximal AM and SR ATPase activities in fast-twitch fibres at 4.3 °C amounted to 1.52 ± 0.07 and $0.58 \pm 0.10 \mu\text{mol s}^{-1} (\text{g dry wt})^{-1}$, respectively (means \pm s.e.m.). The SR ATPase activity was 25.1% of the total ATPase activity. The AM ATPase activity in slow-twitch fibres amounted to $0.74 \pm 0.13 \mu\text{mol s}^{-1} (\text{g dry wt})^{-1}$, i.e. about a factor of two smaller than in fast-twitch fibres. The SR ATPase activity amounted to $0.47 \pm 0.07 \mu\text{mol s}^{-1} (\text{g dry wt})^{-1}$, i.e. rather similar to the value in fast-twitch fibres. The fraction of the SR ATPase activity of the total ATPase activity (40%) was larger than in fast-twitch fibres. The rather small difference in SR ATPase activity between *Xenopus* type 1 and 2 on the one hand and type 3 on the other, is compatible with the small difference in Ca^{2+} -ATPase content found in mammalian type IIB and IIA fibres (Leberer & Pette, 1986)

In a previous study in our laboratory (Lännergren *et al.* 1993), it was also found that within fast-twitch fibres differences were observed in the AM ATPase activity of type 1 and type 2 fibres, while the SR ATPase activity in these two fibre types was fairly similar. The present results in fact extend these observations and show that in slow-twitch fibres the AM ATPase activity is lower than in fast-twitch fibres but the SR ATPase activity is very similar. These observations seem to be at variance with previous activation heat measurements, which indicated that the AM and SR ATPase activities co-vary with a sixtyfold variation in energy expenditure (Homsher & Kean, 1978). It should be realized though that the AM ATPase in the fibres of *Xenopus* studied varied by a factor of only 2–3. In fact the ratio of the SR ATPase activity and the total (AM + SR) ATPase activity is still within or close to the range of values found previously (30–50%). Our observations are also different from results for oxygen consumption and lactate production in fast and slow mammalian (mouse) muscles (Crow & Kushmerick, 1983). During short tetani the total energy cost of contraction of slow muscles was about 3 times smaller than in fast muscles whereas the filament overlap-independent energy consumption was a constant percentage (31%) of the total energy cost.

Although we did not cover the wide range described by Homsher & Kean (1978), our measurement suggest that co-variation between the AM and SR ATPase activity is not a fixed general rule.

The calcium requirements inside muscle cells are fairly similar but the calcium buffering inside the cytosol in fast-twitch *Xenopus* fibres varies as a result of differences in the concentration of calcium binding proteins such as parvalbumin (Lännergren *et al.* 1993). Biochemical determinations of SR Ca^{2+} -ATPase and parvalbumin content (Leberer & Pette, 1986) showed a good correlation. Therefore, we consider it likely that the variability in SR ATPase activity found between fibres of the same type is due to differences in content or density which result from adaptation to differences in buffering power.

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