J. Physiol. (1987), **390**, *pp.* 285–293
With 5 text-figures
Printed in Great Britain

THE TEMPERATURE DEPENDENCE OF ISOMETRIC CONTRACTIONS OF SINGLE, INTACT FIBRES DISSECTED FROM A MOUSE FOOT MUSCLE

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(*Received 4 December 1986*)

SUMMARY

1. Isometric tension responses to electrical stimulation have been studied at 7·5–37·5 °C in single, intact fibres of the flexor digitorum brevis muscle of the mouse. A large number of reproducible tetani could be obtained at temperatures $\leqslant$ 35 °C.

2. The tetanic force per cross-sectional area generated at 25·0 °C was 375 ± 56 kPa (mean ± S.D., $n = 16$).

3. The curve relating maximum tetanic tension to temperature exhibited a transition between a level of almost unaltered force (25·0–32·5 °C) and a marked force decline ($\leqslant$20·0 °C). At temperatures higher than 35·0 °C force production was markedly depressed and this reduction was in some cases irreversible.

4. Twitch tension showed less regular dependence on temperature; it was reduced less than tetanic tension at low temperatures. Thus, the twitch/tetanus tension ratio was higher at low temperatures.

5. The times for twitch contraction and for twitch half-relaxation (i) ranged from 7 to 14 ms and from 6 to 15 ms at 35·0 °C and (ii) exhibited Q_{10} values of 3·2 ± 0·4 and 4·0 ± 0·6, respectively.

6. It is concluded that it is possible to use intact, single fibres dissected from mammalian skeletal muscle in physiological studies. Our results are close to previous results obtained from mammalian muscles except that the tetanic tension per cross-sectional area was found to be higher than commonly reported.

INTRODUCTION

For certain studies of muscle function the use of isolated fibres is advantageous or even necessary, e.g. when precise measurements and control of sarcomere length are required, when force output per cross-sectional area is to be determined and when the effects of changes of the external solution are to be studied. Further, to determine some type-specific properties single-fibre preparations may be needed, because even small fibre bundles are rarely homogeneous in their fibre composition (cf. Luff, 1985). The first successful studies on single-muscle-cell preparations were performed relatively early on fibres dissected from frog muscles (Brown & Sichel, 1930; Asmussen, 1932) and since then isolated frog muscle fibres have frequently been used in detailed studies of muscle function.

With mammalian muscle the dissection of intact, single fibres has proved to be difficult and to our knowledge no results from such preparations are available. This fact probably contributes to the uncertainties about some basic properties of mammalian muscle, e.g. force per cross-sectional area, for which divergent values have been reported: (i) *ca.* 180 and 270 kPa for slow and fast whole-muscle preparations, respectively (Close & Hoh, 1968), (ii) *ca.* 60 and 250 kPa for slow and fast motor units, respectively (Burke & Tsairis, 1973), (iii) 250–300 kPa for small fibre bundles (ter Keurs, Luff & Luff, 1984) and (iv) 240–400 kPa for skinned fibres (Sexton, 1967; Stephenson & Williams, 1981).

We now report that intact, single fibres can be prepared from mouse foot muscles. Besides obtaining values for maximum specific force and twitch parameters we were interested in effects of altered temperature, primarily to find a suitable temperature range for further studies with this preparation. We also considered it to be of special interest for a muscle which is superficially located and which may be subjected to large temperature differences in the natural environment of the animal. A preliminary report of the results has been published in abstract form (Westerblad & Lännergren, 1987).

METHODS

Fibre dissection and mounting

Male mice (NMRI strain, 2–3 months of age) were killed by rapid neck disarticulation. Single fibres were dissected from the distal part of the flexor brevis muscle of the foot. The fibres in this pennate muscle are short (about 0·6–1·0 mm) and have a diameter of 20–50 μm. The dissection was made with a pair of scissors and forceps under a stereo-microscope with dark-field illumination at 150× magnification. Towards the end of the dissection the ability to twitch of the two or three remaining fibres was tested by focal electrical stimulation. One fibre was then selected and the other carefully pinched in several places. During this late part of the dissection the preparation proved to be very sensitive to stretch, hence all pieces of the damaged fibres could not be removed.

After this, the trimmed-down tendons of the fibre were gripped by platinum-foil micro-clips and the preparation transferred to the perfusion channel of the experimental chamber. This had a glass bottom and housed a horizontally mounted Akers AE 801 semiconductor force transducer, provided with a glass tube extension 3·8 mm long with a fine hook at the end. The compliance of the force transducer was 5·6 μm mN^{-1}, the resonance frequency 5 kHz. One tendon clip was attached to the transducer hook, the other to an adjustable holder, allowing the fibre to be suspended horizontally and stretched to a sarcomere length giving maximum tetanic force. The preparation was illuminated from below with a hollow cone of light, creating dark-field illumination also in the experimental chamber.

Stimulation

Transverse stimulation was employed; the fibre was flanked by bright platinum-plate electrodes, 0·4 × 4·0 mm, set 1·8 mm apart. Biphasic, rectangular pulses (total duration 1·2 ms at $T \leqslant 15{\cdot}0$ °C and 0·4 ms at $T > 15{\cdot}0$ °C), giving a field strength of about 8 V cm^{-1} (1·5 × threshold) were used. Tetanic stimulation frequency was selected to give a just-fused tetanus at each temperature and the train duration was made just long enough for maximum tension to develop. These stimulation parameters then ranged from about 15 Hz and 1600 ms (7·5 °C) to about 200 Hz and 340 ms (30·0–37·5 °C), respectively.

Recordings and measurements

Signals from the force transducer were displayed on a storage oscilloscope (Tektronix 5111A, U.S.A.) and also on one channel of a strip-chart recorder (Hewlett–Packard 7402A, U.S.A.).

Measurements of tetanic tension were made from Polaroid photographs of the oscilloscope screen (1:1) and from pen records; the accuracy of these measurements was approximately 1% of

maximum tension level. Measurements of twitch contraction (t_c) and half-relaxation ($t_{0.5r}$) times were made from Polaroid photographs of the oscilloscope screen.

The cross-sectional area of the fibre was measured in the dissection microscope and in a high-magnification microscope at 150 and 500× magnification, respectively. The fibre was held at a sarcomere length of about 2·4 μm, and by turning it, the largest (a) and smallest (b) diameter was measured at two places. Cross-sectional area was calculated as $a\,b\,\pi/4$ for each place and the mean was taken. The values obtained from the two methods differed at most by 15%. In early experiments fibre diameters were measured only under the dissection microscope.

Values are given as mean ± S.D. Linear regression was calculated with the least-square method.

Solution and temperature control

Fibres were dissected at room temperature in a solution with the following composition (mM): NaCl, 136·5; KCl, 5·0; $CaCl_2$, 1·8; $MgCl_2$, 0·5; NaH_2PO_4, 0·4; and $NaHCO_3$, 11·9. When mounted in the experimental chamber (capacity 0·3 ml) the preparation was superfused at a rate of about 1·5 ml min^{-1} by a slightly different solution (mM): NaCl, 121; KCl, 5·0; $CaCl_2$, 1·8; $MgCl_2$, 0·5; NaH_2PO_4, 0·4; and $NaHCO_3$, 24·0; which was bubbled with a mixture of 5% CO_2 and 95% O_2 at room temperature (pH 7·40). After a considerable number of unsuccessful experiments, in which the force production became low and irregular as the temperature was increased above 30 °C, we decided to include fetal calf serum (Gibco, U.K.) in the perfusion solution (cf. Boyd & Ward, 1975). The first experiment with this modified solution containing about 0·2% serum was successful and we have used it since. However, a systematic study on the importance of including calf serum for fibre survival has not yet been performed.

Solution temperature, monitored close to the fibre, was regulated to within 0·1 °C by passing the solution through a temperature-controlled heat exchanger just before it entered the perfusion channel. Due to the restricted area of the perfusion channel the thermistor probe caused some disturbance of the solution flow which might have led to minor errors in temperature monitoring.

Experimental procedure

A typical experiment started at 25·0 °C after adjusting the length of the fibre to obtain maximum tetanic tension. The temperature was then first reduced to 20·0 °C, after that in steps of 2·5 °C down to 10·0 or 7·5 °C and then, after return to 25·0 °C, increased in the same way to 35·0 or 37·5 °C. The fibre was allowed to rest for 10 min at each temperature and was then stimulated first by a single current pulse for measurement of t_c and $t_{0.5r}$ and then by another single pulse followed by a pulse train.

RESULTS

Fig. 1 illustrates the frequency–tension relation of a fibre stimulated at 30·0 °C. Similar tests were done at each temperature to find the optimum frequency for tetanic stimulation.

There was little tendency for the force-generating ability of the preparations to decline with time, irrespective of the temperature, if they were not exposed to temperatures higher than 35·0 °C (see below). Control tetani at 25·0 °C after temperature decrease and increase periods produced 98·3 ± 5·1 and 98·2 ± 4·7% of the original force, respectively. In some cases fibres were tested at about 1 h intervals after the temperature experiments had been finished and found to give undiminished tetanic tension responses for up to 8 h.

Tetanic force per cross-sectional area

The tetanic force produced at 25·0 °C was 391 ± 59 kPa ($n = 8$); the mean cross-sectional area of these fibres was 765 μm^2 (range 343–1206 μm^2). At 25·0 °C the fibres produced about 92% of maximum tetanic tension (see below) but we chose to give

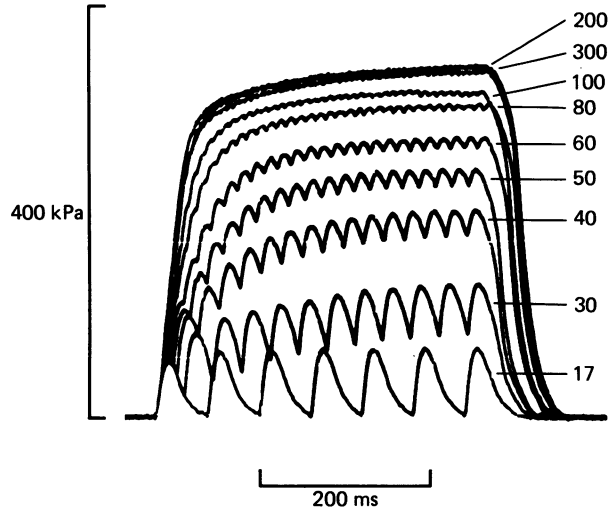

Fig. 1. Superimposed oscilloscope records of tension responses of one fibre to 400 ms stimulation trains at 17 to 300 Hz (30·0 °C).

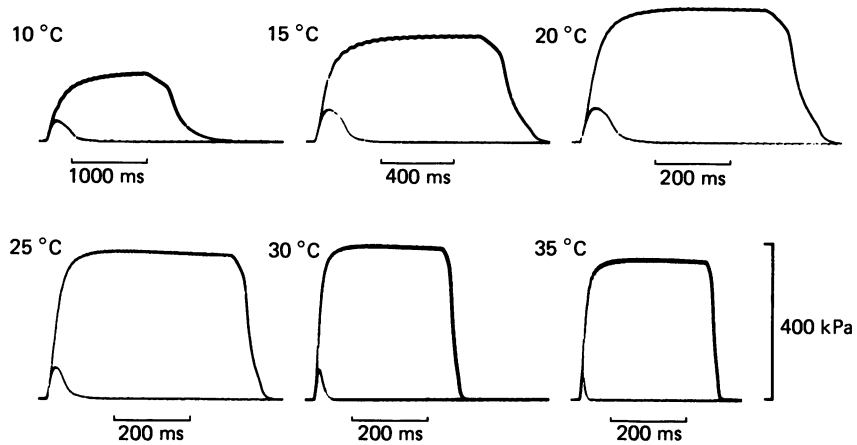

Fig. 2. Oscilloscope records of the tension response to single and train stimulation at 10·0–35·0 °C. The stimulation frequency of trains ranged from 15 Hz (10·0 °C) to 200 Hz (35·0 °C).

the results at this temperature because not all fibres were tested at higher temperatures. If the fibres from early experiments, in which the cross-sectional area was measured in the dissection microscope only (see Methods), are also included the tetanic tension produced was 375 ± 56 kPa ($n = 16$).

Temperature dependence of isometric force

Fig. 2 displays oscilloscope records of twitches and tetani obtained from one fibre at 10·0–35·0 °C. In Fig. 3*A* the relation between relative tetanic force and temperature of six fibres is plotted; the maximum force obtained from each fibre is set

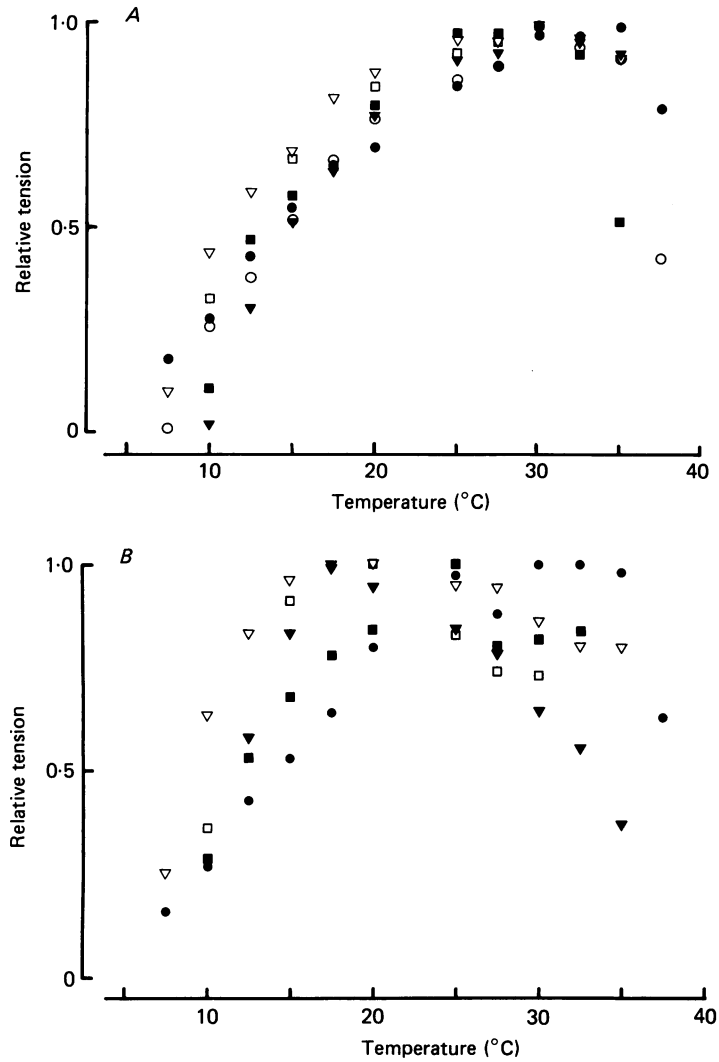

Fig. 3. Relative tension *vs.* temperature for tetani (*A*) and twitches (*B*). One type of symbol refers to the same fibre.

as 1·0. There was a transition between a level of almost unaltered force (25·0–32·5 °C) and a marked force depression ($\leqslant$ 20 °C). This is further illustrated by the tetanic force values: 24·0 $\pm$ 15·2, 79·7 $\pm$ 6·3, 91·6 $\pm$ 5·0 and 95·8 $\pm$ 1·9 % at 10·0, 20·0, 25·0 and 32·5 °C, respectively. At 37·5 °C the tetanic force was markedly decreased (in one fibre already at 35·0 °C). It appeared that fibres were vulnerable at those higher temperatures and some fibres (not included here) showed irreversible failure after they had been exposed to 37·5 °C. In late experiments the temperature increase therefore terminated at 35·0 °C.

The relation between twitch tension and temperature is plotted in Fig. 3*B* (n = 5; one of the fibres above was excluded because the set of twitch recordings was

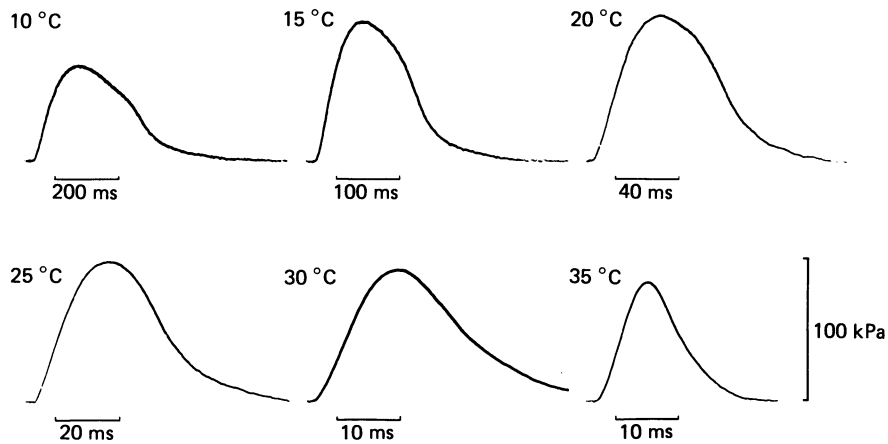

Fig. 4. Oscilloscope records of twitches obtained at 10·0–35·0 °C; records from the same fibre as in Fig. 2.

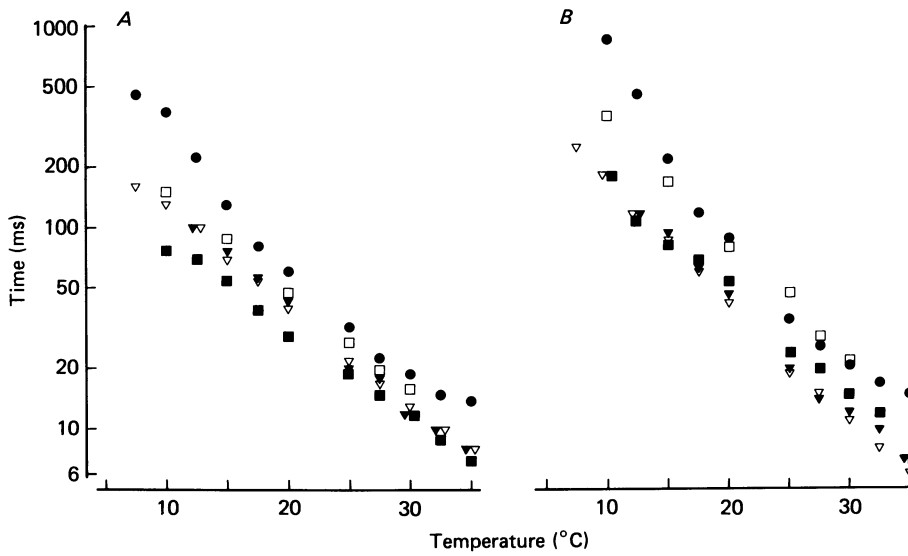

Fig. 5. Semilogarithmic plots illustrating the exponential temperature dependence of twitch contraction (*A*) and half-relaxation (*B*) times ($r > 0{\cdot}98$ for each fibre, respectively). One type of symbol refers to the same fibre.

incomplete). The optimum temperature for twitch tension varied considerably among the fibres, e.g. maximum twitch force was obtained at 17·5 and 32·5 °C in two fibres. A general pattern however was that twitch tension decreased less than tetanic tension at low temperatures which led to an increase of the twitch/tetanic tension ratio from $0{\cdot}16 \pm 0{\cdot}03$ at 30·0 °C to $0{\cdot}27 \pm 0{\cdot}11$ at 15·0 °C.

The temperature dependence of twitch contraction and half-relaxation times

Oscilloscope records of twitches obtained from one fibre at 10·0–35·0 °C are displayed in Fig. 4 and collected data of twitch parameters of five fibres (same fibres as

in Fig. 3*B*) are plotted in Fig. 5. The contraction (t_c) and half-relaxation ($t_{0.5r}$) times ranged from 7 to 14 ms and 6 to 15 ms at 35·0 °C, respectively. In semilogarithmic plots both parameters increased with decreasing temperature, following straight lines ($r > 0.98$ for each fibre, respectively). Q_{10} values calculated from these lines were 3.2 ± 0.4 for t_c and 4.0 ± 0.6 for $t_{0.5r}$.

General features of the preparation

Two well-known features of frog muscles could be demonstrated in this preparation also. First, in Fig. 2 it can be noted that the relaxation phase after tetanic stimulation at 10·0–20·0 °C exhibited a clear shoulder, that is a transition from an almost linear, slow phase to a faster, nearly exponential phase (Hartree & Hill, 1921). Secondly, traces of latency relaxation (Rauh, 1922; Sandow, 1944) were commonly observed in twitch recordings. An example of this is the record obtained at 35·0 °C in Fig. 4.

DISCUSSION

Tetanic force per cross-sectional area

The specific tetanic force generated by our preparations ranged between 300 and 480 kPa. In most studies of mammalian muscle tetanic tension values of less than about 300 kPa have been obtained (for review see Close, 1972) and to our knowledge only Stephenson & Williams (1981) using skinned rat fibres have obtained values comparable to ours (*ca.* 300 kPa for slow fibres and 400 kPa for fast fibres at 35 °C). The reason for this discrepancy is not clear. The following causes for underestimated specific tension values in multi-fibre preparations may be suggested: (i) some fibres might be damaged, (ii) some fibres might not be activated, and (iii) tension might be measured in a direction partly different from that in which force is generated.

Temperature dependence of isometric contraction

One aim of the present investigation was to study the temperature dependence of force production of our preparation and to compare it with that of other mammalian muscles. This is of some interest because it might be expected that fibres of the flexor brevis muscle, located close to the plantar surface of the foot, experience much larger temperature differences than, say, calf muscles. Intact as well as skinned mammalian muscle fibres, unlike amphibian fibres, have been shown to respond with markedly diminishing tetanic tension production when temperature is decreased below 20 °C (intact multi-fibre preparations: Isaacson, Hinkes & Taylor, 1970; Stein, Gordon & Shriver, 1982; Ranatunga & Wylie, 1983; skinned fibres: Stephenson & Williams, 1981). One of our aims was then to see if this depression was less marked in our preparation. However, rather the opposite was found, e.g. at 10·0 °C only about 25% of maximum tetanic tension was produced as compared with about 50% in the investigations above (no tests were done at this temperature by Isaacson *et al.* (1970)). Since the temperature–tension relation is relatively steep in this range the difference may not be important and corresponds to a shift of 2–3 deg only.

The tension-generating ability of our preparation was depressed at temperatures above 35·0 °C and this depression was in some cases irreversible. The appearance of the tetani at 37·5 °C (rapid tension alterations during stimulation) suggests that the

low tension might be the result of impaired excitation–contraction coupling. A less pronounced reduction of tetanic tension at high temperatures has been reported by Stein *et al.* (1982) and this reduction was partly irreversible at temperatures over 40 °C. Moreover, above 37 °C protein conformational changes resulting in dysfunction of the sarcoplasmic reticulum have been reported (Inesi, Millman & Eletr, 1973). Thus, 37 °C appears to be relatively close to the limit for normal muscle function; the reason why our preparations began to fail at slightly lower temperatures is unclear at present.

Since force output is markedly reduced at temperatures below 20 °C and problems with stability appeared at high temperatures the most suitable temperature range for experiments, in which reproducible, high-force production is required, would be 20–30 °C. Preliminary fatigue studies in this temperature interval have already been performed (Westerblad & Lännergren, 1987) in which we found complete tension recovery after fatiguing stimulation, which brought tension down to 10–20% of the original.

The temperature dependence of both contraction (t_c) and half-relaxation ($t_{0.5r}$) times closely followed exponential curves ($r > 0.98$) which indicates that the respective rate-determining reaction is the same at all temperatures; no sign of a break-point in the curves at about 20 °C (Stein *et al.* 1982; Ranatunga & Wylie, 1983) was obtained. The Q_{10} value of t_c (3.2 ± 0.4) differed from that of $t_{0.5r}$ (4.0 ± 0.6), thus two separate rate-determining reactions are plausible. Both Q_{10} values are slightly higher than normally obtained from mammalian muscle preparations, i.e. *ca.* 2.5 for t_c and 3.0 for $t_{0.5r}$ (e.g. Close & Hoh, 1968; Ranatunga, 1980).

Fibre types

At 35.0 °C t_c and $t_{0.5r}$ of one fibre separated from the other three; t_c was 14 ms ($t_{0.5r}$ 15 ms) against 7–8 ms ($t_{0.5r}$ 6–7 ms) for the others. These times are consistent with previous results from fast and slow mouse muscles (Ranatunga, 1980; Stein *et al.* 1982; Gordon & Stein, 1985). Other properties of the slower fibre also differed, which suggests that it was a slow-twitch fibre while the others were fast-twitch fibres: (i) the twitch tension produced did not increase as the temperature was lowered from 35 °C to about 25 °C (Fig. 3*B*), which only fast muscles have been reported to do (Close & Hoh, 1968; Buller, Kean, Ranatunga & Smith, 1984); (ii) the maximum tetanic tension generated was relatively low, which is in agreement with most studies comparing fast-twitch and slow-twitch preparations (e.g. Close & Hoh, 1968; Stephenson & Williams, 1981). With the present approach it then seems practical to obtain intact single fibres of different types, which would make detailed, type-specific studies of e.g. the force–velocity relation and fatigue properties possible.

In summary, we have shown that it is possible to perform physiological studies using intact, single fibres of mammalian skeletal muscle. The tetanic tension per cross-sectional area was higher whereas values for other isometric contraction parameters were close to those reported in the literature for mammalian muscle.

This study was supported by grants from the Swedish MRC (project no. 3642) and funds at the Karolinska Institutet.

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