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TEMPERATURE-DEPENDENCE OF SHORTENING VELOCITY AND RATE OF ISOMETRIC TENSION DEVELOPMENT IN RAT SKELETAL MUSCLE

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SUMMARY

1. The temperature-dependence of the maximum velocity of shortening and of the maximum rate of isometric tension development were examined in a rat fast twitch muscle, extensor digitorum longus (e.d.l.), and a slow twitch muscle, soleus, *in vitro* and with direct stimulation. The temperature range was 35–20 °C.

2. The maximum velocity of shortening and the maximum rate of tension development decreased with cooling in both muscles. The decrease was such that their logarithms were linearly related to the reciprocal of the absolute temperature over the temperature range of 35–20 °C in e.d.l. and 35–25 °C in soleus.

3. The calculated Arrhenius activation energy for maximum velocity of shortening was around 40–45 kJ in both muscles. The activation energies for maximum rate of tension development were 48 kJ in e.d.l. and 56 kJ in soleus. Similar analyses made on the time to peak and time to half-relaxation of the isometric twitch showed that their activation energies were higher, between 60–80 kJ, in both muscles.

4. The results are examined in relation to biochemical findings and discussed in relation to A. F. Huxley's (1957) cross-bridge theory.

INTRODUCTION

The influence of temperature on the isometric twitch contraction of rat and mouse skeletal muscles has been investigated in several previous studies (Close & Hoh, 1968; Hoh, 1974; Krarup, 1981; Ranatunga, 1977*a*, 1980). These studies have shown that the time to peak and the time to half-relaxation of the isometric twitch increased with a temperature co-efficient (Q_{10}) of 2·1–3·2 in cooling from 35 to 20 °C. The temperature-dependence of the velocity of after-loaded isotonic shortening and of the rate of isometric tension development in the rat muscle are examined in the investigation reported in this paper. Some observations on shortening velocity measurements made by isotonic release method are also reported.

A brief report of part of this work has been published (Ranatunga, 1981).

METHODS

Experiments were performed *in vitro* on extensor digitorum longus (e.d.l., a fast-twitch muscle) and soleus (a slow twitch muscle) isolated from male rats of approximately 4 weeks of age. Animals were anaesthetized with an intra-peritoneal injection (50 mg/kg of body weight) of Pentobarbitone Sodium (Sagatal) and were kept under anaesthesia during the dissection procedure. During an experiment a muscle lay horizontal in a Perspex chamber of about 17 ml in volume and was bathed in physiological saline solution (composition: NaCl, 115 mM; KCl, 5 mM; $CaCl_2$, 4 mM; $MgCl_2$, 1 mM; NaH_2PO_4, 1 mM; glucose, 11 mM; $NaHCO_3$, 24 mM; tubocurarine chloride, 0·02 g l^{-1}) bubbled continuously with a mixture of 95% O_2 and 5% CO_2.

Mechanical apparatus

The system used is shown schematically in Fig. 1. It consisted of two tension transducers, one for recording muscle tension and a second for monitoring the isotonic tension to be applied to the muscle, and two vertical levers. The two levers, the primary and the secondary, were assembled so that their movements were in the same vertical plane and they were connected to each other (as shown) by a plaited silk thread. The primary lever was made of magnesium with a piece of stainless steel tube fixed to its tip with epoxy adhesive (Araldite). The stainless steel tube was bent into a hook for attachment of muscle tendon. The lever was mounted on the spindle of a commercial isotonic transducer (Type T1, George Washington Ltd.). The lever movement, however, was not monitored by its transducer mechanism (see below). A block of Perspex fixed to the muscle chamber acted as an after-stop and movement could be prevented by a screw-stop (see Fig. 1), for isometric recording. The secondary lever was a Palmer crank lever made of brass (commonly used with Starling's myograph in class experiments). Its lever arm was lengthened to about 100 mm with a strip (5 mm wide) of glass microscope slide. The primary lever was after-loaded with metal extension springs applied via the secondary lever. The combined lever ratio was approximately 43 : 1. The springs consisted of ten to twelve individual light duty springs (RS Components Ltd.) in series. Four such sets of springs were assembled to cover overlapping ranges of tension between 0·6 and 17·5 N. A spring set was connected between the secondary lever and the second tension transducer (Statham G1-64-675) which was mounted on a rigid stand having a magnetic base. The amplified output of the transducer was displayed on one channel of a digital voltmeter (Solartron, see below), and was used to monitor the spring tension in setting the isotonic tension level. By this method a required isotonic tension in the range of approximately 15–400 mN could be conveniently set by selecting the spring set and by moving the transducer stand away from or closer to the lever assembly. When the tension developed at the tip of the primary lever was monitored (isotonic tension in an experiment) by connecting it to the muscle tension transducer, its tension was found to remain constant within $\pm 3\%$ for a movement of up to 5 mm away from the after-stop (i.e. in the direction of muscle shortening in an experiment). The equivalent mass of the lever system was about 240 mg, as estimated from the acceleration produced when the afterloaded primary lever was suddenly released.

The movement of the primary lever was monitored by a photodiode with integral amplifier (308-067, RS Components Ltd.). A rectangular piece of photographic film stuck to the lever interrupted a beam of red light from a light emitting diodode reaching the photodiode. After appropriate biasing and amplification, the photodiode output was displayed on one of the four channels of a storage cathode ray oscilloscope (D 13, Tektronix).

Most of the experiments reported in this paper were carried out using the lever system detailed above. The lever system was completely rebuilt at a later stage, however, and some of the experiments were done using this new system. Its design was essentially the same as that described above and illustrated in Fig. 1. The differences in the new system were the following. The primary lever had no side arms and was built of wood (cocktail sticks) fixed together with Araldite. The secondary lever was made out of titanium and had several alternative points for attaching a spring set. Each lever was mounted on a stainless steel pivot, 11 mm long and 1·5 mm in diameter. The two ends of the pivot were ground and honed and were held by two 'travelling alarm clock' pivot bearings. The combined lever ratio could be readily altered by changing the point of attachment of the spring set to the secondary lever. The ratio used in most recordings was around 72 : 1. The values obtained for equivalent mass of the lever system were around the same range as for the earlier

system. For isometric recording, the lever movement was prevented by placing a thick glass rod horizontally and at right angles to the primary lever, approximately at the vertical position indicated by the filled triangle in Fig. 1. The glass rod was rigidly clamped to a magnetic stand and was so placed that the primary lever was firmly held between it and the after-stop. Experimental results obtained using this lever system were comparable to and pooled with the other results.

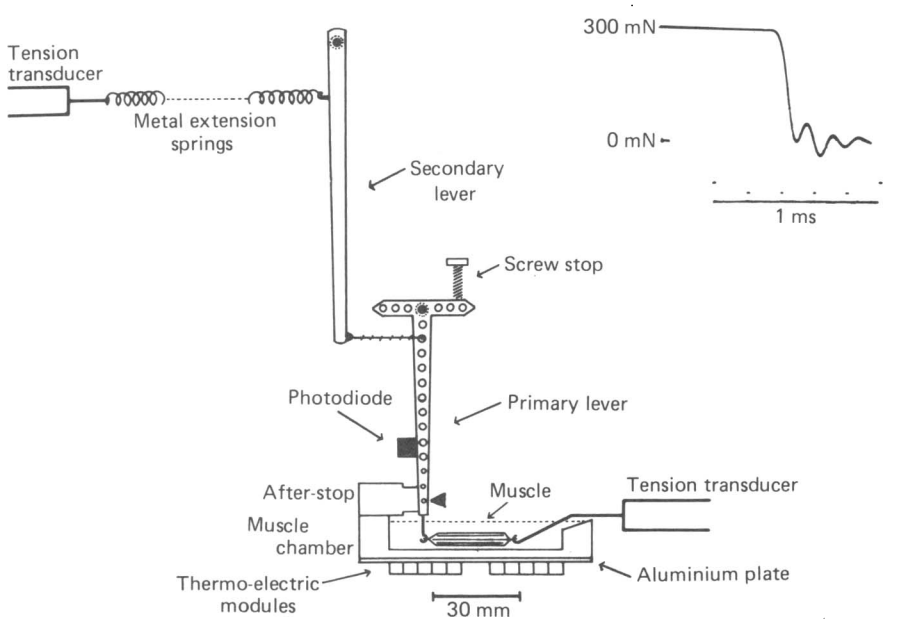

Fig. 1. A diagrammatic representation of the first lever system used for the experiments. The primary lever was made of magnesium and was mounted on the spindle of a Washington T1 isotonic transducer. The secondary lever was a Palmer crank lever with an extended lever arm. The primary lever movement was prevented by the screw stop for isometric recording. The diagram also represents schematically the second lever system. In this the primary lever was made of wood and had no side arms. Its movement was prevented by a horizontally placed glass rod (near filled triangle) for isometric recording. The secondary lever was made of titanium. Both levers were mounted on stainless steel pivots held by 'travelling alarm clock' pivot bearings. With both lever systems, the primary lever movement was monitored photo-electrically and after-loading was done by metal extension springs applied, as shown, via the secondary lever. Isotonic release experiments were done using the second lever system. The horizontal glass rod was replaced with a third lever moving in the horizontal plane. It was mounted on a d.c. motor whose activation held the primary lever against the after-stop and deactivation released the lever. When the horizontal lever was connected to the muscle tension transducer (with an effective lever arm length as used in an experiment), the transducer recorded a constant tension of 300 mN following the motor activation. The tension fell to near zero level in less that a ms when the motor was deactivated. The inset in the upper right corner is a record of the fall in tension, where the oscillations represent the natural resonant frequency of the muscle tension transducer.

In a separate series of experiments the shortening velocity was estimated by the method of isotonic release during the plateau of an isometric tetanus (Jewell & Wilkie, 1958). These experiments were done using the second lever system. The primary lever was held, about 10 mm above its tip (to which one muscle tendon was attached), by a (third) horizontal lever (i.e. perpendicular to the plane of the page in Fig. 1). This lever (built of wood) was mounted on the

spindle of a d.c. motor (335–292, RS Components Ltd.) so that activation of the motor (via a fast relay) held the primary lever against the after-stop (indicated by the filled triangle in Fig. 1), resisting a pulling force of up to 300 mN, and its de-activation released the primary lever. When tested directly against the arm of the muscle tension transducer (see inset in Fig. 1), the motor deactivation was found to release the lever in less than 1·0 ms. During an experiment the motor was activated some 200 ms prior to, and was deactivated a certain set time after, the beginning of stimulation. The timing was determined by a gate output from a digital timer (Digitimer, Devices).

Tension recording

The muscle tension transducer was a resistance-wire bridge strain gauge (Statham G1-32-350) which could measure tensions up to a maximum of around 9 N. Its response was linear to within $\pm 2\%$ over the entire tension range and its natural resonant frequency was 1·25 kHz. In early experiments a Fenlow A2 amplifier was used with the tension transducer, but this was later replaced with a bridge balance d.c. amplifier built using a strain gauge amplifier module (RS Components Ltd.). The noise on the tension record amounted to less than 0·3 mN and the base line (or d.c.) drift of tension during a recording was estimated to be no more than 0·2 mN/s. After amplification the peak tension was measured by means of a peak reading voltmeter (Model 440A, Control Data Corporation). The output of the peak voltmeter was fed via a scanner (LU 1461, Solartron) into one channel of a digital voltmeter (LM 1420, Solartron). The peak voltmeter output voltage was so adjusted that the digital voltmeter display of that channel during an experiment provided the peak tension in mN. Calibration of this tension recording system, by using triangular and half-sine wave pulses with rise times and peak amplitudes corresponding to those obtained in an experiment, indicated that tension measurements during these experiments should have been accurate to within $\pm 3\%$. The peak voltmeter was reset to zero once during each cycle of operation by a pulse from the master timer (Digitimer, Devices). The time of resetting was so adjusted that in the isometric mode (see below) it monitored the peak (isometric) tension and in the isotonic mode it recorded the steady isotonic tension, i.e. it was reset after the initial tension oscillations.

The tension records were examined on a storage cathode ray oscilloscope screen (D 13, Tektronix) by displaying the input to the peak voltmeter on one of the four vertical input channels of the oscilloscope. A second channel displayed a time scale output (see Fig. 3) from the Digitimer. The time to peak and the time to half-relaxation of the isometric twitch were estimated from such traces stored on the screen.

The tension signal was differentiated using an operational amplifier (Nexus SA-2). In most experiments a time constant of 0·6 ms was used. The positive peak of the differentiated signal was measured using a second peak recording voltmeter (440A, Control Data Corporation), the output of which was fed via the scanner to a second channel of the Solartron digital voltmeter. With suitable gain adjustment to the peak voltmeter output, the digital voltmeter display of this channel provided a measurement of the maximum rate of tension development in mN/ms during an experiment. The inaccuracy associated with the measurement of the maximum rate of tension development in a contraction was found to be not greater than $\pm 5\%$. The amplified differentiated tension record was displayed on a third channel of the cathode ray oscilloscope and examination of this record provided a useful guide to determining the fusion frequency for tetanic stimulation.

Muscle preparations

Most experiments were done on whole soleus and e.d.l. muscle preparations. One muscle tendon was attached to the arm of the muscle tendon transducer and the other to the hook of the primary lever (see Fig. 1). Each attachment was made by a small loop (of about 2·0 mm in diameter) made of non-absorbable, braided, silk thread tied securely to a muscle tendon, as close to the end of the muscle fibres as possible. The total compliance associated with these connections was estimated to be about 0·5 μm/mN. This amount of compliance would have resulted in approximately 3 to 4 % shortening in muscle fibre length in e.d.l. and 1 to 2 % shortening of muscle fibre length in soleus during an isometric tetanus at 35 °C.

Whole muscles could not be used with the isotonic release method since they developed too high tetanic tensions. The preparations used in this method were a muscle fibre bundle from e.d.l. muscle and a longitudinal muscle strip from soleus muscle. At least in the case of e.d.l. such a preparation had significantly less tendon elasticity in series with muscle fibres. For example, the ratio of muscle

fibre length to muscle length determined from surface measurements in the present study was 0·57 (±0·01 S.E. of mean, $n = 10$) from whole e.d.l. muscles, whereas, the corresponding ratio from fibre bundles was 0·77 (±0·02, S.E. of mean, $n = 4$). There was no such difference in soleus, although the muscle fibre to muscle length ratio was typically higher than in e.d.l. in the whole muscle: the ratios were 0·72 (±0·01, S.E. of mean, $n = 11$) for whole muscle and 0·77 (±0·01 S.E. of mean, $n = 4$) for muscle strips. In different experiments, the maximum tetanic tensions (P_0) of the preparations ranged between 40 and 180 mN. In so far as the decrease in P_0 during a recording session at a given temperature was not greater than in whole muscle experiments (see below), the performance of the preparations was considered satisfactory for the present purpose. The mean (±S.E. of mean) percentage isometric tetanic tension recorded at the end (from all the experiments) was 102·5 (±2·1, $n = 13$) from e.d.l. fibre bundles and it was 99·3 (±2·9, $n = 7$) from soleus strip preparations (temperature range 20–35 °C).

Temperature control, variation and measurement

The Perspex muscle chamber was mounted on an aluminium plate. The temperature control and variation was done by means of thermoelectric modules (Mectron-Frigister Ltd.) which were attached to the underside of the aluminium plate (see Fig. 1). The heating or cooling produced by the modules was under feed-back control from a thermistor placed in the chamber. The temperature of the saline bathing the muscle was monitored by a separate thermistor connected to a digital thermometer (Digitec United Systems Corporation). During an experiment a temperature change of 5 °C was effected in about 10 min and recording at the new temperature was done after about 15 min of equilibration. The saline solution in the chamber was replaced at least once in between recording at two different temperatues. The temperature variation during a series of contractions was typically less than ±1 °C.

Stimulation

The muscle was stimulated directly by applying voltage pulses to two stainless steel plate electrodes placed 10 mm apart and on either side of the muscle. Each electrode was about 20 mm long and 7 mm wide. Unipolar stimulating pulses were obtained from an electronic square wave stimulator (Palmer Ltd.) in the early experiments and from a high voltage stimulator (Type 3072, Devices) in the other experiments. In either case, the stimulator output was connected to the electrodes via a step-down transformer (approximately 5 : 1). Duration of the stimulating pulses was from 0·2 to 0·6 ms.

Considerable care was taken in adjusting the stimulus parameters during an experiment. Whereas the stimulus intensity used was clearly supramaximal and within the mid-range of intensities which produced maximal twitch tension development, it was lower than those which resulted in 'repetitive firing'. A clear, sudden, increase in the time to peak of the twitch for a given step increase intensity, was taken as a sign of the occurrence of 'repetitive firing'. The amplitudes of the supramaximal stimuli used in these experiments were estimated to be in the range of 20–35 V in the muscle chamber. The polarity of the stimulus was reversed at infrequent intervals and no obvious indications of electrode polarization were observed during the time course of an experiment.

The stimulator was triggered by a Devices 'gated pulse generator', which was used to obtain the required stimulus frequencies.

A muscle was stimulated once every 30 s for setting stimulus parameters and muscle length. The interval between single stimuli was then increased to 60–90 s. Tetanic contractions were elicited at intervals no shorter than 3 min.

The appropriate frequencies for eliciting tetani at different temperatures were examined in a preliminary series of experiments. The change produced in the tetanic frequency *versus* peak tension relation when the temperature is lowered from 35–20 °C is illustrated in Fig. 2. On the basis of these observations the range of frequency employed for tetanic stimulation at 35, 30, 25 and 20 °C (±1·0 °C) were, respectively, 300–500, 200–400, 150–350, and 100–300 Hz for e.d.l. and 200–350, 150–300, 100–200 and 60–100 Hz for soleus. The frequency used for determining isometric tetanic tension (P_0) was nearer the lower end of a range and the duration was sufficiently long to record the tension plateau. The frequencies used for recording the maximum rate of development of tension resulted in near fusion of mechanical responses as seen in the differentiated tension record (Buller & Lewis, 1965). The frequencies used in isotonic recording at 35 °C were comparable to, but slightly higher than, those employed by Close (1964) for shortening velocity measurements in rat muscles at 35 °C.

Experimental procedure

Typically a muscle was set for recording isometrically at 35 °C and the stimulus parameters and the muscle length were adjusted for recording maximal twitch tension. About three tetanic contractions were elicited using frequencies and durations suited for recording maximal isometric tetanic tension and maximum rate of tension development. The recording was then changed to the isotonic mode and 7–10 isotonic tetanic contractions at different after-loads were recorded. The isometric tetanic tension was checked halfway through and at the end of an isotonic series. The

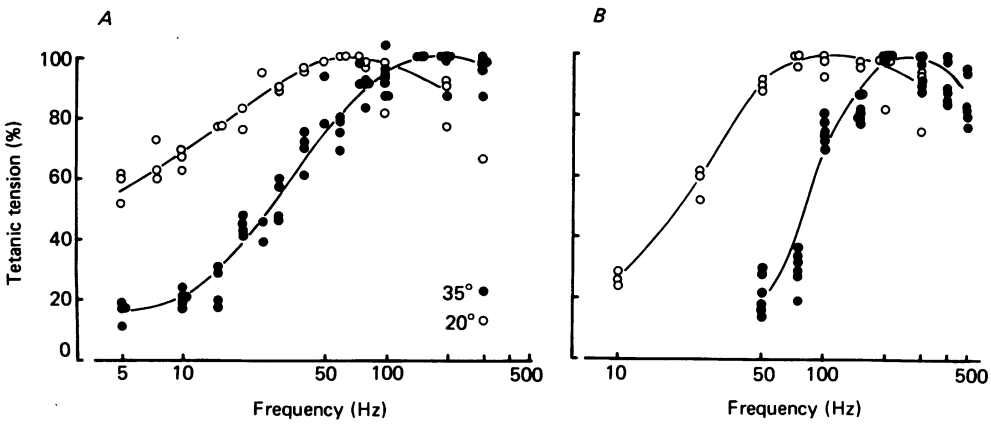

Fig. 2. Dependence on stimulation frequency of isometric tetanic tension in soleus (*A*) and e.d.l. (*B*) muscles at 35 °C (filled symbols) and 20 °C (open symbols). Results are from three e.d.l. and three soleus muscles. In each muscle, a complete relation was established at 35 °C, at 20 °C and finally at 35 °C after rewarming. The tension is plotted as a percentage of the maximum tetanic tension recorded at that temperature in each muscle.

mean (±S.E. of mean) isometric tetanic tension recorded at the end of a series (as a % initial tension) was 91·5 (±2·0), 99·8 (±1·4), 95 (±0·7), 98 (±0·6) for e.d.l. and 93·9 (±1·2), 97·4 (±1·5), 103 (±1·1), 103 (±2·3) for soleus at 35, 30, 25 and 20 °C respectively. The temperature was then changed to 30, 25, or 20 °C and a procedure similar to the above was repeated.

Analysis of results

Hill's equation (Hill, 1938), $(P+a)V = b(P_0-P)$, in which P = isotonic tension, V = speed of shortening, P_0 = maximum isometric tetanic tension and a and b are constants, was fitted to the tension and velocity measurements obtained from a muscle at a given temperature. Constants a and b were calculated by plotting P as ordinate against $(P_0-P)/V$ as abscissa. A straight line was fitted to the data by the method of least squares and a was estimated as the intercept on the ordinate and b as the slope of the line. P was represented as a ratio of the average P_0 recorded prior to and after the isotonic contractions.

The temperature dependence of different parameters was examined using the Arrhenius equation: Rate $= A \cdot e^{-E/RT}$ where A = constant, E = Arrhenius activation energy, $R = 8{\cdot}317\ \mathrm{J \cdot K^{-1}}$, and T = absolute temperature, in degrees Kelvin (°K).

Some general considerations

One major problem encountered in *in vitro* physiological studies on mammalian muscle is muscle fatigue (Ranatunga, 1979, 1980). The fatigue is greater at higher temperatures, is enhanced by repetitive stimulation and is more pronounced in fast twitch muscle (Brust, 1976; Ranatunga, 1979). Consideration had to be given, therefore, as to what extent the measurements taken for Arrhenius analyses in the present study were affected by muscle fatigue.

The experimental procedure listed above was followed strictly in experiments on eleven e.d.l. and on sixteen soleus muscles. In each experiment two sessions of recording were made at 35 °C,

the first at the beginning of the experiment prior to cooling and the second at the end after rewarming. An average total of around forty tetani (39·3 ± 3·0, S.E. of mean, in e.d.l. and 42·1 ± 3·3, S.E. of mean, in soleus) were elicited in each muscle, of which twenty-five tetani (24·7 ± 1·4, S.E. of mean, in e.d.l. and 24·6 ± 1·6, S.E. of mean, in soleus) were elicited at 35 °C. From these experiments, the peak tension in the last isometric tetanus (i.e. from the second session at 35 °C) which was considered in the analyses was represented as a percentage of that recorded at 35 °C at the beginning of the first session. Mean (±S.E. of mean) percentage was 85·7 (±3·5) from e.d.l. and 91·2 (±2·0) from soleus experiments. Presented in the same manner, the mean percentage ratios (±S.E. of mean) for the rate of tension development and the maximum velocity of shortening values respectively were 104·8 (±3·1) and 96·4 (±3·5) from e.d.l. and 98·8 (±2·2) and 102 (±4·1) from soleus muscles. Paired comparisons of values obtained in the two sessions indicated no significant difference ($P > 0.05$) for rate of tension development and for maximum shortening velocity but a significant difference ($P < 0.01$) for tetanic tension in both muscle types. Examination of tetanic tension (P_0) data showed that much of the tension fatigue occurred during recording at 35 °C. Therefore, in estimating the depression of tetanic tension induced by cooling, the P_0 recorded at lower temperatures were represented as a percentage of the average of the P_0 recorded at the end of first session and at the beginning of the second session at 35 °C. The mean (±S.E. of mean) of average P_0 at 35 °C was 97·9 (±1·3) in e.d.l. and 98·8 (±0·56) in soleus muscles. The P_0 data at other temperatures are given in Table 1 and they are similar to those reported in previous studies in which muscles were not exposed to as much repetitive tetanic stimulation as was necessary in the present study (Close & Hoh, 1968; Ranatunga, 1977*a*, 1980).

Similar calculations were also done on the peak tension, the time to peak and the time to half relaxation of isometric twitch contractions recorded in the two sessions at 35 °C. The mean (±S.E. of mean) percentages were, respectively, 75·5 (±7·4), 92·2 (±2·6), and 98·0 (±2·9) from e.d.l. and 103 (±5·3), 96·6 (±1·7) and 96·2 (±3·1) from soleus experiments. Paired comparisons between values obtained from the two sessions showed no significant difference ($P > 0.05$) for twitch tension, time to peak and time to half-relaxation in soleus and for half-relaxation in e.d.l. The differences in twitch tension and time to peak measurements in e.d.l. were found to be significant ($P < 0.02$).

On the basis of above considerations, it appears that – excepting time to peak measurements in e.d.l. muscle – the different measurements taken for Arrhenius analyses were not excessively affected as a result of muscle fatigue in either muscle type. It will be seen also that the temperature sensitivity determined from time to peak measurements of e.d.l. is not outside the range obtained in previous studies (Close & Hoh, 1968; Ranatunga, 1977*a*, 1980) in which fatigue was minimized by avoiding repetitive stimulation.

RESULTS

A representative set of oscilloscope records from a soleus muscle is illustrated in Fig. 3. A twitch and a tetanus recorded in the isometric mode are illustrated in the upper frames, and isotonic tetani at two different afterloads are shown in the lower frames. The bottom trace in each frame represents the photodiode output monitoring the muscle shortening and the one above represents muscle tension. Muscle tension (isometric and isotonic) and the maximum rate of isometric tetanic tension development (from the peak of the differentiated tension record; see upper right frame) were measured by using peak reading voltmeters. The time to peak and time to half-relaxation and the velocity of isotonic shortening were estimated by inspection of records (such as the ones illustrated in Fig. 3) stored on an oscilloscope screen (see Methods).

The tensions per cross-sectional area and the twitch tension: tetanic tension ratios recorded at 35 °C in the present study were comparable to values obtained in a separate previous study (Ranatunga, 1979). As reported in previous studies on mammalian fast and slow twitch muscles at 35–38 °C (Wells, 1965; Close, 1969; Buller

& Pope, 1977), the values of the constant a in Hill's equation and the maximum velocity of shortening for e.d.l. muscle were considerably higher than those for soleus muscle (see Table 1).

Velocity of shortening

Force and velocity measurements made from an e.d.l. muscle at three different temperatures (at 35, 30 and 20 °C) are shown in Fig. 4*A*. Force, as a percentage of the isometric tetanic tension, is plotted along the abscissa and velocity (mm/s) along

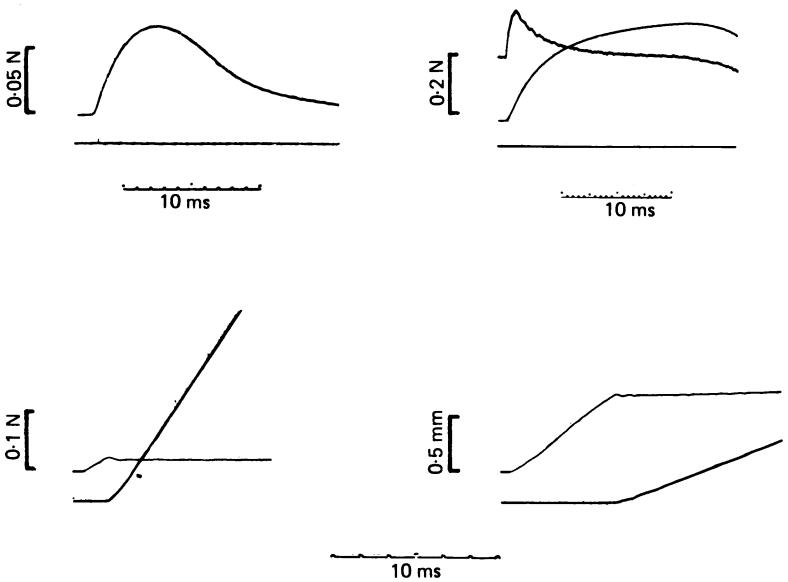

Fig. 3. A typical set of records (from a soleus muscle at 25 °C). An isometric twitch (left) and an isometric tetanus (right) are shown in the upper frames and two after-loaded isotonic contractions in the lower frames. The lower trace in each frame represents muscle shortening and the other, the muscle tension. The top trace in the upper right frame is the electronically differentiated tension, the peak of which was measured as the maximum rate of isometric tension development.

the ordinate. The line drawn through each set of points represents that fitted using Hill's equation (Hill, 1938). The linear plots used in the determination of the constants a and b in Hill's equation are shown in Fig. 4*B*. These results illustrate the major temperature-dependent changes which occur in the force–velocity relation of both muscle types. Cooling results in a marked reduction in the maximum velocity of shortening (velocity at zero load: see Fig. 4*A*) associated with a reduction in the constant b (slopes of linear plots: see Fig. 4*B*) and in a less marked reduction in the constant a (intercepts in Fig. 4*B*). These findings are qualitatively similar to the observations made on frog muscle (Katz, 1939). Summary of the data is given in Table 1.

Maximum velocity of shortening estimated from a total of ten e.d.l. muscles and thirteen soleus muscles are presented in the form of an Arrhenius plot in Fig. 5*A*. Maximum velocity (mm/s) is plotted along a logarithmic ordinate and the reciprocal

of the absolute temperature, $(1/T) \times 10^3$, along the abscissa. Logarithm of the maximum velocity of e.d.l. (open circles) decreased linearly with $1/T$ over the entire temperature range. The line drawn through the points represents the calculated regression for all the data points, and its slope corresponds to a temperature co-efficient (Q_{10}, as velocity at 35 °C divided by velocity at 25 °C) of 1·76 and an

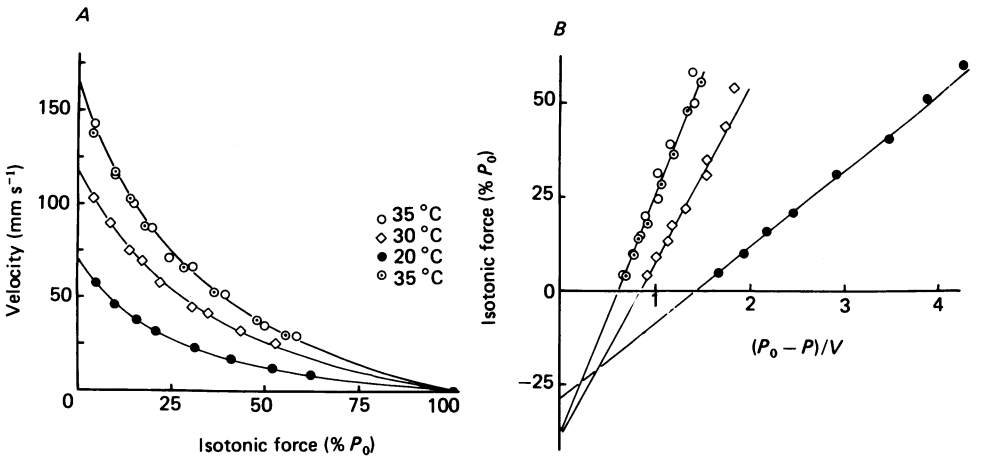

Fig. 4. Force–velocity relations at different temperatures. *A*, force–velocity relations at three different temperatures from one e.d.l. muscle. Force is represented as a percentage of isometric tetanic tension at that temperature. The sequence in which data were collected was 35, 30, 20 and 35 °C (circles with a dot). Curves drawn through points represent those calculated using Hill's equation (Hill, 1938). *B*, plot of force (ordinate) versus $(P_0 - P)/V$ for the same data as in *A*. Straight lines represent linear regressions calculated for the data points. Intercept was taken as constant *a* and slope as constant *b* of Hill's equation.

Arrhenius activation energy (E) of 43·3 kJ. Inspection of the soleus data indicated that there was some uncertainty as to whether its results around 20 °C should be included in such an analysis. The continuous line drawn through soleus data is the calculated regression excluding data at around 20 °C. The Q_{10} estimated from the regression was 1·74 and the activation energy was 42·1 kJ.

When the above type of analysis was made for maximum velocity data normalized to muscle length, E was found to be 42·9 kJ for e.d.l. and 40·9 kJ for soleus. Values of E estimated from data of individual muscles ranged from 39·9 to 50·5 kJ (mean ± S.E. of mean 44·8 ± 1·3, $n = 9$) in e.d.l. and from 36·7 to 41·6 kJ (mean ± S.E. of mean 40·1 ± 0·6, $n = 7$) in soleus muscles.

Values of E estimated from velocities calculated to correspond to 10 % P_0 were 46·2 kJ and 48·4 kJ respectively for e.d.l. and soleus. A similar calculation on actual velocity measurements made with a 9–11 % P_0 isotonic force (eight e.d.l. muscles, $n = 13$ and twelve soleus muscles, $n = 17$) yielded similar E values of 46·3 kJ (e.d.l.) and 44·4 kJ (soleus).

In all the above analyses, the soleus data at around 20 °C were excluded. Reasons for exclusion of these data were the following.

(a) The mean Q_{10} for maximum volocity of shortening, calculated from individual soleus muscle

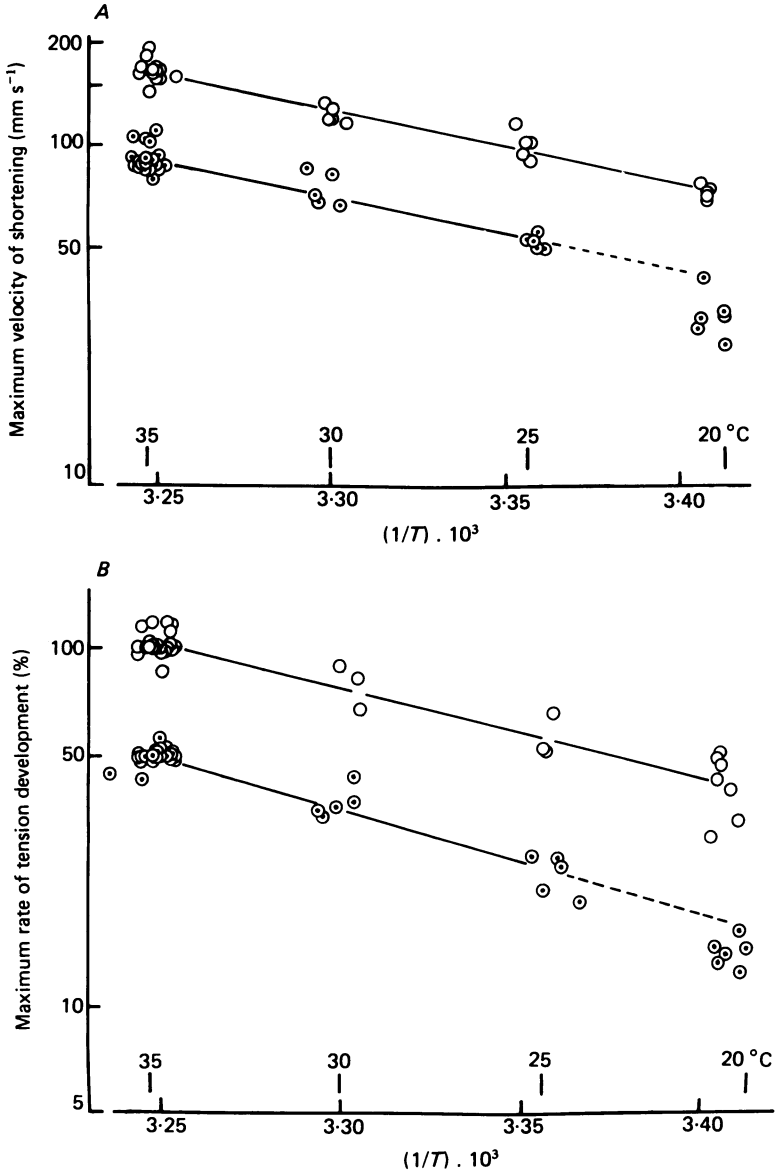

Fig. 5. Dependence on temperature of maximum velocity of shortening and of maximum rate of tension development. *A*, the maximum velocity of shortening in mm s^{-1} is plotted along a logarithmic ordinate and reciprocal of the absolute temperature, $(1/T) \times 10^3$, along the abscissa (an Arrhenius plot). The abscissa is also labelled in °C. E.d.l. data are shown with open symbols. The continuous line through each set of points is the calculated regression for all the data points in e.d.l. and excluding data points at 20 °C for soleus. Correlation co-efficient was 0·98 ($n = 28$) for e.d.l. and 0·93 ($n = 29$) for soleus: the activation energies determined from their slopes were 43·3 kJ (e.d.l.) and 42·1 kJ (soleus). *B*, Arrhenius plot for maximum rate of isometric tension development. The rate of tension development is plotted, as a percentage of that at 35 °C, along a logarithmic ordinate. (For soleus, 100 % is plotted as equal to 50.) Correlation co-efficients were 0·95 ($n = 35$) for e.d.l. and 0·96 ($n = 26$) for soleus and their activation energies in kJ were 48·3 (e.d.l.) and 55·7 (soleus). Soleus data at 20 °C were excluded.

data was $1{\cdot}68 \pm 0{\cdot}01$ S.E. ($n = 8$) when data at 20 °C were excluded: this was significantly ($P < 0{\cdot}002$) different from the mean Q_{10} of $1{\cdot}93 \pm 0{\cdot}09$ S.E. ($n = 11$) determined for all the data. The comparable calculation on e.d.l. results indicated no significant difference.

(b) Inclusion of data at 20 °C in a regression analysis as in Fig. 5 resulted in a clear increase in the slope in the case of soleus, whereas it produced only a little change in the case of e.d.l. For instance, the activation energy of the maximum velocity of shortening for e.d.l. was 43·3 kJ from all the data (see Fig. 5*A*) and it was 41·9 kJ when data at 20 °C were excluded. The same calculation for the maximum velocity of shortening of soleus yielded a value of 52·1 kJ from all results and a value of 42·1 kJ without data at 20 °C (see Fig. 5*A*).

(c) All the other measurements (e.g. rate of tension development, time to peak twitch tension and time to half-relaxation) made from soleus muscles at 20 °C showed evidence of deviation from the expected regression (see below).

In the above experiments the shortening velocities were estimated from the initial phase of after-loaded isotonic contractions. A more appropriate way to estimate shortening velocity is to perform isotonic releases during the plateau of an isometric tetanus (Jewell & Wilkie, 1958). A series of observations were made with this method. These experiments were done on muscle strips (in soleus) and muscle fibre bundles in e.d.l. (see Methods section).

A representative set of records taken from an experiment on a soleus muscle strip at 25 °C are illustrated in Fig. 6. An isotonic release during one tetanic contraction is shown in the upper left frame, where the upper trace is the tension and the lower is the length record. It is seen that following the release, the tension reduced from an isometric value of 100 mN to an isotonic tension of around 25 mN. The length record illustrates a rapid shortening followed by a slower and steady shortening phase (Jewell & Wilkie, 1958). In each record the transition from one phase to the other is obscured by oscillations. Although the origin of these oscillations is most likely to be in the lever system (especially in the spring loading system) no attempt was made during these experiments to damp them.

In a few experiments, the isotonic release was done at different delays after the beginning of tetanic contraction. The five pairs of superimposed tension and length records shown in upper right frame of Fig. 6 illustrate the essential observation made: there was little if any consistent change in the steady shortening velocity. It is of interest to note, however, that the amplitude of the rapid shortening appeared to scale down with the pre-release isometric tension.

In experiments on six preparations (two e.d.l. and four soleus) a total of thirteen force–velocity relations at different temperatures were determined employing both methods. It was found that in a given preparation, at a given temperature, and with the same isotonic force, the steady shortening velocities determined by the two methods were similar, as had been noted in frog muscle (Jewell & Wilkie, 1958). The superimposed pairs of tension and length records shown in the lower three frames of Fig. 6 were obtained with the two techniques using three different after-loads. They illustrate the extent of similarity (or difference) seen. Indeed no significant difference was found ($P > 0{\cdot}1$) when paired comparisons were made between values obtained with the two methods, either for maximum shortening velocity or for a/P_0 ratio, in both e.d.l. and soleus preparations. Arrhenius activation energies were also calculated on pooled maximum velocity of shortening data collected with the isotonic release method: they were 44·5 kJ ($n = 13$) for e.d.l. and 45·1 kJ ($n = 5$) for soleus (excluding soleus data below 22 °C).

The data presented in Fig. 5*A* and in the preceding paragraphs and the analyses referred to indicate that various techniques and types of data analyses yield somewhat different values for the Arrhenius energy of maximum velocity of shortening. However, all the mean estimates fall within the range of 40·1–45·1 kJ.

Rate of isometric tension development

The positive peak of the differentiated tetanic tension record was measured as representing the maximum rate of tension development (Buller & Lewis, 1965). Variation with temperature of this parameter is shown as an Arrhenius plot in

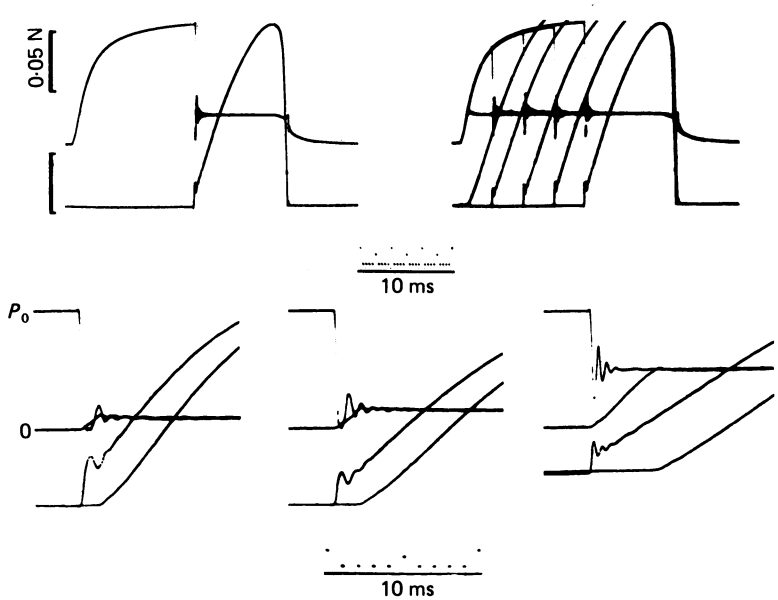

Fig. 6. Representative experimental records obtained in a soleus muscle (strip) experiment at 25 °C using the isotonic release method. In each frame, the upper trace represents tension and the lower the length. Upper left: an isotonic release during the plateau of an isometric tetanus is shown. Note that tension drops to about 25 % P_0 and the length record shows two clear phases: the transitions from one phase to the other are masked by oscillations. Upper right: five pairs of superimposed tension and length records illustrating isotonic releases at different times after the beginning of a tetanus. The steady shortening velocity remains similar but initial rapid shortening scales down with the tension before the release. Lower frames: a pair of superimposed tension and length records comparing after-loaded shortening with shortening following isotonic release, at three different isotonic tensions (equal to the afterloads). The unlabelled vertical calibration bar represents 1 mm of shortening for all the records except lower right in which it represents 0·4 mm.

Fig. 5*B*, where the maximum rate of tension development is plotted as a percentage of that recorded in the same muscle at 35 °C prior to cooling. The activation energies calculated from the regressions on these pooled data were 48·3 kJ ($n = 35$; $Q_{10} = 1·88$) for e.d.l. and 55·7 kJ ($n = 26$; $Q_{10} = 2·07$) for soleus, excluding soleus data at around 20 °C. When activation energy was estimated for each individual muscle experiment,

the mean (±S.E. of mean) was 47·6 (±2·3, $n = 11$) kJ in e.d.l. and was 55·2 (±3·3, $n = 8$) kJ in soleus. It appears from the above values that the activation energy of the maximum rate of tension development is somewhat higher than that of the maximum velocity of shortening.

There was a wide variability in the values of E determined from individual muscle experiments. The values of e.d.l. muscles ranged from 39·9 to 64·5 kJ and those of soleus from 39·4 to 67·1 kJ in different experiments. Furthermore, when paired comparisons were made between E values of maximum velocity of shortening and rate of tension development obtained from the same muscles (eight e.d.l. and seven soleus), their difference was found to be significant ($P < 0·01$) only in the case of soleus.

The question may be raised as to whether the maximum rate of tension development was obtained in the present experiments, since the existence of a considerable series compliance (including the tendon elasticity) may have resulted in a certain degree of muscle fibre shortening during isometric recording (see Methods). What effect this may have had on the estimate of temperature sensitivity for the rate of tension development remains uncertain. An equally important consideration in this connection concerns the pronounced frequency dependence of the rate of tension development in mammalian muscle fibres at 35–38 °C. As was first reported by Buller & Lewis (1965), the rate of tension development increases with stimulus frequency, and the highest rate is recorded at a frequency which is near the limit set by the refractoriness of the muscle fibre membrane. Thus, there is considerable uncertainty as to whether the maximum rate of tension development of the contractile apparatus can be achieved in mammalian muscle contraction at 35–38 °C. Cooling appears to decrease the degree of frequency dependence of the rate of tension development in mammalian muscle (Ranatunga, 1977*b*). The implication from the above considerations is that the temperature sensitivity of the rate of tension development obtained in the present experiments may not represent an over-estimate.

The usual way of analysing the rate of tension development in mammalian muscle contraction was to represent it as a percentage of tetanic tension (as % P_0 ms^{-1}): such normalization allows pooling of data from muscles developing different tensions and, thus, comparison of the rates in muscles with different speeds of contraction (Buller & Lewis, 1965). The mean rates of tension development (at different temperatures) represented in this manner are given in Table 1. Such normalization was not appropriate in the context of the analyses made in the present study, since the tetanic tension in a given muscle tended to decrease with temperature. When the analyses were made following normalization of the maximum rate of tension development to P_0 at each temperature, the temperature sensitivity was found to be similar to that of the maximum velocity of shortening for both muscles (E was 40 kJ for e.d.l. and 47·2 kJ for soleus). This was the method of analysis adopted in the brief report already published (Ranatunga, 1981).

Isometric twitch

The measurements made from the same set of muscles for the time to peak tension and the time to half-relaxation in the isometric twitch myogram are presented as Arrhenius plots in Fig. 7. These results are essentially similar to previous observations (Hoh, 1974; Ranatunga, 1977*a*, 1980) and they are presented here for comparison with those for maximum velocity of shortening (Fig. 5*A*). One may note three relevant points. Firstly, to a first approximation, there is a linear relation between logarithms of the time to peak and of time to half-relaxation and $1/T$, for both muscles. Secondly, as in the case of velocity of shortening and rate of tension development a fair number of these values of soleus at around 20 °C are somewhat lower than expected from a

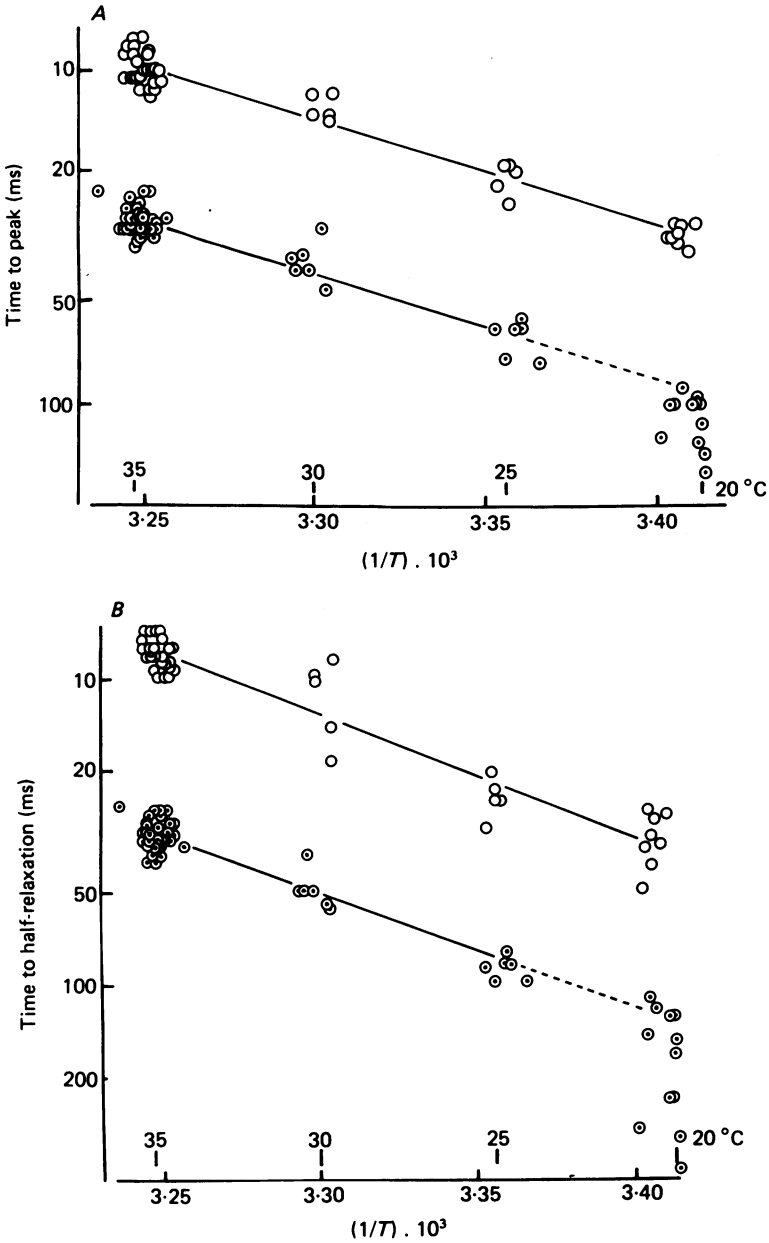

Fig. 7. Temperature-dependence of the time to peak and the time to half-relaxation of the isometric twitch. *A*, Arrhenius plots for time to peak tension of isometric twitch. Time to peak in ms is plotted along a reciprocal logarithmic ordinate. Correlation co-efficients for the regressions were 0·98 ($n = 43$) in e.d.l. and 0·93 ($n = 44$) in soleus (excluding data at 20 °C). The activation energies in kJ were 62·2 (e.d.l.) and 60·0 (soleus). *B*, Arrhenius plots for time to half-relaxation of isometric twitch. Presentation and analysis are similar to *A*. Correlation co-efficients were 0·96 ($n = 43$) for e.d.l. and 0·94 ($n = 44$) for soleus, with activation energies in kJ of 78·2 (e.d.l.) and 72·5 (soleus).

single regression. Thirdly, the slope of each regression is clearly higher than that determined for maximum velocity of shortening. Thus, the values obtained for E (excluding soleus data at 20 °C) were 62·2 and 60·0 kJ from time to peak measurements, and they were 78·2 and 72·5 kJ from time to half-relaxation measurements, respectively for e.d.l. and soleus. The Q_{10}s were 2·26 and 2·20 for time to peak and 2·79 and 2·59 for time to half-relaxation measurements.

TABLE 1. Summary of the data from a total of seventeen soleus (A) and fourteen extensor digitorum longus (e.d.l., (B)) muscles of 4-week-old male rats

	(A) Soleus data			
	35 °C	30 °C	25 °C	20 °C
Tetanic tension	98·8 ± 0·56 (32)	99·6 ± 0·9 (5)	95·5 ± 2·3 (6)	86·1 ± 2·4 (11)
Twitch–tetanus tension ratio	0·21 ± 0·01 (32)	0·231 ± 0·01 (6)	0·254 ± 0·02 (7)	0·247 ± 0·01 (12)
Maximum rate of tetanic tension development	2·01 ± 0·05 (22)	1·58 ± 0·04 (6)	1·14 ± 0·06 (6)	0·6 ± 0·04 (7)
a/P_0	0·212 ± 0·01 (19)	0·18 ± 0·02 (5)	0·157 ± 0·01 (5)	0·137 ± 0·01 (6)
Maximum velocity of shortening	4·65 ± 0·11 (19)	3·76 ± 0·32 (5)	2·66 ± 0·11 (5)	1·58 ± 0·13 (6)
	(B) E.d.l. data			
	35 °C	30 °C	25 °C	20 °C
Tetanic tension	97·9 ± 1·3 (22)	98·2 ± 6·8 (3)	95·8 ± 3·4 (3)	89·8 ± 2·9 (7)
Twitch–tetanus tension ratio	0·146 ± 0·01 (24)	0·153 ± 0·01 (5)	0·257 ± 0·01 (5)	0·264 ± 0·02 (8)
Maximum rate of tetanic tension development	4·06 ± 0·14 (24)	2·93 ± 0·12 (5)	2·39 ± 0·15 (5)	1·83 ± 0·08 (8)
a/P_0	0·415 ± 0·01 (13)	0·429 ± 0·02 (5)	0·385 ± 0·03 (5)	0·283 ± 0·01 (5)
Maximum velocity of shortening	7·71 ± 0·22 (13)	5·75 ± 0·24 (5)	4·61 ± 0·29 (5)	3·4 ± 0·18 (5)

Each value is given as a mean ± S.E. of mean with number of experiments in brackets. The left-most column pools the data obtained at 35 °C, prior to cooling, and after rewarming. The data listed are the maximum tetanic tension (P_0) as a percentage of that recorded at 35 °C (see Methods), the twitch tension–tetanic tension ratio, the maximum rate of tetanic tension development as % P_0/ms, the constant a (as a/P_0) in Hill's equation, and the maximum velocity of shortening in muscle lengths/s.

The mean (± S.E. of mean) muscle lengths were 19·4 ± 0·46 mm ($n = 13$) for soleus and 21·8 ± 0·44 mm ($n = 12$) for e.d.l., and their weights were 39·6 ± 3·5 mg ($n = 9$) and 43·9 ± 3·5 mg ($n = 11$). Tensions per cross-sectional area calculated using these measurements were $2{\cdot}23 \pm 0{\cdot}18 \times 10^5$ N . m^{-2} for soleus and $3{\cdot}6 \pm 0{\cdot}14 \times 10^5$ N . m^{-2} for e.d.l. at 35 °C.

DISCUSSION

An attempt was made in the present study to obtain quantitative data for the temperature sensitivity of maximum velocity of shortening, maximum rate of isometric tension development, isometric twitch time to peak and isometric twitch time to half-relaxation in rat muscle. These measurements are commonly recorded in experiments on mammalian skeletal muscle. There were three findings which may prove to be of general interest. Firstly, the Arrhenius plots were, to a first

approximation, linear within the temperature range of 35–20 °C in e.d.l. and 35–25 °C in soleus, with respect of all the measurements. The simplest interpretation of this observation would be (see Crozier, 1924; Levy, Sharon & Koshland, 1959; Gutfreund, 1972) that the underlying basis of each parameter remains the same within that temperature range. Secondly, as shown by their different Arrhenius energies (E), the parameters differed in the degree of temperature sensitivity. The maximum velocity of shortening showed the least, and time to half-relaxation of twitch showed the highest sensitivity to a temperature change. The implication here is that their rate limiting processes are not the same. Thirdly, it may be argued that the results from the fast e.d.l. and the slow soleus muscles were similar. Thus, the sequence in which the degree of temperature sensitivity increased was from velocity of shortening – rate of tension development – time to peak – time to half-relaxation in both muscles. Furthermore, the Arrhenius energies for velocity of shortening, time to peak, and time to half-relaxation, were also similar between the two muscle types. This is indicative of the fact that although soleus and e.d.l. differ in the absolute speed of their responses, the underlying events of their contractions are of the same type. However, it should be noted that the temperature sensitivities of maximum rate of tension development, a/P_0 and b (see Table 1) appear to be different in the two muscles (see below).

A good correlation has been found between the maximum velocity of shortening and actomyosin ATPase activity in muscles having different speeds of contraction (Barany, 1967) implying that the biochemical basis of maximum shortening velocity in a muscle may be its actomyosin ATPase activity (see also Podolsky & Tawada, 1980). The activation energy of actomyosin ATPase activity of rabbit muscle myosin, in the neighbourhood of 25 °C, has been estimated to be around 50 kJ (Levy *et al.* 1959), which is somewhat higher than the average values of 40–45 kJ obtained for the maximum velocity of shortening in the present study. It is noteworthy that E values for maximum rate of tension development were also close to 50 kJ, around 48·0 kJ in e.d.l. and 56 kJ in soleus. The E values for time to peak and time to half-relaxation of the isometric twitch on the other hand, were higher, between 60 and 80 kJ. Clearly the time course of the isometric twitch in mammalian muscle is not determined by its intrinsic speed of shortening (Close, 1965). The activation energy of time to peak (60 kJ) obtained here, is comparable to that determined for initial calcium uptake by isolated sarcoplasmic reticular vesicles of rabbit muscle by Inesi & Watanabe (1967) (between 54·0 and 67·0 kJ), but it is much higher than the values of about 46·0 kJ reported from a similar study by Sreter (1969).

The data presented in this paper may be of interest in relation to A. F. Huxley's (1957) cross-bridge theory. On the basis of these formulations (see Simmons & Jewell, 1974) (*a*) the maximum velocity of shortening is principally dependent on the rate of cross-bridge detachment during shortening (rate constant g_2); (*b*) the rising phase of an isometric tetanus (and hence, the maximum rate of tension development) is determined by f_1+g_1 – the net rate of cross-bridge attachment – where f_1 represents the rate of cross-bridge attachment and g_1, the rate of isometric cross-bridge detachment; and (*c*) the shape of the force–velocity relation is largely determined by $(f_1+g_1)/g_2$ where a decrease in the ratio represents an increase in the curvature.

The present results show that, in the case of e.d.l. muscle, the curvature of the

force–velocity relation remains largely unchanged between 35 and 25 °C (see a/P_0 ratios in Table 1). A plausible interpretation of this finding is that the temperature sensitivity of g_2 and of (f_1+g_1) is similar. It appears therefore that the temperature sensitivities (E values) of the maximum velocity of shortening (g_2) and maximum rate of tension development (f_1+g_1) of e.d.l. are expected to be of the same order of magnitude for the temperature range of 35–25 °C, as was found in the present study. In contrast to e.d.l., the curvature of the force–velocity relation of soleus increases in cooling (see Table 1), indicating that (f_1+g_1) is more temperature sensitive than g_2. Consequently, the finding that the temperature sensitivity of the rate of tension development in soleus is clearly higher than that of the maximum velocity of shortening is also as expected on the basis of the cross-bridge theory.

The results also show that, in cooling from 35 to 25 °C, there is a small decrease in the maximum tetanic tension (P_0). P_0 is given by $C\,(f_1/(f_1+g_1))$ in the cross-bridge theory, where C is a constant incorporating bridge stiffness and density of bridges and sites. On the assumption that there is no change in the constant C, the relatively small decrease in P_0 implies that the temperature sensitivities of f_1 (cross-bridge attachment) and (f_1+g_1) are approximately similar for both muscles.

An apparent change in the temperature sensitivity of the contractile process is indicated in some of the results from both muscles, in cooling below 25 °C. Thus, cooling below 25 °C results in a definite decrease in P_0 in both muscles, an increased curvature of the force–velocity relation of e.d.l. and an increased temperature sensitivity of most contraction parameters of soleus muscle. It is of interest to note in this connection that Stephenson & Williams (1981) have obtained evidence suggestive of a considerable decrease in the number of cross-bridge interacting sites in rat fast and slow skinned muscle fibres at temperatures below 25 °C. A detailed analysis should await experimentation at temperatures below 20 °C.

The above considerations show that, to a first approximation, the observations reported in the present study are qualitatively explicable on the basis of A. F. Huxley's (1957) cross-bridge theory formulations. It is relevant to note, however, that the temperature sensitivities of the maximum velocity of shortening and rate of tension development in e.d.l. were not significantly different and that they were within the same range for both muscles when the maximum rate of tension development was normalized to P_0. An interpretation of these findings may be that the rate-limiting process underlying the maximum shortening velocity and maximum rate of tension development is the same, which would be in keeping with the suggestion made by Podolsky & Tawada (1980) – from a different type of experiment – that there is one rate-limiting step in muscle contraction. The experiments reported here were made on whole muscle preparations and no account was taken of the many uncertainties inherent in the use of such preparations. These include the existence of a considerable series (tendon) elasticity and the heterogeneity of the muscle fibre type composition in the two muscles. Thus, the interpretations given for these observations and the conclusions made should be considered as tentative. However, the observations have been sufficiently informative to suggest that definition of temperature sensitivity of contraction measurements in physiological experiments may provide valuable insight into the rate limiting events in muscle contraction.

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