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TEMPERATURE-DEPENDENT TRANSITIONS IN ISOMETRIC CONTRACTIONS OF RAT MUSCLE

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SUMMARY

1. The effect of temperature on tetanic tension development was examined in extensor digitorum longus (fast-twitch) and soleus (slow-twitch) muscles of the rat, *in vitro* and with direct stimulation. The temperature range was from 35 to 10 °C.

2. The maximum tetanic tension decreased slightly on cooling from 35 to 25 °C. Cooling below 20 °C resulted in a marked depression of tetanic tension. The results were similar in the two muscles.

3. Analysis (in the form of Arrhenius plots) of the rate of tetanic tension development and relaxation clearly showed the occurrence of two phases in their temperature dependence, due to an increased temperature sensitivity below about 25 °C. Arrhenius activation energy estimates for temperatures lower than 21 °C were around twice as high as those for temperatures higher than 24 °C in both muscles.

INTRODUCTION

The temperature-dependent variation of the shortening velocity, the rate of isometric tension development and the time course of the isometric twitch in the rat fast- and slow-twitch skeletal muscles were examined in a previous study (Ranatunga, 1982). The temperature range employed in that study was from 35 to 20 °C, and in the results from both muscle types there was an indication of an increase in the temperature sensitivity of several contraction parameters in cooling below about 25 °C. It may be argued that such an increase in temperature sensitivity implies a change in an underlying rate-limiting process (Crozier, 1924). With a view to extending those observations, we have now examined the effect of temperature over a wider temperature range from 35 to 10 °C on the isometric tetanic tension development in rat extensor digitorum longus (fast), and soleus (slow) muscles.

Our results show that the cooling depression of tetanic tension is biphasic, due to an increased temperature sensitivity in cooling below 25 °C. The existence of two phases is established by analyses of rate and time measurements of the isometric contraction.

Some aspects of this study have been reported briefly (Ranatunga & Wylie, 1982).

METHODS

Experiments were performed *in vitro* on extensor digitorum longus (e.d.l., fast-twitch) and soleus (slow-twitch) muscles isolated from rats 27–29 days old. Animals were anaesthetized with an intraperitoneal injection (50 mg kg^{-1} body weight) of pentobarbitone sodium (Sagatal, May & Baker Ltd.) and were kept under anaesthesia during the dissection procedure. A muscle was set up horizontally in a Perspex muscle chamber of 15–20 ml in volume for isometric tension recording. One muscle tendon was tied to the hook of a thick glass rod which was rigidly clamped to a sturdy magnetic stand and the other was tied to the arm of the tension transducer (Statham Gl-32-350). The chamber was filled with physiological saline containing 115 mM-NaCl, 5 mM-KCl, 4 mM-$CaCl_2$, 1 mM-$MgCl_2$, 1 mM-NaH_2PO_4, 24 mM-$NaHCO_3$, 11 mM-glucose and 10–20 mg tubocurarine chloride l^{-1}. The solution was bubbled continuously with a mixture of 95% O_2 and 5% CO_2. In many experiments, the solution was replaced continuously at the rate of about 1 ml min^{-1}. In other

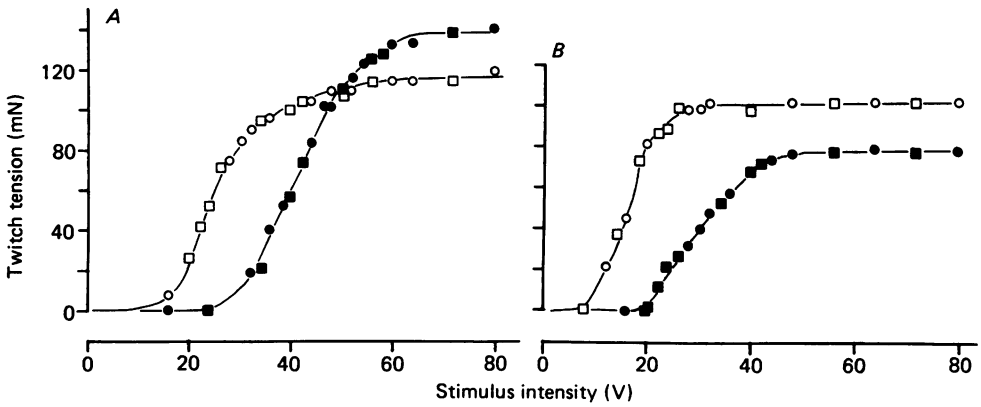

Fig. 1. The relation between stimulus intensity and twitch tension at 30 °C (open symbols) and at 10 °C (filled symbols), *A*, e.d.l. muscle; *B*, soleus muscle. Each relation was established by increasing (circles) followed by decreasing (squares) the stimulus intensity. Stimulus duration was 0·5 ms. There was negligible variation in the time course of the twitch within the stimulus intensity range in which twitch tension was maximal.

experiments the saline solution in the muscle chamber was replaced once with saline from a closed chamber in the same assembly, i.e. at approximately the same temperature: see Ranatunga, 1979), in between recordings at two different temperatures (Ranatunga, 1982). The pH of the solution was around 7·4 at room temperature. The estimated pH change for the temperature range from 35 to 10 °C was less than 0·3 pH units (see Isaacson, Hinkes & Taylor, 1970).

Other relevant information regarding the method of stimulation, the measurement of peak tension and maximum rate of tension development, as well as the control, variation and measurement of saline temperature are given in the previous paper (Ranatunga, 1982).

A preliminary series of experiments was carried out to determine optimal conditions and a suitable protocol for the final experiments. The stimulus parameters required for supramaximal direct muscle stimulation, the stimulation frequency and duration required for maximal tetanic tension development, and the initial muscle length optimal for twitch tension development were examined in these experiments at temperatures ranging from 35 to 10 °C. The change typically seen in the stimulus intensity *versus* twitch tension relation in cooling from 30 to 10 °C is illustrated by the results shown in Fig. 1*A* (from an e.d.l. muscle) and Fig. 1*B* (from a soleus muscle). The time course of the twitch contraction remained virtually the same in the plateau phase at each temperature. Such a complete relation was not established at each new temperature in the final series of experiments but the adequacy of a stimulus was checked by recording twitch contractions with stimulus intensities lower and higher than that used at the preceding temperature. A stimulus of 200–500 μs duration and 30–60 V was found to be adequate for supramaximal stimulation at all temperatures.

Stimulation frequency and duration required for producing nearly fused tetanic contractions varied markedly with temperature, as shown in the previous study (Ranatunga, 1982). The frequencies in Hz (and durations in ms) used in the present study were 400 (100), 300 (100), 250 (200), 100 (400), 60 (600) and 30 (1000) for e.d.l. muscle and 200–300 (100), 200 (200), 150 (300), 60 (400), 50 (1000), 20 (1500) for soleus muscle at 35, 30, 25, 20, 15 and 10 °C respectively. They resulted in nearly fused contractions with a distinct tension plateau at each temperature.

With change of temperature there was only minimal variation (typically less than 1·0 mm) of the initial muscle length required for maximal twitch tension development. In five experiments, the optimal muscle length for isometric tetanic tension development was also examined after re-warming to 35 °C. As reported by Close & Hoh (1968*b*) for rat e.d.l. muscle, it was very close (within $\pm 1{\cdot}0$ mm) to the value optimal for twitch tension. Additionally, Cullingham, Lind & Morton (1960) showed that changes in muscle temperature (range 40–20 °C) did not alter the length/tetanic tension relation in cat muscle. In our final experiments, the initial muscle length was set for maximum twitch tension development, prior to cooling at 35 °C.

Experimental procedure

In each experiment, a muscle was set up for recording first at 35 ± 1 °C. The stimulus parameters and the initial muscle length were adjusted to obtain maximal twitch tension, and one or two tetanic contractions were recorded. The saline temperature was then lowered, (in sequence) to 30, 25, 20, 15 and 10 ± 2 °C, and was subsequently increased, (in the reverse sequence) back to 35 °C. A temperature change of 5 °C was made in about 10–15 min and a recording was made after allowing about 10 min of equilibration. At each new temperature the adequacy of a stimulus was checked and twitch contractions and one or two tetanic contractions were recorded using appropriate frequencies and durations.

The interval between contractions adopted during adjustment of stimulus parameters and muscle length was 30 s and this was raised to 60 s during recording of tetanic contractions. Stimulation was stopped during a temperature change.

Measurements and their analyses

The peak tension and the maximum rate of tension development were recorded from each tetanic contraction (see Ranatunga, 1982). Additionally, the time to half-rise of tension and time to half-relaxation of tension were estimated from traces stored on a cathode-ray oscilloscope screen. The time to half-relaxation was measured from the end of the tetanic train of stimuli to the time when tension was half the isometric value. The tetanic tensions were represented as percentages of that recorded from each muscle at 35 °C prior to cooling. The data for maximum rate of tension development, time to half-rise of tension and time to half-relaxation of tension were analysed using the Arrhenius equation: rate $= A \exp(-E/RT)$ where A is a constant, R is the universal gas constant ($=8{\cdot}317$ J °K^{-1}), T is the absolute temperature in degrees Kelvin (°K) and E is the Arrhenius activation energy. The Arrhenius activation energy characterizing the temperature sensitivity of a rate is given in k J °K^{-1}. Since it is a common practice in physiology to represent temperature sensitivity of a process in the form of a temperature coefficient (Q_{10}), Q_{10} values are also given where appropriate. It is however clear that, on the basis of the Arrhenius equation, Q_{10} is expected to be different for different temperature ranges. The Q_{10} values given in this paper refer to the ratio of the rate at 35 °C to the rate at 25 °C.

RESULTS

Fig. 2 shows isometric tetanic contractions recorded from an e.d.l. muscle at different temperatures. The response at the upper left was recorded at 35 °C prior to cooling and that at the lower right was the response recorded after re-warming back to 35 °C. The tension recorded after re-warming was 98·6 % of that recorded at 35 °C prior to cooling. The other five responses were recorded during re-warming from 10 to 35 °C (see Methods). It is seen that cooling from 35 to 10 °C (compare the two contractions on the left in the upper row) resulted in a substantial depression (around

40 %) of tetanic tension, and much of the recovery of tension during subsequent re-warming occurred between 10 and 25 °C.

The pooled tetanic tension data from different experiments on each muscle type are illustrated in Fig. 3*A* (e.d.l.) and Fig. 3*B* (soleus). The tetanic tension is plotted along the ordinate (as a percentage of the tension recorded at 35 °C prior to cooling, and the reciprocal of the absolute temperature is plotted along the abscissa. The results include tensions recorded during the cooling and re-warming procedures. The tetanic tension recorded at 35 °C after re-warming was typically within 10 % of that recorded prior to cooling. As found in our previous studies (Ranatunga, 1980, 1982),

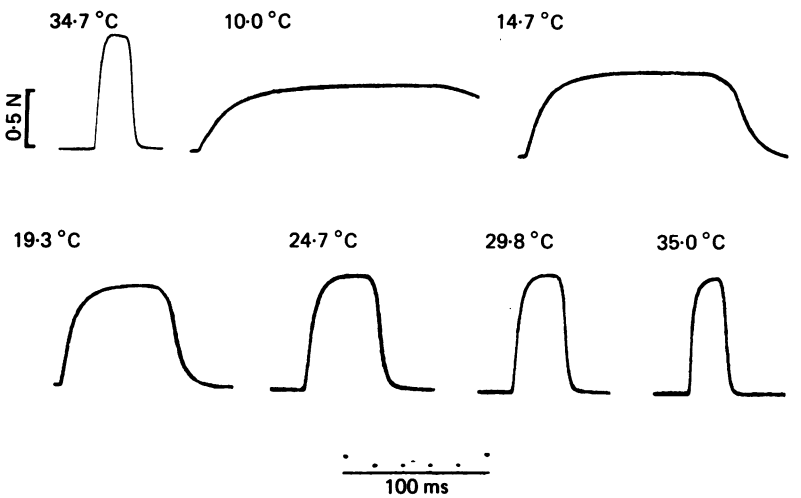

Fig. 2. Isometric tetanic contractions of an e.d.l. muscle at temperatures shown above each record. The top left contraction was recorded at 35 °C prior to cooling. Saline temperature was then lowered in 5° steps to 10 °C (see Methods). Other records were made during re-warming from 10 to 35 °C. Total duration of an experiment was 5–6 h.

however, the recovery was less complete in the fast e.d.l. than in the slow soleus muscle. The mean (±S.E. of the mean) recovered tension was 91·2±3·9 % for e.d.l. muscles and it was 104±2·3 % for the soleus muscles. Disregarding this difference, which may be related to different fatiguability properties of their muscle fibres, it appears from Fig. 3 that the temperature-dependent variation in tetanic tension is similar in the two muscles. To a first approximation, the depression of tetanic tension during cooling is rather less marked from 35 to 25 °C and it is more pronounced from 20 to 10 °C. The mean (±S.E. of mean) percentage tensions for 35, 25 and 10 °C respectively were 95·6 (±2·2), 89·5 (±2·0), and 58·3 (±3·0) for eight e.d.l. muscles; they were 102 (±1·2), 92·8 (±1·5) and 58·7 (±2·0) for seven soleus muscles. The data for the higher temperature range are essentially similar to those reported in several previous studies on rat muscles (Close & Hoh, 1968*a*; Ranatunga, 1977, 1982), on mouse muscles (Ranatunga, 1980), and on cat muscles (Cullingham *et al.* 1960; Ranatunga, 1969), where a temperature range from 35–38 °C to 20 °C was employed. The increased depression of tension on cooling below about 25 °C is similar to the results obtained by Hajdu (1951) from rat diaphragm muscle strips. The biphasic

nature of the temperature dependence agrees with the data reported by Stephenson & Williams (1981) from calcium-activated skinned fibres of rat muscle.

The maximum rate of tension development was recorded from the same tetanic contractions by measuring the positive peak of the electronically differentiated tension record (Buller & Lewis, 1965). The data obtained from the two muscle types are shown in the form of Arrhenius plots in Fig. 4. The rate of tension development as a percentage of the tetanic tension is plotted along a logarithmic ordinate. The results show that the cooling depression of the rate of tension development exhibits two phases in both muscles. The temperature sensitivity of the rate appears to

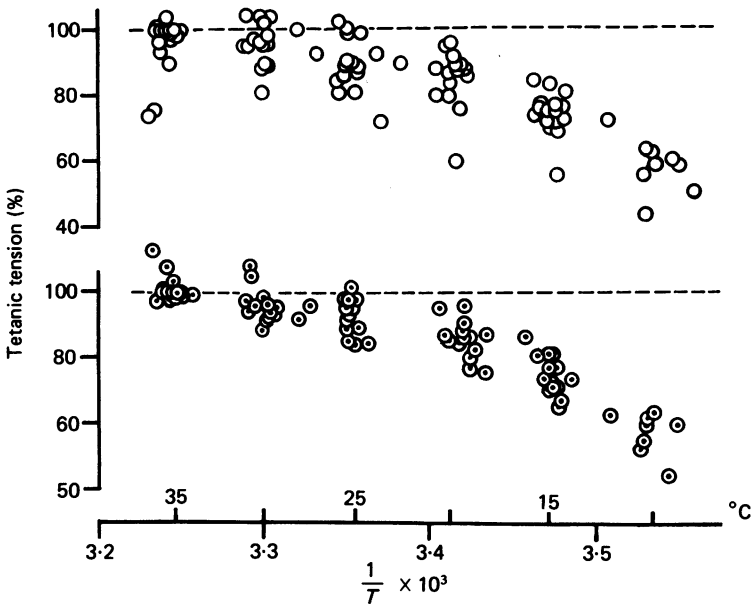

Fig. 3. Variation with temperature of the isometric tetanic tension in eight e.d.l. (upper) and seven soleus (lower) muscles. Tensions were represented as percentages of those recorded from each muscle at 35 °C prior to cooling (dotted line). Abscissa is the reciprocal of absolute temperature ($1/T\ 10^3$) and is labelled also in °C. Results include tensions recorded during cooling and re-warming.

increase on cooling below about 25 °C. In an attempt to obtain a quantitative estimate for the change in the temperature sensitivity, the data from the two muscle types were fitted with two regressions: one for data at temperatures higher than 24 °C and the other for data at temperatures below 22 °C. The straight lines drawn through the sets of points represent these regressions. The Arrhenius activation energies estimated from the slope of the regressions were 34·9 and 61·1 kJ for e.d.l. and 43·8 and 80·7 kJ for soleus, respectively, for the higher and the lower temperature ranges. The data for the rate of tension rise were also analysed after each measurement of the rate had been normalized to that recorded prior to cooling at 35 °C (i.e. without normalizing to tetanic tension). This procedure gave higher estimates of Arrhenius activation energy and a more pronounced increase of temperature sensitivity below about 25 °C for both muscles (see Table 1, first row).

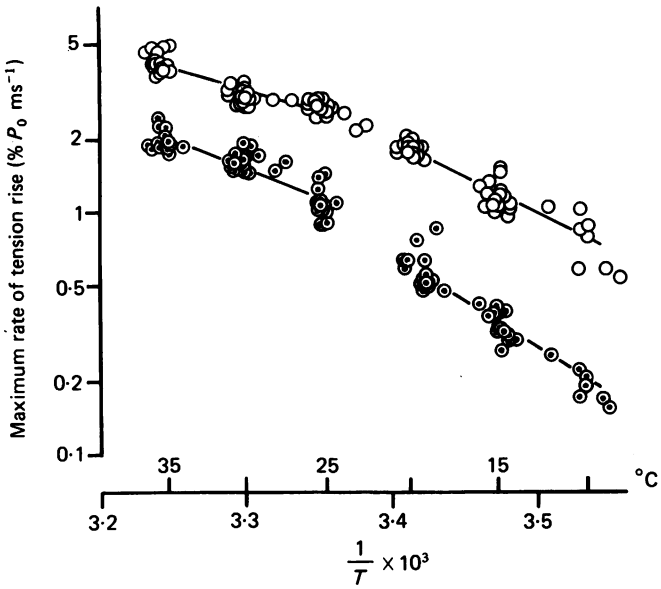

Fig. 4. Arrhenius plots for rate of tetanic tension development. Rate of tension development, as a percentage of the tetanic tension (i.e. as % P_0 ms^{-1}), is plotted along a logarithmic ordinate. Abscissa is the reciprocal absolute temperature as in Fig. 3. E.d.l. data are shown by open symbols. Results from each muscle type are fitted with two regressions ($r > 0{\cdot}87$, $n > 35$), one for the temperature range from 24 to 36 °C and the other for temperatures from 9 to 21 °C. Arrhenius activation energies estimated from the slopes were (kJ) 34·9 and 61·1 for e.d.l. and 43·8 and 80·7 for soleus, respectively, for higher and lower temperature ranges.

TABLE 1. Summary of data. Each value gives the Arrhenius activation energy (kJ °K^{-1}) and Q_{10} in parentheses (see Methods). These were determined from the slope of the regressions as in Fig. 4. The correlation coefficients (r) for regressions were higher than 0·86 ($n > 30$). All the data except those in the bottom row refer to tetanic contraction

	E.d.l.		Soleus	
Temperature range (°C)	36–24	21–9	36–24	21–9
Rate of tetanic tension development	39·2 (1·67)	86·2 (3·09)	51·3 (1·96)	113·2 (4·4)
Rate of tetanic tension development (% P_0 ms^{-1})	34·9 (1·58)	61·1 (2·24)	43·8 (1·78)	80·7 (2·89)
Time to half-tension rise	33·8 (1·56)	74·3 (2·65)	49·7 (1·92)	74·7 (2·66)
Time to half-relaxation of tetanic tension	43·4 (1·77)	79·2 (2·82)	42·0 (1·74)	105·9 (4·01)
Time to half-relaxation of twitch tension	72·0 (2·55)	87·8 (3·16)	64·6 (2·33)	121·1 (4·88)

The measurements made at different temperatures of the time to half-rise of tension and of the time to half-relaxation of tension in the tetanic contraction were also analysed in the form of Arrhenius plots. The reciprocals of the time measurements were plotted along a logarithmic vertical axis against the reciprocal absolute temperature. There was an absolute difference between the two muscles with respect to both time measurements, which was correlated with the difference in the speed of their contractions. The over-all form of the temperature dependence, however, was essentially similar to that depicted in Fig. 4. The biphasic nature of the temperature dependence, with an increased temperature sensitivity below about 25 °C, was clearly evident. This is apparent from the Arrhenius activation energies for the higher and the lower temperature ranges given in the summary table (Table 1). Our estimates of Arrhenius activation energy for the time to half-relaxation are similar to those reported by Sandow & Zeman (1979) for the exponentially falling phase of tetanic relaxation in mouse e.d.l. muscle.

Arrhenius activation energies for the rate of rise, the time to half-rise and the time to half-relaxation of tetanic tension were calculated from each muscle experiment, and paired comparisons were made between the mean estimates for the higher and lower temperature ranges. The difference between the mean activation energies was found to be significant ($P < 0{\cdot}01$) in both muscle types. Furthermore, analyses of variances showed that two regressions, as drawn in Fig. 4, fit the data significantly better ($P < 0{\cdot}05$) than a single regression through all the points for all the measurements except the time to half-rise of tension in soleus and the rate of tension rise when represented as a percentage of tetanic tension in e.d.l.

It is of interest that, in the higher temperature range of 24–36 °C, the rising phase of the tetanus is more temperature-sensitive in the slow soleus than in the fast e.d.l. muscle. The relaxation phase, on the other hand, appears to be sensitive to a similar extent in both muscles, as characterized by Arrhenius activation energies in the range of 40–45 kJ for both muscles. It is relevant to note, also, that no obvious difference was seen between the two muscles in the temperature sensitivity of the time to half-relaxation of twitch tension (see Ranatunga, 1982). For comparison, the Arrhenius activation energies obtained for this measurement in the present study are also given in Table 1. It appears that the relaxation of twitch tension is more temperature-sensitive than the relaxation of tetanic tension in both muscles in the temperature range of 24–36 °C.

DISCUSSION

Our results show that the temperature dependence of the plateau tension, the maximum rate of tension development, the time to half-rise of tension and the time to half-relaxation of tension in the isometric tetanus in mammalian (rat) skeletal muscle exhibit two distinct phases within the range of 10–36 °C. Analysis of the rate and the time measurements in the form of Arrhenius plots illustrated the occurrence of two linear segments with an apparent transition (an abrupt change in temperature sensitivity) at around 22–23 °C. The change in temperature sensitivity was so marked that the Arrhenius activation energy calculated for the lower temperature range (10–21 °C) was typically twice as high as that calculated for the higher range (24–36 °C). Results from two muscles (fast and slow) having distinctly different absolute speeds

of contraction were basically similar, and the apparent transition temperature was approximately the same (21–24 °C) for different contraction measurements.

The temperature sensitivity (Arrhenius activation energy) of a reaction, or a process, is thought to characterize its rate-determining step, and a temperature-induced transition may be considered to represent a change in the rate-determining step (Crozier, 1924; Levy, Sharon & Koshland, 1959). The data obtained in this study for the rates of rise and relaxation of tension are not strictly quantitative because of the presence of an unknown amount of series (tendon) compliance in the whole muscle preparations used. Further, it is not possible to assign the changes in these rates with temperature to particular transitions, whether in the activation pathway or in the cross-bridge cycle. Nevertheless the results show clearly that one, or more, of the underlying processes in mammalian muscle contraction undergoes an abrupt and marked change in cooling below about 22–23 °C.

Celio & Heizmann (1982) have shown recently that the calcium-binding protein parvalbumin is located exclusively in type II (fast-twitch) mammalian muscle fibres. Thus, most muscle fibres of rat e.d.l. contain parvalbumin whereas those of rat soleus do not, and Celio & Heizmann (1982) suggested that parvalbumin may be concerned with rapid tension relaxation in the fast muscle. Our results show that, despite having a faster tension relaxation, the Arrhenius activation energy for tension relaxation in fast e.d.l. muscle (in the higher temperature range) is similar to that of soleus muscle, both for the twitch and for the tetanus (see Table 1). If parvalbumin plays a rate-determining role in the tension relaxation in e.d.l. muscle and a similar mechanism is non-existant in soleus muscle, then our findings are not readily explicable. The simplest interpretation of our observations would be that similar rate-limiting events underlie tension relaxation in the two muscles: the events may, however, be different in the twitch and in the tetanus.

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