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TEMPERATURE-DEPENDENT CALCIUM SENSITIVITY CHANGES IN SKINNED MUSCLE FIBRES OF RAT AND TOAD

BY D. G. STEPHENSON AND D. A. WILLIAMS*†
From the Zoology Department, La Trobe University, Bundoora, Victoria, Australia

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SUMMARY

1. Single mechanically skinned muscle fibres of different types (fast- and slow-twitch mammalian; slow and twitch amphibian) were successively activated in solutions of various Ca^{2+} concentrations at different temperatures.

2. An increase in temperature from 5 to 22 °C reversibly shifted the isometric steady-state force–pCa curves towards higher Ca^{2+} concentration for individual fibres of each of the muscle types. A further increase in temperature to 35 °C in mammalian fibres resulted in an additional decrease in Ca^{2+} sensitivity.

3. The temperature dependence of Ca^{2+} sensitivity was greater in the 'faster' fibre types: fast-twitch > slow-twitch; twitch > slow.

4. The maximum isometric force response, P_0, of both rat and toad skinned fibres was found to be strongly dependent on temperature below 22 °C. No detectable force could be induced by Ca^{2+} in mammalian muscle fibres at 0–1 °C while in toad fibres P_0 decreased by about 90% when temperature dropped from 20 to 0 °C. Since in mechanically skinned fibres of other amphibians (*Bufo bufo*, *Rana* species) P_0 is only marginally affected it is likely that the P_0–temperature relations are indicative of the range of temperature over which the muscles are normally functional.

5. The P_0–temperature relations of skinned muscle fibres closely resembled the P_0–temperature relations of tetanically stimulated intact muscle preparations from the same species of animals suggesting that the contractile apparatus is mainly responsible for the variation of force response with temperature in intact muscle.

INTRODUCTION

Recent studies on fast-twitch mammalian (Stephenson & Williams, 1981) and twitch amphibian (Godt & Lindley, 1982) muscle fibres have clearly indicated that the sensitivity of the contractile apparatus to Ca^{2+} decreased by a factor of about 2·2–2·5 when the temperature was increased from 5 °C to about 22 °C. However, no statistically significant differences in Ca^{2+} sensitivity could be shown to exist between data obtained at different temperatures with different slow-twitch mammalian muscle preparations when using similar sample sizes as for fast-twitch fibres

* To whom reprint requests should be addressed.
† Present address: Department of Physiology, University of Massachusetts Medical School, Worcester, MA 01605, U.S.A.

(Stephenson & Williams, 1981). These observations could be interpreted as indicating fundamentally different properties of the Ca^{2+}-regulatory system in these muscle fibre types and were imparted physiological significance (Godt & Lindley, 1982). There was however, the distinct possibility that the more pronounced biological variability in Ca^{2+} sensitivity reported for slow-twitch muscle fibres compared to the fast-twitch fibres (Stephenson & Williams, 1981) could have masked the temperature effect on the Ca^{2+} sensitivity of the former but not that of the latter mammalian fibre type.

In an attempt to clarify this important point, an experimental system was developed to activate the same individual skinned fibres at different Ca^{2+} concentrations and temperatures, such as to allow a self-paired analysis of the results to be made. The study was also extended to include not only twitch mammalian but also twitch and slow fibres from an amphibian. The results show that changes in temperature affect the maximum Ca^{2+}-activated force response and the Ca^{2+} sensitivity of the contractile apparatus in a similar manner in all these muscle fibre types, albeit to different degrees. Interesting interspecific differences in the temperature dependence of the maximum force response of amphibian muscle preparations were recorded. The largest temperature effect on Ca^{2+} sensitivity was observed with twitch amphibian and fast-twitch mammalian muscle fibres while the slow-twitch mammalian and the slow amphibian muscle displayed a substantial, but less pronounced, temperature dependence of Ca^{2+} sensitivity.

METHODS

Experiments were performed on muscle preparations obtained from adult Hooded Wistar rats of either sex and cane toads (*Bufo marinus*). The soleus and extensor digitorum longus (e.d.l.) were ablated from rats after deep ether anaesthesia. The pyriformis and iliofibularis muscles were removed from the hind limbs of the toad sacrificed by double pithing of the central nervous system.

Single muscle fibres were dissected and subsequently mechanically skinned under paraffin oil using the standard techniques employed in this laboratory (Ashley & Moisescu, 1977; Stephenson & Williams, 1981). The rat e.d.l. and soleus muscles were used as a source of fast- and slow-twitch muscles as has previously been described (Stephenson & Williams, 1981, 1982). Fibres displaying intermediate contractile characteristics (Stephenson & Williams, 1981) were not used in these experiments.

Twitch and slow (tonic) muscle fibres were isolated from both the iliofibularis and pyriformis muscles. Slow fibres were identified by a series of criteria including: (i) their unusually high transparency in aqueous solution in comparison to twitch fibres, (ii) their relatively smaller diameter, (iii) their inability to twitch following single-pulse electrical stimulation, (iv) the ability to undergo localized contraction induced by choline chloride.

The apparatus for measuring the force produced by single skinned muscle fibres and the specifications of this system are described elsewhere (Stephenson & Williams, 1981). The sarcomere length of each fibre was determined using a He–Ne laser (Spectra-Physics 136-04) and adjusted to 2·20–2·33 μm in amphibian and 2·69–2·81 μm in mammalian fibres. This avoided the sarcomere-length-dependent changes in Ca^{2+} sensitivity which occur in these muscles (Moisescu & Thieleczek, 1979; Stephenson & Williams, 1982, 1983; Williams, 1982).

The bathing solutions were identical to those used in the previous study (Stephenson & Williams, 1981) and can be summarized briefly. All solutions contained (mM): K^+, 117; Na^+, 36; 2-(2-hydroxy-1,1-bis(hydroxymethyl) ethyl) aminoethane sulphonic acid (TES^-), 17; Mg^{2+}, 1; total ATP, 8; ethyleneglycol-bis(β-aminoethyl ether)*N*,*N*′-tetraacetic acid ($EGTA^{2-}$) + $CaEGTA^{2-}$ + hexamethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid ($HDTA^{2-}$), 50; creatine phosphate, 10; creatine phosphokinase, 15 u.ml^{-1}. The pH of all solutions was adjusted to 7·10 ± 0·01 at the temperatures used such that the variation in K^+ concentration was less than 1 mM. All apparent affinity constants (K_{app}s) were experimentally determined at each temperature as

previously described (Moisescu & Thieleczek, 1979; Stephenson & Williams, 1981). The K_{app} of Ca^{2+} to EGTA at pH 7·10 and in the presence of 1 mM-Mg^{2+} was taken as $4{\cdot}88 \times 10^6$ M^{-1} for 5 °C and as $4{\cdot}78 \times 10^6$ M^{-1} for 22 and 35 °C (see Stephenson & Williams, 1981). Ionic strength in all solutions was close to 225 mM. Due to the temperature dependence of the pK of the pH buffer used, the total concentration of TES was 140 mM in solutions at 5 °C, 60 mM in solutions at room temperature and 40 mM in solutions at 35 °C. All solutions contained caffeine (10 mM) and azide (1 mM) to minimize membranous Ca^{2+} movements within the fibres. Solutions containing 50 mM-EGTA with no added Ca^{2+} are referred to as solutions of type B, those containing equimolar concentrations of Ca^{2+} and EGTA are referred to as solutions of type A and those containing 50 mM-HDTA are referred to as solutions of type H (Stephenson & Williams, 1981).

Protocol for data collection. Three identical solution-containing chambers, each interchangeable within the transducer and force recording system were required for these experiments. After the skinned muscle preparation was attached to the force transducer and adjusted to the standard sarcomere length, a complete set of contraction–relaxation cycles was performed at room temperature (20–22 °C) in solutions of type A′/B′/H′. The experimental apparatus, comprising the transducer system with attached fibre, bridge circuit and related electronics and chart recorder was then moved in its entirety to a thermostatically controlled cold room at 5 °C. A second solution chamber containing bathing solutions of type A/B/H at 5 °C was then used to complete a similar activation cycle in the same preparation after the fibre and the apparatus had been pre-equilibrated at the 'new' temperature for 30–60 min. The apparatus was then returned to room temperature and solutions of type A″/B″/H″, which were contained in a third chamber were used to complete an activation cycle at 35 °C. In this case a water/glycerol mixture was circulated around the activating solutions and maintained at $35 \pm 0{\cdot}5$ °C in a thermostatically controlled central reservoir. The entire activation protocol was then repeated in an alternate order. All force responses were obtained using the 'Ca^{2+}-jump' technique (Moisescu, 1976; Stephenson & Williams, 1981) and were corrected for time-dependent force deterioration especially evident at high temperature by normalizing to interpolated control contractions using the method of Julian (1971). This activation procedure aids in maintaining the sarcomere length homogeneity of each preparation throughout the experiments (Julian & Moss, 1981).

RESULTS

Temperature dependence of Ca^{2+} sensitivity. Fig. 1 illustrates the dependence of the isometric force–pCa relation on temperature (5, 22 and 35 °C) in a mammalian fast- (e.d.l.) and slow-twitch (soleus) preparation. The curves have been fitted to the experimental points by eye. The force–pCa curve is shifted progressively in a parallel fashion towards higher Ca^{2+} concentration as temperature is increased from 5 to 22 °C and then to 35 °C within each individual preparation. The Ca^{2+} sensitivity is decreased by a factor of 2·3 for the e.d.l. and 2·1 for the soleus fibre for an increase in temperature from 5 to 22 °C and by a further factor of 1·65 and 1·5 for e.d.l. and soleus respectively when temperature is increased to 35 °C.

The effect of a change in temperature from 5 to 22 °C on the Ca^{2+}-activation curves in a single toad twitch and slow skinned fibre (muscle: pyriformis) is illustrated in Fig. 2. It can be seen that these relations display a similar temperature dependence to that of mammalian fibres, with a higher Ca^{2+} sensitivity at lower temperature in both fibre types. The drop in Ca^{2+} sensitivity following an increase in temperature from 5 to 22 °C is more pronounced in the twitch fibre (decreased by a factor of 2·40) than in the slow fibre (decreased by a factor of 1·62). Similar results were obtained from twitch and slow fibres isolated from the toad iliofibularis muscle.

Unlike mammalian skinned fibres it was not possible to obtain stable force responses at 35 °C with toad muscle preparations. At this temperature the force response rapidly increased following Ca^{2+}-jump activation but decayed almost as

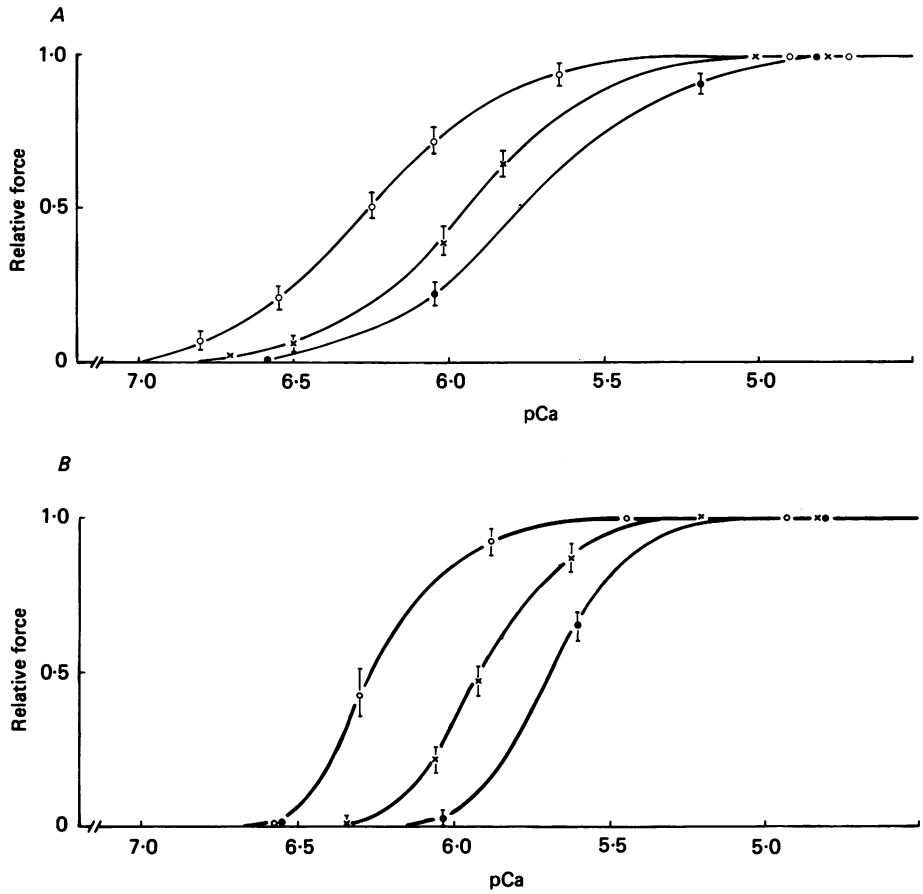

Fig. 1. The steady-state relative force–pCa relations of an individual slow-twitch (*A*) and fast-twitch (*B*) skinned muscle fibre isolated from the soleus and e.d.l. muscles respectively, of the same rat, as a function of temperature. Temperatures: *A* and *B*: ○, 5 °C; ×, 22 °C; ●, 35°C. Each data point represents the average from at least six exposures of a single fibre to a particular activating solution. In all cases no increase in maximum force was observed when Ca^{2+} concentration was decreased below pCa 5·0. The absolute range of the observations is indicated by the vertical lines. The continuous lines were drawn by fitting the data points by eye. Sarcomere length: *A*, 2·73 μm; *B*, 2·76 μm.

quickly to a much lower level. Subsequent force responses at lower temperatures were not affected by this decay suggesting that activation at 35 °C did not result in irreversible damage or denaturation within the preparation.

Fig. 3 represents a plot of the Ca^{2+} concentration required for half-maximal activation (pCa_{50}) as a function of $1/T$ in the four individual fibres presented in Figs. 1 and 2. The relations between $\ln Ca_{50}$ and $1/T$ are steeper for the fast (twitch and fast-twitch) than the slow (slow-twitch and tonic) fibre types. If one regards the pCa_{50} values as representing the sensitivity of the respective muscle fibres to Ca^{2+}, then the Ca^{2+} sensitivities of the amphibian fibres, which were isolated from the same muscle of the same animal, would appear to be similar around 13 °C whilst those of the mammalian fibres (isolated from muscles from the same animal) would be

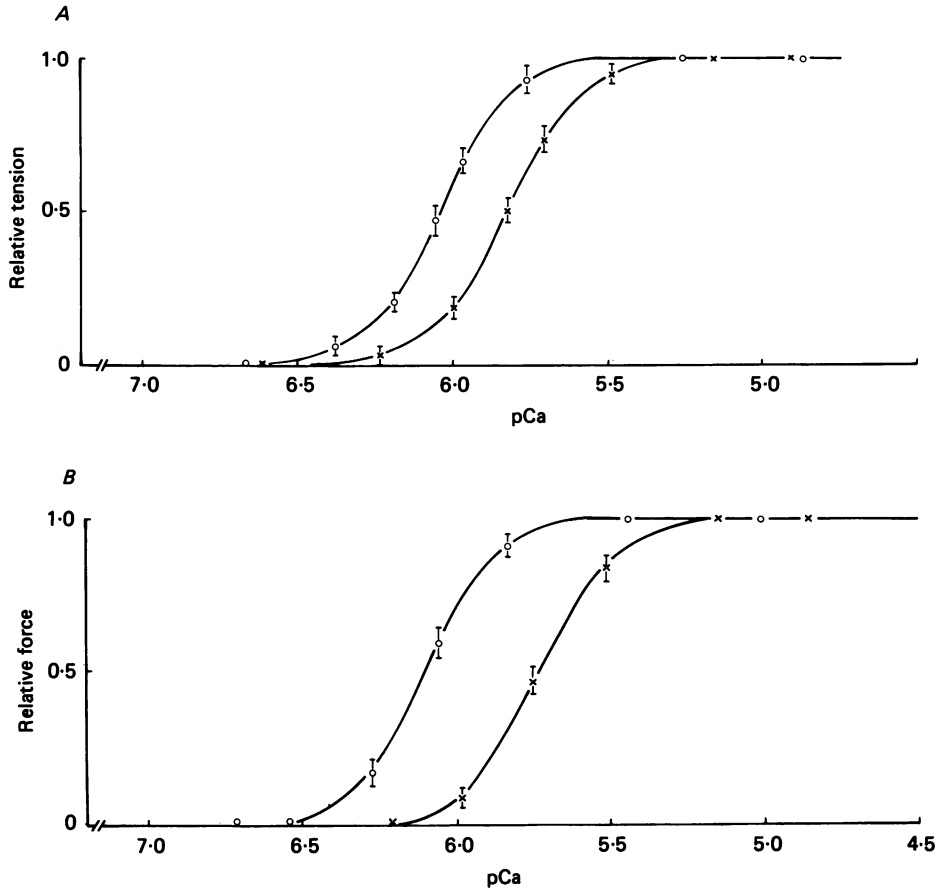

Fig. 2. The effect of temperature on the steady-state isometric force–pCa relations of a single slow (*A*) and twitch (*B*) amphibian skinned fibre isolated from the same muscle (pyriformis) of a cane toad. Temperatures: ○, 5 °C; ×, 22 °C. Each data point is the average value obtained from at least six contractile responses of the same single fibre elicited by each particular activating solution. The continuous lines were fitted to the data by eye and the vertical bars indicate the range of observations within each fibre. Sarcomere length: *A*, 2·29 μm; *B*, 2·21 μm.

equivalent at 15 °C. At 5 °C the Ca^{2+} sensitivity order is: mammalian fast-twitch > slow-twitch whilst for amphibian muscles it is twitch > slow. As temperature is increased the relative Ca^{2+} sensitivities of the four fibres are differentially affected with the order becoming: mammalian slow-twitch > fast-twitch and amphibian slow > twitch at 22 °C. Due to the variation in pCa_{50} values obtained for different preparations from different muscles and different animals, it is not possible to comment on the relative Ca^{2+} sensitivities between for example, one amphibian and one mammalian preparation especially if they differ by less than the range of such variations (see Table 1).

The major benefit derived from using paired data from individual fibres in comparative studies as opposed to grouped and averaged data is apparent in the

results presented in Table 1. All mean ΔpCa_{50} values were tested for statistical difference using a standard t test. The paired results obtained with individual fibres show that a temperature increase from 5 to 22 °C or from 22 to 35 °C results in a significant ($P < 0.01$, t test) decrease in Ca^{2+} sensitivity (decreased pCa_{50}) for each fibre type investigated. It is evident that the mean change in pCa_{50} values over the temperature range 5–22 °C is largest in the 'faster' fibre types (fast-twitch mammalian and twitch amphibian).

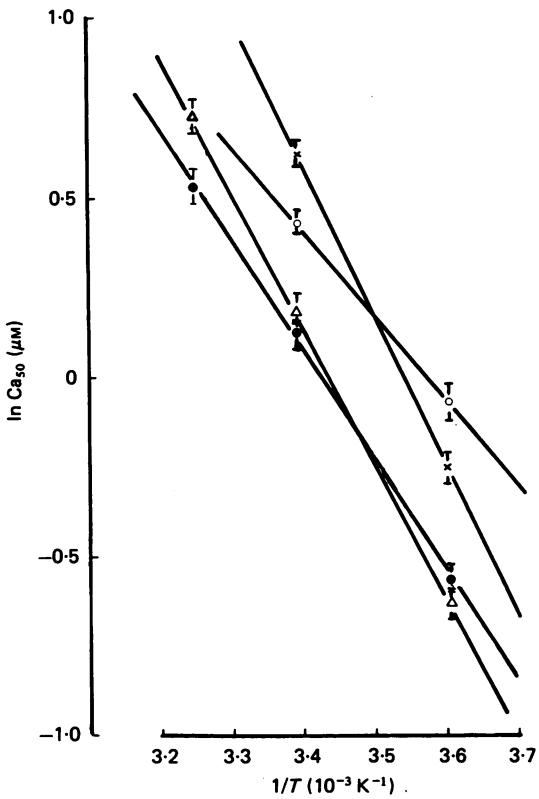

Fig. 3. The effect of temperature on the Ca^{2+} concentration required for half-maximal activation (Ca_{50}) of each fibre type. The data points shown represent the Ca_{50} values taken from the force–pCa curves of the individual fibres presented in Figs. 1 and 2. ○, slow amphibian; ×, twitch amphibian; ●, soleus; △, e.d.l.

The averaged Ca^{2+} sensitivity data are a compilation of the data presented here and those presented in the previous temperature studies on mammalian and amphibian fibres (Stephenson & Williams, 1981, 1983). This averaged data still does not clearly elucidate the temperature-dependent changes that occur in each fibre type despite significantly larger sample sizes. For example there appears to be little difference in Ca^{2+} sensitivity at 5 and 22 °C in the averaged data for both mammalian slow-twitch and amphibian slow muscle fibres ($P > 0.1$, t test) but this is due exclusively to the relatively large range of pCa_{50} values which occur in these fibres under the respective conditions.

TABLE 1. Summary of the Ca^{2+}-activation data from the four fibre types as a function of temperature

Fibre type	Sarcomere length (μm)	Temp. (°C)	All fibres: pCa_{50} Mean ± S.D. (range)	All fibres: ΔpCa_{50} 5–22 °C	All fibres: ΔpCa_{50} 22–35 °C	Paired data: ΔpCa_{50} mean ± S.D. (range) 5–22 °C	Paired data: ΔpCa_{50} mean ± S.D. (range) 22–35 °C	n*
Mammalian								
Fast-twitch	2·69–2·80	5	6·25 ± 0·08, n = 16 (6·11–6·36)	0·31†	0·05‡	0·33§ ± 0·07, n = 5 (0·21–0·37)	0·18§ ± 0·04, n = 5 (0·13–0·21)	4
	2·60–2·80	22	5·94 ± 0·06, n = 14 (5·85–6·05)					4
	2·60–2·80	35	5·89 ± 0·15, n = 15 (5·62–6·02)					4
Slow-twitch	2·70–2·81	5	6·20 ± 0·12, n = 15 (6·01–6·40)	0·01‡	0·21†	0·21§ ± 0·03, n = 4 (0·19–0·25)	0·20§ ± 0·06, n = 4 (0·14–0·27)	2
	2·65–2·81	22	6·19 ± 0·20, n = 16 (5·85–6·60)					2
	2·65–2·81	35	5·98 ± 0·14, n = 13 (5·70–6·12)					2
Amphibian								
Twitch	2·21–2·33	5	6·10 ± 0·09, n = 22 (5·99–6·36)	0·36†		0·40§ ± 0·09, n = 5 (0·27–0·49)		4
	2·21–2·33	22	5·74 ± 0·06, n = 22 (5·66–5·86)					4
Slow	2·20–2·31	5	5·93 ± 0·22, n = 22 (5·66–6·35)	0·08‡		0·20§ ± 0·05, n = 5 (0·14–0·27)		4
	2·20–2·31	22	5·85 ± 0·14, n = 22 (5·58–6·02)					4

n* is the closest integer value for the Hill coefficient from equation $P_r = K[Ca^{2+}]^n/(1 + K[Ca^{2+}]^n)$ where P_r represents the fraction of the maximum force response P_0, and K is a constant.

† $P < 0·01$, t test; ‡ not significant, t test ($P > 0·1$). § Results significantly different from zero (t test, $P < 0·001$).

Temperature dependence of maximum isometric force. The maximum Ca^{2+}-activated force response in individual amphibian and mammalian skinned fibres displays a strong temperature dependence in the range 0–22 °C. Fig. 4 shows the relation between maximum isometric force response (P_0) and temperature for mammalian (*A*) and amphibian (*B*) skinned and intact muscle fibres. A decrease in temperature from 22 to 5 °C resulted in a decrease in P_0 by 73–85 % in *skinned* fast- and slow-twitch mammalian fibres. A similar temperature decrease in *intact* fibres of the same type also resulted in a very pronounced decrease in force. At 0–1 and 4 °C in skinned and intact mammalian fibres respectively, the ability to produce force was reversibly abolished.

The P_0–temperature relations of amphibian intact and skinned fibres of different amphibian species vary markedly. Previous studies have shown that the maximum force response of *Bufo* and *Rana* species is relatively temperature independent between 0 and 20 °C whilst skinned fibres from frogs (*Rana*) also showed high tension at low temperatures (Fig. 4). The data obtained in this study using the cane toad *B. marinus* indicate however, that there is a strong temperature dependence of maximum isometric force in skinned fibres from this amphibian as P_0 falls steeply to zero as temperature is lowered to approximately −2 to −3 °C. Preliminary results obtained using intact iliofibularis whole muscles from *B. marinus* have also displayed a steep P_0–temperature relation. A decrease in temperature from 22 to 11 °C decreased maximum tetanic force by about 50 % as indicated in Fig. 4.

DISCUSSION

It is evident from the results presented here that the Ca^{2+} sensitivity of the contractile apparatus is decreased with increasing temperature for the four vertebrate muscle fibre types used in this study. Although vertebrate fibres differ with respect to features such as their comparative Ca^{2+}/Sr^{2+} sensitivities (Takagi & Endo, 1977; Secrist & Kerrick, 1980; Williams, 1982), speed of contraction (Costantin, Podolsky & Tice, 1967; Close, 1972; Lännergren, 1978) innervation and filament structure and organization (Page, 1965; Hess, 1970), the process underlying the temperature dependence of Ca^{2+} sensitivity is a basic one and is likely to be common to all vertebrate muscle fibre types.

A likely mechanism for this dependence is that temperature affects the binding of Ca ions to troponin-C. An increase in temperature was found to lead to a decreased Ca^{2+} affinity for fast-twitch mammalian troponin-C because the enthalpy of this reaction is negative (Potter, Hsu & Pownall, 1977; Godt & Lindley, 1982). However, it is also possible that other Ca^{2+}-binding sites that may be involved in regulation are similarly affected by temperature. The binding of Ca^{2+} to troponin-C is a mechanism inherent in the contractile processes of the four major vertebrate fibre types investigated and the negative enthalpy of the binding reaction would explain the qualitative similarity in the temperature dependence of Ca^{2+} sensitivity between each fibre type. It would also be interesting to determine whether the binding of Ca^{2+} to troponin-C from the different muscle types also shows a similar quantitative difference in behaviour.

The most direct comparisons can be drawn with our earlier report (Stephenson &

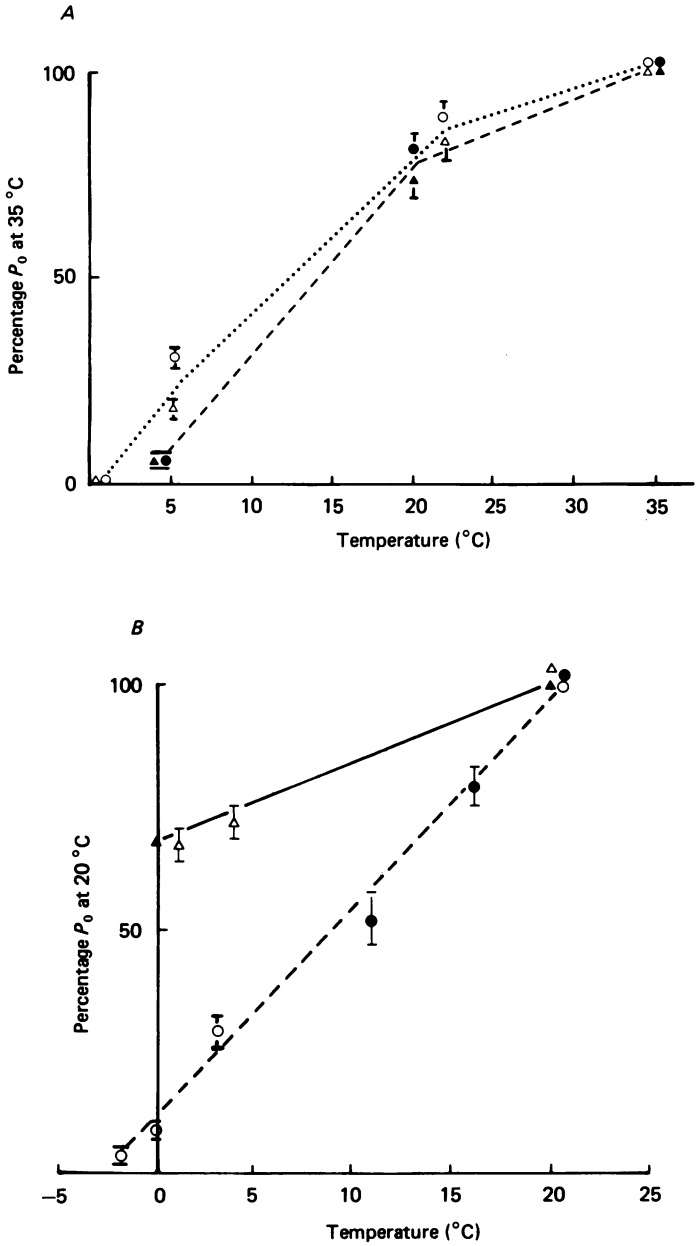

Fig. 4. The dependence of the maximum isometric force response of intact and mechanically skinned muscle fibres of rat (*A*) and various amphibians (*B*) on temperature. *A*: intact fibres: ▲, e.d.l.; ●, soleus. Skinned fibres: △, e.d.l.; ○, soleus. Force responses are normalized to the response produced at 35 °C in the same preparation. Data for intact muscle preparations were taken from the work of Isaacson *et al.* (1970). *B*: skinned fibres: ○, *B. marinus*; △, *Rana esculenta*. Intact fibres: ●, *B. marinus* (iliofibularis muscle); ▲, *B. bufo*. All amphibian data were plotted as a percentage of the maximum force response obtained in the same preparation at 20–22 °C. The data for *B. bufo* and *R. esculenta* are those of Bressler (1981) and Moisescu (1976) respectively.

Williams, 1981) and more recent observations using frog twitch fibres (Godt & Lindley, 1982). Both groups used similar values for the apparent affinity constants of CaEGTA and MgATP to those used in this study. Godt & Lindley (1982) reported a shift in the force–pCa relation by 0·4 log units towards higher Ca^{2+} concentration when temperature was increased from 4 to 22 °C in skinned frog twitch muscle fibres. This compares favourably with the 0·40 and 0·36 log unit shifts apparent using individual (Fig. 2) and averaged (Table 1, see also Stephenson & Williams, 1983) data respectively over the same temperature range for toad twitch fibres. Although no statistically significant difference was evident when averaged data from different preparations activated at different temperatures were used to investigate the temperature dependence of Ca^{2+} sensitivity in slow-twitch mammalian fibres (Stephenson & Williams, 1981), a significant difference (0·21 log units) can be noticed using a self-paired analysis of individual data (see Table 1).

The existence of a primary effect of temperature on the Ca^{2+}-regulatory system in vertebrate muscle may aid in explaining some effects of temperature in intact muscle preparations. Some of these implications have been discussed previously (Stephenson & Williams, 1981). Here we wish only to mention additional points based on the new information available through this study. Hill (1972) found that resting tension increased markedly in the intact cat soleus muscle upon cooling from 22 to 4 °C but varied little in the e.d.l. If it is remembered (see Fig. 1) that the force–pCa relation of the soleus muscle is much flatter than that of the e.d.l. in the rat (and presumably the cat) and we assume an equivalent resting Ca^{2+} concentration in each muscle type which is just below the contraction threshold in the soleus muscle, then a decrease in temperature will result in a significant increase in Ca^{2+} sensitivity in both the soleus and e.d.l. muscle, but residual force may result only in the soleus muscle at the resting Ca^{2+} concentration.

The 'rapid cooling contractures' of Sakai & Kurihara (1974) are similarly likely to be due to a combination of increased Ca^{2+} sensitivity due to a rapid left shift of the force–pCa curve and a further Ca^{2+} sensitivity increase due to the presence of caffeine (Williams, 1982; Wendt & Stephenson, 1983).

The self-paired individual results obtained confirm our previous observations on mammalian fast- and slow-twitch fibres (Stephenson & Williams, 1980, 1981) which indicate that the temperature dependence of maximum isometric force (P_0) reported in intact fibres (Cullingham, Lind & Morton, 1960; Close & Hoh, 1968; Isaacson, Hinkes & Taylor, 1970; Ranatunga & Wylie, 1982, 1983) can largely be determined by a direct effect on the contractile apparatus. The very steep temperature dependence has important implications for maximum force production in both intact and skinned fibres particularly below 10 °C. In this range a 1 °C variation in temperature may produce a 20 % change in maximum force which places great importance on the need for a temperature control system with an accuracy greater than ± 1 °C in this range.

The amphibian fibre data show interesting interspecific differences in the temperature dependence of maximum force. Skinned and intact muscle preparations from the toad (*Bufo bufo*) and frogs (*Rana* species) show a relative temperature independence for maximum force between 0 and 20 °C. Close & Hoh (1968) reported that P_0 in frog sartorius muscle increased from 2·38 to 3·5 kg cm^{-1} when temperature was increased from 0 to 20 °C whilst Bressler (1981) found a 32 % increase in P_0 for

frog and toad (*B. bufo*) sartorius over the same temperature range. The data of Moisescu (1976) show relatively high tension at 4 °C in skinned fibres from frogs. The maximum force responses of skinned and intact muscle preparations from the cane toad, however, are strongly dependent on temperature in the same range.

This difference may be due to the rearing conditions of the animals. The tropical cane toad *B. marinus* rarely encounters extremes of low temperature whereas the European toad *B. bufo* would be expected to encounter a much lower temperature range. Although interspecific variation is likely, the different temperature dependencies mainly reflect the variation in temperature range over which the muscles of the respective toads are normally functional rather than major differences in the effects of temperature on excitation–contraction coupling and the contractile apparatus. Both intact and skinned muscle preparations from the various amphibians may therefore display similar temperature dependencies for maximum force production, as is evident in mammalian muscle fibres, with the P_0–temperature relation being displaced to a lower temperature range in cold-adapted animals.

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