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DIFFERENT ISOMETRIC FORCE - [Ca^{2+}] RELATIONSHIPS IN SLOW AND FAST TWITCH SKINNED MUSCLE FIBRES OF THE RAT

D.G. STEPHENSON* and Q.G. FORREST

Department of Zoology, La Trobe University, Bundoora, Victoria 3083 (Australia)

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Summary

Skinned muscle fibres prepared from fast and slow twitch muscles of rat have been activated in Ca^{2+}-buffered solutions using a new activation procedure (Moisescu, D.G. and Thieleczek, R. (1978) J. Physiol. 275, 241—262). The results indicate that (i) the Ca^{2+} activation curve is less steep for slow fibres, (ii) physiologically relevant force levels are attained considerably faster at constant [Ca^{2+}] in fast fibres, and (iii) active force becomes noticeable at lower [Ca^{2+}], but reaches saturation at higher [Ca^{2+}] for slow fibres.

Slow and fast twitch muscle fibres from rat have been extensively used for comparative physiological, histochemical and morphological experiments (for reviews see Refs. 1 and 2). However, no data have been published on physiological differences in Ca^{2+}-activated force in these muscle fibres. Comparative Ca-activation curves have been reported for slow and fast twitch skinned muscle fibres from two other mammals: rabbit [3—5] and guinea pig [6] but the results differ quantitatively and therefore they cannot be simply extrapolated to all mammals.

A recently developed activation procedure [7, 8] enabled us to use single skinned muscle preparations to construct whole Ca^{2+} activation curves with a higher degree of accuracy than previously done [9]. The application of this technique to investigate on a quantitative basis the differences in the Ca^{2+} activation properties between the fast and slow twitch muscle fibres is

*Correspondence should be addressed to: Dr. D.G. Stephenson (previously Moisescu), La Trobe University, Department of Zoology, Bundoora, Victoria, Australia 3083.

Abbreviations: Tes, 2-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)amino)-ethanesulfonic acid; HDTA, hexamethylenediamine-*N,N'*-tetraacetic acid: EGTA, ethylene glycol bis (β-aminoethyl ether)-*N,N'*-tetraacetic acid; pCa = — log [Ca^{2+}].

critical since it is known [1] that albeit some muscles contain predominantly fibres of one type, all skeletal mammalian muscles are made of a mixture of muscle fibre types. As a result, precious information is lost by averaging observations from many unidentified muscle fibres. With this activation technique we were also able to observe for the first time the rate of force development in both muscle fibre types at essentially constant $[Ca^{2+}]$.

Skinned muscle fibres have been prepared from the extensor digitorum longus (predominantly fast) and from the soleus (predominantly slow) muscles of adult rats using a procedure described earlier [10]. The muscles were dissected immediately after the killing of the animal and kept under parraffin oil at 5° C until freshly skinned muscle fibres were prepared for experiments. Isometric force was measured with a semiconductor force transducer (Aksjeselskapet, AE 875) connected to a ToshinElectron chart recorder. The average sarcomere length was calculated from the diffraction pattern of a He-Ne laser (Spectra Physcis 136-04) beam crossing the preparation. The sarcomere length was adjusted in these experiments to a value of approx. 2.7 μm, which corresponds to optimum tetanic contractions in these fibres [1]. Otherwise, the experimental set-up and the preparation of the solutions were as previously described [7, 8, 10]. The solutions used for these experiments contained (mM); $[Ca\text{-}EGTA^{2-}]$ + $[EGTA^{2-}]$, 50; disodium creatine phosphate, 10; Na_2-ATP, 8; caffeine, 10; NaN_3, 1; Tes, 140. Creatine kinase 15 U · ml^{-1}. The pH was adjusted with KOH to 7.10 ± 0.01 at 2° C so that the final [K] was 127 ± 2 mM. The level of ionized Mg in all solutions was 0.1 mM and it was calculated based on the measured apparent affinity constants for our conditions [7—9]. Caffeine and azide were added to diminish the ability of the sarcoplasmic reticulum [8] and mitochondria [11] to oppose the fast equilibration of $[Ca^{2+}]$ in the preparations [8]. The experiments were performed at a low temperature (3.5° C) in order to prolong the life of the muscle fibres as well as to improve the time resolution between force development and $[Ca^{2+}]$ equilibration in the preparations [8]. Fig. 1 illustrates the relationships between the relative isometric force response and $[Ca^{2+}]$

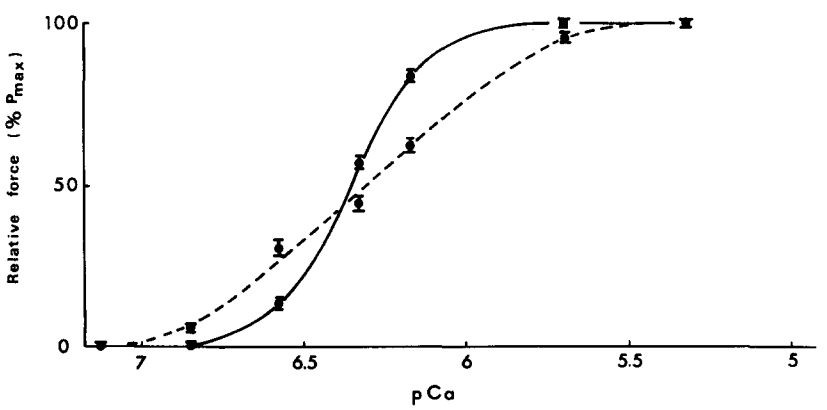

Fig. 1. Relative force responses (expressed in % of maximum force, P_{max}) as function of pCa for steady-state conditions in a skinned slow twitch muscle fibre from soleus (●- - -●); diameter 30 μm, length 0.65 mm, sarcomere length 2.7 μm) and a skinned fast twitch muscle fibre from extensor digitorum longus (● - - - ●); diameter 25 μm, length 2.25 mm sarcomere length 2.79 μm) of the same rat. The absolute scatter of the results is indicated by the vertical bars. The curves have been fitted by eye.

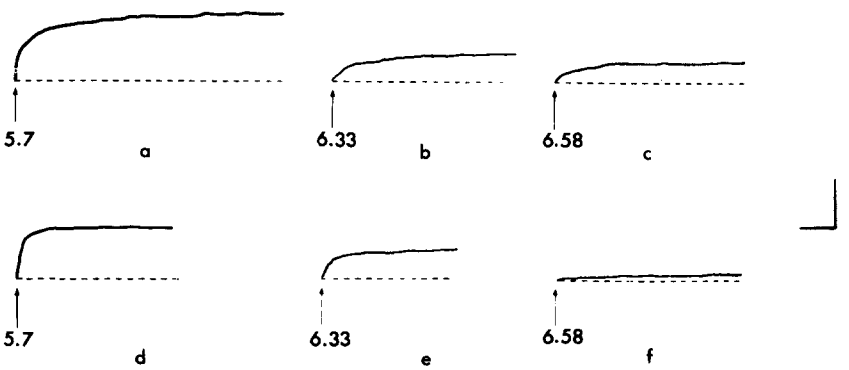

Fig. 2. Time course of isometric force responses in the same skinned muscle fibres as for Fig. 1 when activated (see arrows) in three solutions with pCa values 5.7, 6.33 and 6.58, respectively. Traces a—c represent force responses in the slow muscle preparation from soleus and traces d—f show force responses in the fast muscle preparation from the extensor digitorum longus muscle. Before activation the preparations were equilibrated in an ionically balanced solution which contained 49.9 mM $HDTA^{2-}$ and 0.1 mM $EGTA^{2-}$. Under these conditions $[Ca^{2+}]$ should have changed from approx. 10^{-8} M to the indicated pCa value within 0.15 s in both preparations [8]. The baseline represents zero active force. Calibration bars: vertical 50 μN; horizontal 60 s for a—c and 12 s for d—f.

in a fast and a slow skinned muscle fibre of the same rat using the same batch of solutions. Results showing similar relationships to those presented in Fig. 1 were obtained with 10 extensor digitorum longus and 12 soleus preparations from 5 rats. From Fig. 1 one can observe that the Ca^{2+}-activation curve for the fast fibre type is steeper than that for the slow fibre type. Thus Ca^{2+} must be increased only 3 times in the fast fibre to produce a rise of the relative force from 10% to 90% but 11 times in the slow fibre for the same effect. This finding could be important in explaining the higher rate of force development and/or relaxation in fast twitch than in slow twitch muscle fibres, since a 3-fold change in $[Ca^{2+}]$ should be potentially faster achieved than an 11-fold change. In addition, the lower $[Ca^{2+}]$ threshold for either activation or relaxation in slow fibres (Fig. 1 and Fig. 2) could explain the higher resting force observed previously [12] in intact soleus than in intact extensor digitorum longus muscle at low temperatures, when the level of $[Ca^{2+}]$ in the sarcoplasm was believed to have increased [13]. The lower Ca^{2+}-activation/relaxation threshold for the slow than for the fast twitch muscle fibres could also account for the discrepancy, reported previously [14], between the relative Ca^{2+} uptake capabilities of the sarcoplasmic reticulum and the half relaxation times in these muscles, as well as for the similar delays observed [15] in the onset of force in intact extensor digitoum longus and soleus muscles following stimulation, although force develops considerably slower in the latter muscle (Fig. 2). The wider range of $[Ca^{2+}]$ over which detectable submaximal force is noticed in the slow muscle fibre type could also partly explain the longer duration of the twitch in the soleus fibres [15].

It is important to note that one other extensor digitorum longus and one other soleus preparation had both the Ca^{2+}-activivation curve and the time course of force development (see Fig. 2) of the types characteristic to

the soleus and extensor digitorum longus muscles respectively. This correlates well with the probability of about 0.1 to find slow muscle fibres in the extensor digitorum longus of the rat and fast muscle fibres in the rat soleus muscle. Had it been necessary to pool all the individual results obtained on the extensor digitorum longus or on the soleus muscle, we would not have been able to notice a significant difference in the Ca^{2+} activation curves shown in Fig. 1.

We were also interested to compare the rate of force development at essentially constant $[Ca^{2+}]$ for conditions in which the steady-state force response was either similar to the height of a twitch or to the tetanic response [1]. In Fig. 2 are shown typical responses from a slow twitch (traces a—c) and a fast twitch muscle fibre (d—f) when the preparations were suddenly activated (see legend) in identical solutions at three different Ca^{2+} concentrations: one corresponding to the maximum force response in the preparations (traces a,d), one corresponding to about 50% relative force response for both muscle fibre types (traces b,e), and one close to the threshold for activation of the fast fibre type (traces c,f). The much steeper relationship between relative steady state force and pCa for the fast fibre shown in Fig. 1 is clearly outlined by these traces. One observation is that force develops faster at higher $[Ca^{2+}]$ in each fibre type, and this observation was also reported for skinned muscle fibres of frog [7]. However, there is a significant difference in the time course of force development in these mammalian muscle fibres when compared to that in frog muscle fibres since the maximum rate of force development is attained immediately after increasing $[Ca^{2+}]$ in the rat muscle preparations, but later in the frog fibres. The force responses from fast and slow muscle preparations are apparently similar in shape if the time scale is adjusted, and one can use the time required to reach 50% of the steady-state level (t_{50}) as a reliable parameter to compare the average rates of force development in the two fibre types. From traces c,f in Fig. 2 one can estimate that the value of t_{50} for the fast muscle fibre is about half that for the slow muscle fibre. However, when the steady-state force response in both fibre types is in a range of physiological importance (e.g. twitch to tetanus height), then the value of t_{50} for the fast muscle type is considerally shorter than that for the slow fibre type at constant $[Ca^{2+}]$ (the time factor between traces a,d is about 6 and between traces b,e is about 10). This is an important finding since it directly shows that not only the rate of shortening [16], but also the rate of force development at constant $[Ca^{2+}]$ depends upon the fibre type. Then, it is quite possible that the longer time to peak in the intact slow muscle than in the intact fast twitch muscle fibre is mainly due to other differences in the properties of the two fibre types rather than in the free Ca^{2+} transient in vivo [15]. From the steepness of the activation curves it was calculated [9, 17] that the apparent number of Ca ions involved per functional unit of the regulatory system to produce force is two in the slow muscle fibre and four in the fast muscle fibre type. Thus the Ca^{2+} regulatory systems in the two muscle fibre types have different basic properties. In this context it is interesting to point out that amino acid sequencing of the troponins have indicated a lower number of Ca binding sites for the cardiac muscle [18] than for the fast skeletal muscle. From our results it ap-

pears likely that troponin in the slow muscle type should also involve a lesser number of Ca^{2+} ions in regulation. However, our data do not rule out the possibility of an additional, myosin-linked regulatory system in the vertebrate skeletal muscle [7].

Our results also indicate that the Ca^{2+}-threshold for contraction is lower for the slow muscle fibre, and in this respect are in line with both other physiological studies on mammalian skeletal muscle [3, 6]. However, biochemical studies suggest a greater Ca^{2+}-sensitivity for fast muscle [19]. This discrepancy is only apparent, since if one uses the 50% force (or ATPase) point to compare different sensitivities, as done in biochemical studies, then it is obvious from Fig. 1 that the fast muscle fibre would be considered more sensitive than the slow muscle fibre. This apparently paradoxical situation arises from the fact that the two activation curves have a significantly different steepness.

It is difficult to compare on a quantitative basis the Ca-activation curves on rat skeletal muscles with those reported on guinea pig [6] and rabbit [3], partly because the experimental conditions were different and partly because the error involved in constructing the curves was different. However, with these observations in mind and allowing for the different values used for the absolute affinity of Ca^{2+} to EGTA, it appears that rat muscles have properties closer related to guinea pig muscles [6] rather than to rabbit muscles [3].

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References

1 Close, R. (1972) Physiol. Rev. 52, 129—197
2 Hess, A. (1970) Physiol. Rev. 50, 40—62
3 Kerrick, W.G.L., Secrist, D., Coby, R. and Lucas, S. (1976) Nature 260, 440—441
4 Secrist, J.D., Kerrick, W.G.L., Coby, R., Hoar, P., Lucas, S. and Spaulding, B. (1976) Biophys. J. 16, 73a
5 Donaldson, S.K.B. and Hermansen, L. (1977) Biophys. J. 17, 201a
6 Takagi, A. and Endo, M. (1977) Exp. Neurol. 55, 95—101
7 Moisescu, D.G. (1976) Nature 262, 610—613
8 Moisescu, D.G. and Thieleczek, R. (1978) J. Physiol. Lond. 275, 241—262
9 Moisescu, D.G. and Thieleczek, R. (1979) Biochim. Biophys. Acta 546, 64—76
10 Ashley, C.C. and Moisescu, D.G. (1977) J. Physiol. Lond. 270, 627—652
11 Jewell, B.R. and Rüegg, J.C. (1966) Proc. Roy. Soc. B 164, 428—459
12 Hill, D.K. (1972) J. Physiol. Lond. 221, 161—171
13 Martonosi, A. and Ferentos, R. (1964) J. Biol. Chem. 239, 648—658
14 Briggs, F.N., Poland, J.L. and Solaro, R.J. (1977) J. Physiol. Lond. 266, 587—594
15 Close, R. (1969) J. Physiol. Lond. 204, 331—346
16 Barany, M. (1967) J. Gen. Physiol. 50, 197—218
17 Moisescu, D.G., Ashley, C.C. and Campbell, A.K. (1975) Biochim. Biophys. Acta, 396, 133—140
18 Van Eerd, J. (1975) Biochem. Biophys. Res. Commun. 64, 122—127
19 Ebashi, S., Endo, M. and Ohtsuki, I. (1969) Q. Rev. Biophys. 2, 351—384