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The effects of vascular smooth muscle caldesmon on force production by 'desensitised' skeletal muscle fibres

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Smooth muscle caldesmon inhibits actomyosin MgATPase in the absence of Ca^{2+} and is the key regulatory component of Ca^{2+}-regulated thin filaments. We now show that caldesmon can affect contractility as well. Glycerinated skeletal muscle fibres were treated so as to produce substantial contractions without Ca^{2+} as an activator. Addition of caldesmon caused reductions in force in a concentration- and time-dependent manner. Perfusion with caldesmon concentrations for < 5 min gave up to 48% reduction in isometric tension with a half-maximal effect at 1.5 μm caldesmon. Perfusion with 15 μM caldesmon for 30 min gave an irreversible tension drop.

Caldesmon; Ca^{2+} regulation; Contraction; (Skeletal muscle, Smooth muscle)

1. INTRODUCTION

The protein caldesmon is present in all vertebrate smooth muscles and has been shown to be exclusively located as a component of the thin filaments of the contractile apparatus [1]. Caldesmon is a potent inhibitor of actin activation of myosin MgATPase and it has been proposed that it is involved in the control of contractility [2,3].

Ca^{2+} controls smooth muscle contractility and in the test tube Ca^{2+} controls the ATPase activity of the contractile proteins, actomyosin, via regulatory mechanisms located both on the thick filaments (Ca^{2+}/calmodulin-dependent myosin phosphorylation) and the thin filaments [4].

Whilst there is considerable evidence linking myosin phosphorylation with both activation of actomyosin MgATPase and initiation of contraction [5] we have less understanding of the role of caldesmon. Recent work using anti-caldesmon antibody has confirmed that caldesmon is essential in the Ca^{2+} control of thin filament activation of myosin MgATPase [6]; in this study we have asked whether caldesmon can regulate muscle contractility as well. In order to obtain an unambiguous answer we have developed a muscle preparation whose own intrinsic regulatory mechanisms are inactivated. This was done by desensitising glycerinated rabbit psoas muscle fibres so that they contract independently of Ca^{2+}. This contractile model provided a suitable system in which to investigate the regulatory functions of exogenous caldesmon.

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2. MATERIALS AND METHODS

Caldesmon was prepared by heat treatment of a sheep aorta homogenate according to [7]. Caldesmon was purified from the heat stable supernatant by the pH 3/35–50% $(NH_4)_2SO_4$ procedure described in [2]. Yield was 0.25 mg protein/g artery. Smooth muscle actin and tropomyosin were prepared as in [8], and skeletal myosin as in [9]. All caldesmon preparations were tested for their ability to inhibit actin-tropomyosin activation of myosin MgATPase as described in [3]. 0.12 mol caldesmon per actin inhibited ATPase by 80 ± 4% (n = 11). All statistical values quoted are mean ± standard deviation.

Rabbit psoas fibres were chemically skinned according to Eastwood et al. [10]. For mechanical measurements 4–6 fibres of 10 mm in length were mounted between a tension transducer (Gould/Statham UC2) and a length drive coupled to a length

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sensor [11]. The mounted fibres were immersed in a temperature-controlled continuous flow chamber and perfused with rigor buffer (5 mM EGTA, 5 mM $MgCl_2$, 50 mM KCl, 1.5 mM NaN_3, 1 mM dithiothreitol) at 1 ml/min. Maximal contractions were produced in 'Ca solution' (rigor + 4 mM MgATP + 4 mM $CaCl_2$ (pCa^{2+} 6.0)) and relaxation in 'EGTA' solution (rigor + 4 mM MgATP (pCa^{2+} 9)).

Desensitisation was based on Meinrenken's method [12]. The mounted muscle fibres were perfused with 50 mM KCl, 20 mM Tris (pH 8.0–8.1) at 39°C for 30 min at zero tension and then returned to rigor buffer at 25°C.

3. RESULTS

3.1. *Desensitisation of rabbit psoas muscle fibres*

The chemically skinned muscle fibres adjusted to zero tension in rigor buffer produced contractions of 512 $\mu N \cdot fibre^{-1}$ ± 4.9 (n = 21) in Ca solution. Fibres relaxed in EGTA solution to a steady force 15 ± 4% (n = 21) of the maximal contractile force. After desensitisation treatment the maximal force in Ca solution was 342 $\mu N \cdot fibre^{-1}$ ± 6 (n = 19) whilst in EGTA solution the force was 68 ± 11% (n = 17) of maximal force (see fig.1a). Desensitised fibres thus produced substantial force in the absence of Ca^{2+} and they remained desensitised during many contractions. SDS-gel electrophoresis of desensitised fibres showed that the protein components had not changed substantially; in particular tropomyosin and the troponin components were all still present.

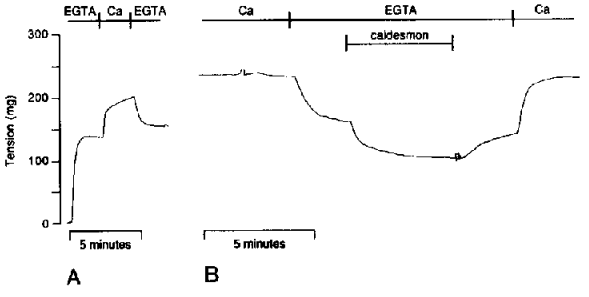

Fig.1. (a) Contraction of desensitised skeletal muscle fibres. 6 psoas fibres were bathed in rigor solution at resting length having been treated with Meinrenken solution as described in section 2. Perfusion with EGTA solution gave an isometric contraction 68% of that obtained in Ca solution. Return to EGTA solution relaxed the muscle to a holding tension 76% of that in Ca solution. (b) Inhibition of isometric force by caldesmon. 6 desensitised psoas fibres were maximally activated in Ca solution. Perfusion with EGTA solution resulted in 71% of isometric force being produced without Ca^{2+}. Addition of 4 μM caldesmon reduced this tension in EGTA solution by 34% in 4.5 min. This tension drop was partly reversed by return to EGTA solution and maximum force was regained upon return to Ca solution.

3.2. *Effect of caldesmon on isometric force*

When caldesmon (4 μM) was added to desensitised fibres in Ca solution there was no change in the isometric force (two preparations), however, caldesmon did reduce the force production in EGTA solution (fig.1b). The reduction in force due to 4 μM caldesmon was 24 ± 5% (n = 9) [13]; with short incubations this inhibition was partly reversed by washing in EGTA solution and fully reversed by a cycle of EGTA, Ca, EGTA solutions. Brief sequential perfusion with increasing caldesmon concentrations showed its inhibitory action to be concentration dependent with a maximal inhibition of force of 39–48% and an average half-maximal inhibition of 1.5 μM caldesmon ± 0.4 (n = 3).

More prolonged incubation with any one caldesmon concentration in EGTA solution resulted in further reduction of the force. For example, in one preparation the drops in tension due to 15 μM caldesmon at 1, 2, 5, 10, 15 and 19 min were 16.5, 20.2, 28.3, 35.1, 36.4 and 39.8%, respectively. Fig.2 shows that the tension drop following lengthy incubations in caldesmon became completely irreversible, even after washing in EGTA, Ca, and EGTA solutions. The reduced force in EGTA solution following long incubations

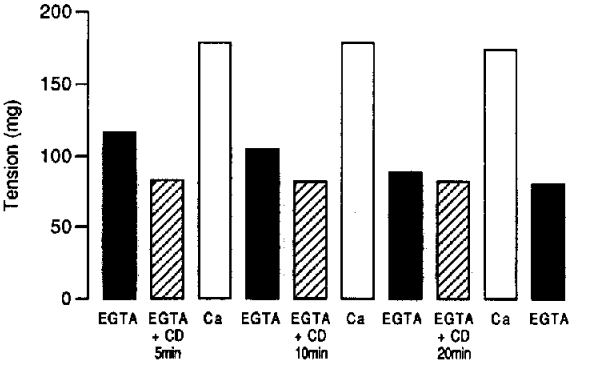

Fig.2. Irreversible drop in tension with increased time of exposure to 15 μM caldesmon. This was a continuous experiment involving a bundle of five desensitised psoas fibres. The inhibition of isometric force caused by caldesmon became progressively less reversible with increased time of perfusion. After a total of 35 min in 15 μM caldesmon the tension drop was irreversible.

in caldesmon was not due to deterioration of the muscle since the maximal force in Ca solution did not change (fig.2). SDS-gel electrophoresis and immunoblots of the fibres showed that in these conditions caldesmon was bound within the fibres at an estimated concentration of 20–50 μM (compare with 750 μM actin) and could not be removed even after extensive washes in EGTA or Ca solutions.

3.3. *Effects of caldesmon on tension transients*

We imposed a rapid shortening of 12 μm (0.2–0.3%) and then held the muscle at constant length for 20 s before slowly stretching it back to its initial length. We recorded tension immediately following the release (T_1) and after 20 s (T_2) and calculated relative muscle stiffness $[(T_1 - T_0)/T_0]$ and force recovery $[(T_2 - T_1)/T_0]$. Relative stiffness did not change significantly following the addition of caldesmon (paired t-test; $p > 0.1$, $n = 6$) but 15 μM caldesmon markedly reduced $[(T_2 - T_1)/T_0]$ in every instance with an average reduction of 25.5%. This reduced amplitude of force recovery did not appear to be due to a change in the rate of tension recovery.

4. DISCUSSION

4.1. *Desensitisation of skeletal muscle fibres*

We have developed a novel method for desensitising glycerinated vertebrate skeletal muscle such that substantial isometric contractions can be obtained in the absence of Ca^{2+} (fig.1a).

The preparation has an advantage over the low salt/high pH method [14] which produces muscles incapable of generating significant tension with or without Ca^{2+}, or Mg-free treatment at low temperature where desensitisation is lost as soon as the fibres are returned to high Mg contracting solutions [11]. Desensitisation did not involve extensive removal of the regulatory proteins from the fibres and yet the preparations remained desensitised in our buffer solutions indefinitely. This contractile model, whose own regulatory system is inactivated, provides an ideal system for investigating the regulatory functions of exogenous proteins.

4.2. *Caldesmon inhibition of contraction*

Caldesmon is present in smooth muscle thin filaments where it is the inhibitory component of the Ca^{2+}-dependent system which regulates thin filament activation of myosin MgATPase. It is an effective inhibitor with both skeletal and smooth muscle actins and myosins [2,3,15] and it also inhibits the MgATPase activity of skeletal muscle desensitised myofibrils (not shown). The work reported in this paper shows, in addition, that it can inhibit muscle isometric contraction thus confirming that caldesmon's inhibition of MgATPase is related to its inhibition of force production.

For short perfusions a half-maximal reduction in force was observed with 1.5 μM caldesmon which compares with a dissociation constant of 0.3 μM for caldesmon binding to pure actin-tropomyosin. Prolonged incubation with high concentrations resulted in irreversible loss of tension accompanied by irreversible binding of an estimated 20–50 μM caldesmon within the fibre in agreement with Galazkiewicz et al. [16].

Caldesmon did not affect relative muscle stiffness but at the highest concentration available, 15 μM, it did reduce the extent of recovery of tension following a quick release. This may be a reflection of the ability of caldesmon to increase skeletal or smooth heavy meromyosin binding to actin-tropomyosin in the presence of MgATP [17], an interaction which may be important in the formation of latch bridges [5] in smooth muscle.

Other lines of research have recently indicated that caldesmon can control contractility or motility [18–20] in agreement with our direct evidence. On this basis the caldesmon present in smooth muscle would be expected to play a role in regulating contractility in vivo. We hope to be able to extend our studies from this simple model system to the greatly more complex intact smooth muscle system in the future.

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REFERENCES

[1] Marston, S.B. and Lehman, W. (1985) Biochem. J. 231, 517–522.
[2] Marston, S.B. and Smith, C.W.J. (1985) J. Muscle Res. Cell Motil. 6, 669–708.
[3] Smith, C.W.J., Pritchard, K. and Marston, S. (1987) J. Biol. Chem. 262, 116–122.
[4] Marston, S.B. (1982) Prog. Biophys. Mol. Biol. 41, 1–41.
[5] Kamm, K.E. and Stull, J.T. (1985) Annu. Rev. Pharmacol. 25, 593–620.

[6] Marston, S.B., Redwood, C. and Lehman, W. (1988) Biochem. Biophys. Res. Commun. 155, 197–202.
[7] Bretscher, A. (1984) J. Biol. Chem. 259, 12873–12880.
[8] Marston, S.B. and Smith, C.W.J. (1984) J. Muscle Res. Cell Motil. 5, 559–575.
[9] Perry, S.V. (1955) Methods Enzymol. 2, 582–588.
[10] Eastwood, A.B., Wood, D.S., Bock, K.L. and Sorenson, M.M. (1979) Tissue Cell 11, 553–566.
[11] Loxdale, H.D. and Tregear, R.T. (1983) J. Muscle Res. Cell Motil. 4, 543–556.
[12] Meinrenken, W. (1969) Pflügers Arch. 311, 243–255.
[13] Taggart, M. and Marston, S. (1988) Trans. Biochem. Soc. 16, 360–361.
[14] Lehman, W. (1977) Nature 274, 80–81.
[15] Dabrowska, R., Goch, A., Galazkiewicz, B. and Osinska, H. (1985) Biochim. Biophys. Acta 842, 70–75.
[16] Galazkiewicz, B., Borovikov, Y.S. and Dabrowska, R. (1987) Biochim. Biophys. Acta 916, 368–375.
[17] Hemric, M.E. and Chalovich, J.M. (1987) J. Biol. Chem. 263, 1878–1885.
[18] Szpacenko, A., Wagner, J., Dabrowska, R. and Ruegg, J.C. (1985) FEBS Lett. 192, 9–12.
[19] Nomura, M. and Sobue, K. (1987) Biochem. Biophys. Res. Commun. 144, 936–943.
[20] Sellers, J.R. and Shirinsky, V.P. (1987) J. Cell. Biol. 105, 194a.