Pflügers Archiv
European Journal of Physiology

Società Italiana di Fisiologia
XXVI Riunione Primaverile
(Spring Meeting)

15–17 February 1999, Firenze, Italy

Abstracts

Springer
Pflügers Arch – Eur J Physiol (1999) 438: R1–R24

Pflügers Archiv
European Journal of Physiology

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KINETICS OF FORCE DECAY IN SINGLE MYOFIBRILS RELAXED BY RAPID SOLUTION CHANGE

S. Nencini, P. Caini, F. Colomo, N. Piroddi, C. Poggesi and C. Tesi

Relaxation of intact skeletal muscle from steady-state force is a poorly understood process that depends on several cellular mechanisms. This study describes an approach that makes it possible to focus on the process of force relaxation at the sarcomere level avoiding the effects of Ca^{2+} diffusion and reuptake by the sarcoplasmic reticulum. Single myofibrils and thin bundles of few myofibrils were isolated by homogenization of glycerinated rabbit psoas muscle and mounted in a force recording apparatus (Colomo et al., J. Physiol. 1997, 500.2, 535-542). Mounted myofibrils were maximally activated (pCa 4.75) and fully relaxed (pCa 8) by rapidly translating the interface between two flowing streams of solution across the preparation (Colomo et al., Biophys. J., 1997, 72, A127). The solution change occurred with a time constant of 2-4 ms and was complete in 10 ms. In agreement with results obtained in skinned fibres relaxed by photolysis of caged Ca^{2+} chelators (Patel et al., Biophys. J., 1998, 74, 360-368; Wahr et al., Am J. Physiol., 1998, 274, C1608-C1615), relaxation started with a slow, almost linear, decay in force followed by a shoulder and then by a fast exponential phase. At 5 °C the slow initial phase of relaxation lasted for about 200 ms and its slope was 0.5 s^{-1} while the rate constant of the fast exponential phase was 10 s^{-1}. At 15 °C the overall kinetics of relaxation was strongly accelerated: Q_{10} values for both the slow and fast rates were higher than 3. As compared to the features of full relaxation from maximal activation it was found that: 1- when partially activated myofibrils (pCa ranging from 5.50 to 6.00) were fully relaxed (pCa 8), the slow phase of force decay was accelerated; 2- when maximally activated myofibrils (pCa 4.75) were only partially relaxed (pCa ranging from 5.50 to 6.00) the rate of the fast phase of force decay was decelerated up to values close to those of the slow phase.

Dipartimento di Scienze Fisiologiche, Università di Firenze, Viale Morgagni 63, I-50134, Firenze

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MUSCLE FREQUENCY RESPONSE INVESTIGATED BY FORCE AND SURFACE MECHANOMYOGRAM

C. Orizio, R. Baratta[1], B.H. Zhou[1], M. Solomonow[1], A. Veicsteinas

Muscle frequency response (FR) is attained by varying the level of activity of the motor units' pool sinusoidally. The input signal to the system is provided to the motor nerve. The muscle transfer function is estimated analysing the amplitude attenuation and the phase lag of the force sinusoidal changes (output signal of the system) with respect to the input signal in the 0.4 - 6 Hz frequency range. Force generation process is accompanied by a muscle surface displacement that is detectable by a laser distance sensor. The related electrical signal is named surface mechanomyogram (MMG). Aims of this study were a) to verify whether from MMG a reliable FR is retrievable and b) to compare the MMG FR with force FR. To this purpose the force and the MMG from the exposed medial gastrocnemius of four cats were investigated. A special apparatus, following the size principle, changed the number of motor units, to be recruited by a 40 Hz train stimuli administered to the motor nerve, sinusoidally. From 0.4 to 6 Hz 14 different sinusoidal inputs were tested. It resulted that the Bode plots of amplitude attenuation and phase lag of the two signals were compatible with the responses of a critically damped second order system with two real double poles (located on the average at 1.83 Hz for force and 2.75 Hz for MMG) and a pure time delay (on the average 22.6 ms for force and 38 ms for MMG). The fact that both the force and MMG FRs could be modelled by a second order system with a pure time delay suggests that MMG may provide data on the muscle transfer function. This is important when force is difficult or impossible to be recorded. On the contrary the differences in the second order system parameters lead to the hypothesis that different components of the muscle mechanical model may specifically influence the force or the MMG properties.

Dip. Scienze Biomediche e Biotec. Università di Brescia. Via Valsabbina, 19; I-25123 Brescia. [1]Bioengineering Lab. Dept Orthopaedics, Louisiana State University, New Orleans.

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FIBRE TYPES AND SHORTENING VELOCITY IN RABBIT MUSCLES: COMPARISON WITH RAT AND HUMAN

M.A.Pellegrino, R.Bottinelli, M.Canepari, R.Rossi and C.Reggiani

Rabbit skeletal muscle fibres have been often used in mechanical and energetical studies, but a reliable analysis of their properties with regard to their myosin heavy chain (MHC) isoform composition is still lacking. In this study unloaded shortening velocity (Vo) and MHC isoform composition of rabbit single muscle fibres were studied and tested for relation. Single fibres were dissected from Soleus, Vastus Lateralis, Psoas and Adductor muscles. The fibres were chemically skinned and Vo was determined by the slack-test technique at 12°C and sarcomere length 2.5 μm. All fibres used were subsequently classified as type 1, 2A, 2X and 2B on the basis of their MHC isoform composition by SDS-PAGE (R.J.Talmadge et al., J.Appl.Physiol. 75(5):2337-2340, 1993). Mean values of Vo were: 0.6 ± 0.205 L/s for slow fibres (n=12), 2.31 ± 0.6 for 2A fibres (n=2), 2.93 ± 0.778 for 2X fibres (n=8), 3.94 ± 0.846 for 2B fibres (n=6). When Vo of fibre types in the rabbit were compared with Vo of homologous fibre types in rat and human, a logaritmic relationship was found between Vo of homologous fibre types and body size of the animal. The larger the body size the lower the Vo for each fibre type. The relatioship was found to be more pronounced for slow fibres than for fast fibres and among fast fibres Vo of type 2B was not or hardly affected by body size. As precise identification of fast muscle fibre types was achieved, these results extend previous observations on the relationship between body size and Vo.

Istituto di Fisiologia Umana, Università di Pavia, via Forlanini 6, I-27100 Pavia

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THE EFFECTS OF PHOSPHATE ON THE CONTRACTION OF SINGLE SKELETAL MYOFIBRILS

N. Piroddi, P. Caini, F. Colomo, S. Nencini, C. Poggesi and C. Tesi

Studies of the effects of phosphate (Pi) on force generation in skinned muscle preparations have been usually restricted to Pi concentrations greater than 150-200 μM because of the inability to buffer the diffusive accumulation of Pi liberated within the fibres (50-100 μm wide) by the actomyosin ATPase. Single myofibrils or thin bundles of few myofibrils (1-3 μm wide) isolated from rabbit psoas have been used here to overcome this limitation. Myofibrils mounted in a force recording apparatus were maximally activated and relaxed at 5 mM MgATP (at 5 °C) by rapidly translating the interface between two continuous streams of relaxing (pCa 8) and activating (pCa 4.75) solutions, with [Pi] ranging from 10 mM to less than 5 μM. Pi concentration was lowered below the contaminant level (about 150μM) by treating the experimental solutions with sucrose phosphorylase-sucrose or nucleoside phosphorylase-7methylguanosine enzyme-substrate systems. Standard colorimetric assays were used to determine the actual Pi concentrations. As previously observed in skinned fibres (Pate et al. Biophys. J. 1998, 74, 369-380) for Pi concentrations in the range 150 μM - 10 mM, isometric force was found to decrease linearly with log [Pi] with a slope of about -0.45/ decade. Below 150μM Pi, a decrease in [Pi] still led to an increase in developed force (about 20 % at 5-10 μM Pi) but the slope of the relation was smaller (about -0.15/decade). In the whole range of concentrations tested, Pi was also found to decrease the half time of force development in a way that closely paralleled the effects of Pi on steady-state force. The results (1) are consistent with cross bridge models in which the release of Pi is involved in the transition from a weakly bound state to a strongly bound state and (2) can be useful to answer questions concerning the dependence of actomyosin mechanics on the free energy of ATP hydrolysis.

Dipartimento di Scienze Fisiologiche, Università di Firenze, Viale Morgagni 63, I-50134, Firenze