Changes in contractile dynamics during the course of a twitch of a frog muscle fibre

P. HAUGEN

Department of General Physiology and Biophysics, University of Copenhagen, The Panum Institute, Blegdamsvej 3C, DK-2200 Copenhagen N, Denmark

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Summary

Single skeletal muscle fibres from the frog were stimulated to produce isometric twitches and released after a delay to shorten isotonically unloaded or against a finite load (P). When varying the delay, the velocity of the initial shortening (V) against a given non-zero load reached its maximum value earlier than the peak of the isometric tension. The velocity of unloaded shortening (V₀, slack test, range: 3.7–5.6 nm ms⁻¹ per half-sarcomere) was independent of the delay of the release. For any given delay, V was hyperbolically related to P, except for the high-load end of the P–V curve at which the velocity took lower values than extrapolated from the hyperbolic relation. The relation between V and the load in units of P₁ (corresponding to V = 1 nm ms⁻¹ per half-sarcomere) coincided in the hyperbolic range with the relations obtained at other delays of the release. P₁ was basically proportional to the maximum power which also had its peak value during the rising phase of the twitch. The quick releases required to reach the non-hyperbolic range of the P–V curves were estimated to be < 9 nm per half-sarcomere irrespective of the delay of the release. At load levels in the non-hyperbolic range V could be increased if the quick release was followed by a brief (2 ms) extra reduction in the load preceding the shortening at isotonic load. The results can be explained if the kinetic properties of the individual strongly bound crossbridges are unaffected by the changing level of activation during the course of the contraction. The time-dependence of the non-hyperbolic range of the P–V relation can be accounted for if crossbridges attached before the release remain attached after the release thus constituting an internal load. The difference in time course of isometric tension as compared to velocity of initial shortening against a given load, P₁, and maximum power may arise as the result of a reduction in the level of activation caused by the release to the isotonic load level.

Introduction

During the course of a twitch the contractile apparatus of a muscle fibre passes through a process of activation and subsequent deactivation which mechanistically is manifested by the time-dependent capacities for carrying a load and for generating mechanical work, i.e. for shortening against a load. Both capacities are generally ascribed to the interaction of myosin crossbridges with the actin molecules of the thin filaments. The time course of the number of attached crossbridges as indicated by the instantaneous stiffness appears roughly proportional to the contractile force under fixed-end conditions, although rising slightly earlier and declining slightly later than the latter (e.g. Haugen & Sten-Knudsen, 1987). On the other hand, it has long been known that during the relaxation period a muscle loses its capacity for shortening against a load, i.e. produce work, while it can still carry a considerable tension under isometric conditions (Jewell & Wilkie, 1960). Does this reflect a change during the course of the contraction of the kinetic properties of the individual crossbridges? This question is related to a controversy yet unresolved, viz. whether the calcium-controlled regulation mechanism functions purely switch-like, i.e. by regulating the number of actin sites available for crossbridge attachment or whether the kinetics of the individual crossbridges are also affected, as discussed in the review by Podolin & Ford (1983).

The ‘classic’ model of the regulatory action of the troponin–tropomyosin system was one of a steric blocking of the myosin-binding sites of the actin filaments preventing the binding of crossbridges when no calcium was bound to the troponin (Haselgrove, 1973; Huxley, 1973; Parry & Squire, 1973). Recent findings from mechanical and biochemical investigations (Brenner et al., 1982; Trueblood et al., 1982, see these papers for further references), however, might indicate that the regulatory mechanism affects the transition from a weakly bound crossbridge state (actomyosin–ADP–Pi) to a strongly bound state. At physiological ionic strengths, however, the weakly bound crossbridges appear not to...
Contractile dynamics during twitch contribute appreciably to force or stiffness (when measured at velocities less than order of magnitude 100 nm ms⁻¹ per half-sarcomere). In the following, such weakly bound states will therefore be considered as (apparently) detached and the strongly bound states as (apparently) attached.

To investigate whether the kinetics of the individual crossbridges in the attached states were influenced by the time after stimulation, i.e. by the level of activation, the force–velocity relation and the velocity of unloaded shortening were studied when the fibre was released at various delays after stimulation. Brief preliminary reports have been presented at meetings of the Scandinavian Physiological Society (Haugen, 1984a, b, 1985).

Methods

Intact single muscle fibres isolated from either head of the anterior tibial muscle of the frog (*Rana temporaria*) were prepared following essentially the same procedure as previously described (Haugen & Sten-Knudsen, 1976, 1987). The experimental set-up was also the same as described in Haugen & Sten-Knudsen (1987) except for the feedback control system which was slightly modified (Fig. 1). The muscle fibre was horizontally suspended (by means of aluminium clips) between a servo motor (PM Minishaker Type 4810, Brüel & Kjaer, Nærum, Denmark) and a tension transducer made from a piezo-resistive transducer element (AE 801, Aksjeselskapet Mikroelektronikk, Horten, Norway). The tension transducer used in the majority of the experiments has been described before (Haugen & Sten-Knudsen, 1987). It had a resonant frequency of ~1.4 kHz and was underdamped with a relaxation time of ~2 ms. In the later experiments, however, the same type element was applied to make a faster version similar to that described by Edman (1979) (resonant frequency ~6.5 kHz, underdamped: relaxation time 1.4 ms).

The response characteristics of the servomotor were controlled in a local feedback circuit where an error signal (the difference between a reference input signal (SMR) and the output of the displacement transducer) and a displacement velocity signal were applied to the amplifier driving the servomotor as described before (Haugen & Sten-Knudsen, 1987).

An overall feedback control system was operated in either length-controlled or force-controlled mode. In the length-controlled mode the length reference input signal

![Diagram](image-url)