

Acting on an impulse (or two): Advantages of high-frequency tetanic onset in skeletal muscle

Richard L. Moss, Thomas L. Lynch IV, and Daniel P. Fitzsimons

Department of Cell and Regenerative Biology, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705

Fast-twitch skeletal muscles are specialized for in vivo motor functions that require high power (mastication and load lifting), high speed (eyelid blink), or a combination of the two (withdrawal reflexes and escape responses). Those functions that rely on high power often involve tetanic stimulation of the effector muscles, resulting in sustained force generation even during tetani that are not fused, which is the physiological norm (McMahon, 1984; Biewener, 2016). Taking escape responses as an example, there is a clear adaptive advantage to rapid initiation of a forceful ballistic contraction, which to a degree is achieved by expression of skeletal muscle myosin isoforms that exhibit fast or super-fast turnover rates in their interactions with actin (Rome and Lindstedt, 1998; Hoh, 2002). Rapid delivery of Ca^{2+} to the myoplasm during excitation–contraction coupling is another mechanism that would presumably speed the rate of force development and contribute to an early increase in the ability of the muscle to generate power. Mechanisms such as these are energetically expensive and are thus evident to lesser degrees in slow-twitch skeletal muscles. They are also regulated properties of cardiac and most smooth muscles, in which Ca^{2+} delivery is modulated by neural activity and circulating levels of cardioactive and vasoactive hormones. In this issue, Bakker et al. examine the mechanism underlying the observation that a short burst of high-frequency action potentials at the onset of tetany results in a greater rate of force development.

Previous studies of the initial stages of contraction have shown that tetanic contractions of fast-twitch motor units in both rodents and humans begin with a high-frequency train of two or three action potentials before settling into a lower frequency of continued stimulation (studies cited in the present paper by Bakker et al. [2017]). Although this might be the response of an under-damped neural control system or a system of more complex design, long-standing work by several groups (review by Binder-Macleod and Kesar [2005] cited by Bakker et al. [2017]) has shown that this initial burst of action potentials contributes to the rate of force development in fast-twitch skeletal muscles. Imposing two or three higher frequency action potentials at the

beginning of tetanic stimulation significantly increases the rate of rise of force, a phenomenon that has been thought to be caused by increased release of Ca^{2+} from the sarcoplasmic reticulum. A study by Cheng et al. (2013), using the Ca^{2+} -sensitive dye indo-1, suggested that a pair of high-frequency action potentials at the beginning of a lower-frequency tetanus evoked an initial Ca^{2+} transient of significantly greater amplitude. Such an increase would explain the faster rate of rise of force associated with the initial doublet of higher-frequency stimuli and was believed by the authors to be caused by greater Ca^{2+} release from the sarcoplasmic reticulum in response to the second stimulus.

The present study by Bakker et al. (2017) further examines the mechanism by which an initial short burst of action potentials alters the time course of intracellular Ca^{2+} in ways that might explain a faster rise of force observed under these same conditions. In this instance, the authors used the Ca^{2+} indicator Mag-Fluo-4, which is faster and has lower affinity than indicators used in previous studies, and laser-scanning microscopy to achieve greater temporal resolution in recordings of intracellular Ca^{2+} concentration during tetanic stimulation. Their results, which are systematic, elegant, and convincing, go a long way toward resolving the basis for the observed greater increase in initial Ca^{2+} concentration caused by insertion of a doublet of high-frequency action potentials.

With the greater time resolution achieved with the combination of Ca^{2+} indicator and recording system used, the authors observe that the amplitude of the Ca^{2+} transient in response to the second stimulus of the doublet is actually less than the first. However, the sustained myoplasmic Ca^{2+} concentration between the first and second stimuli of the doublet is significantly greater than seen when the stimulus frequency during the initial phase is lower, i.e., just sufficient to achieve a fused tetanic contraction. Both the shorter interval and the higher levels of Ca^{2+} between successive Ca^{2+} transients, in response to the higher-frequency doublet, can account for the apparently greater increase in ini-

Correspondence to Richard L. Moss: rlmoss@wisc.edu



tial Ca^{2+} concentration observed previously with a slower indicator and recording system but otherwise similar conditions.

A plausible mechanism for accelerated kinetics of force development during high-frequency stimulation

The sustained initial increase in myoplasmic Ca^{2+} caused by insertion of a high-frequency doublet stimulus at the beginning of a tetanus gives rise to a straightforward mechanism to explain the observed faster rate of rise of tetanic force in the presence of the doublet. As modeled by the authors, sustaining the early increase in Ca^{2+} concentration is predicted to increase the binding of Ca^{2+} to troponin C during the rising phase of tetanic force, which in turn would increase the activation of the thin filament and thus the number of cross-bridges that can bind to actin. As a result, there are increases in the number of cross-bridge binding events and in the absolute rate of force development, in mN/s. However, as important as this mechanism is likely to be, it does not account for all the authors' findings.

When the authors normalized the amplitudes of initial force development with and without the doublet to 100% (Fig. 2 D in Bakker et al. [2017]), they observed the kinetics of force development to be significantly faster when a tetanus was initiated with a higher-frequency stimulus doublet. Because it is unlikely that the kinetics of cross-bridge state transitions vary with the level of thin filament activation by Ca^{2+} , the observed acceleration of contraction kinetics in response to the doublet stimulus must involve another Ca^{2+} -dependent mechanism. In this regard, a conceptual framework first proposed by Campbell (1997) for cardiac muscle, and later expanded to other muscle types by Campbell et al. (2010) and Aboelkassem et al. (2015), is potentially very useful. In this model, the binding of Ca^{2+} to troponin (Tn) activates the associated thin filament regulatory unit comprised of one tropomyosin (Tm) spanning seven actin monomers. Cross-bridges that bind to actin within the activated regulatory unit cooperatively recruit cross-bridges to actins within neighboring regulatory units. This cooperative contribution to activation of the thin filament is presumably the result of movement of the Tn/Tm strand caused by binding of cross-bridges to the thin filament. An earlier review (Fitzsimons and Moss, 2007) summarizes how such a model predicts activation dependence of the rate of force development, which varies ~ 10 -fold between threshold ($P/P_0 = 0$) and maximal ($P/P_0 = 1.0$) Ca^{2+} activation of force (Fig. 1). At high $[\text{Ca}^{2+}]$, when all Tn/Tm regulatory units are effectively "on" because of Ca^{2+} binding to Tn, the rate of force development will be limited by the maximum rate of cross-bridge cycling. At low $[\text{Ca}^{2+}]$, the kinetics of force development are slowed by the cooperative spread of cross-bridge binding and thus the activation of neighboring regulatory units. In

fact, at very low $[\text{Ca}^{2+}]$, activation may involve just one or two regulatory units per thin filament (Fitzsimons and Moss, 2007). Consistent with this idea, the rate of force development can be accelerated by an order of magnitude even at low $[\text{Ca}^{2+}]$ by cooperatively activating the thin filament by infusion of a strong binding, nonforce-generating derivative of the myosin head, N-ethylmaleimide-S1 (Fig. 1).

When the preceding explanation of cooperativity in the activation of the thin filament is taken into account, it becomes evident that the mechanisms involved in the regulation of contraction vary with the extent of Ca^{2+} binding to troponin C. At high concentrations of Ca^{2+} , the near saturation of Ca^{2+} binding to troponin C fully activates the Tn/Tm regulatory strand, so that the intrinsic cross-bridge cycling rate (determined by myosin isoforms and the load on the muscle) is the primary determinant of the rate of force development. In those conditions, the relative values of the rate constants of cross-bridge binding and dissociation determine the steady force developed during a tetanus. At low concentrations of Ca^{2+} , many fewer regulatory units have Ca^{2+} bound to their associated troponins, giving rise to the conditions required for cooperative recruitment of cross-bridges. That is, binding of cross-bridges within a Ca^{2+} -activated functional group further displaces the Tn/Tm regulatory strand, which extends the persistence length of the displacement of the regulatory strand into adjacent regulatory units with no Ca^{2+} bound, allowing cross-bridges to bind despite the absence of Ca^{2+} bound to troponin C. Thus, when Ca^{2+} is low, the rate of force development not only depends on cross-bridge cycling rate, but also the time taken for the spread of cooperative recruitment into adjacent regulatory units. Because of this, the rate of force development is slower at low than at high Ca^{2+} concentrations, which is evident in the order-of-magnitude increase in the rate of force development by skinned skeletal muscle fibers when activation is increased from very low to maximal by increasing the concentration of Ca^{2+} in the activating solution (see "Control" data in Fig. 1 obtained in the absence of NEM-S1). The importance of cooperativity in cross-bridge binding in slowing the rate of rise of force at low levels of activation is evident in the acceleration of force development by the infusion of NEM-S1 at low levels of activation and the near elimination of activation dependence of the rate of force development by infusion of greater concentrations of NEM-S1 (Fig. 1). In contrast, the infusion of NEM-S1 has virtually no effect at maximal activation with Ca^{2+} ($P/P_0 = 1.0$), confirming the absence of cooperative effects on the activation of force development in skeletal muscle thin filaments in which Ca^{2+} binding to troponin C is saturated.

In the context of the study by Bakker et al. (2017), a high-frequency doublet stimulus that sustains the initial

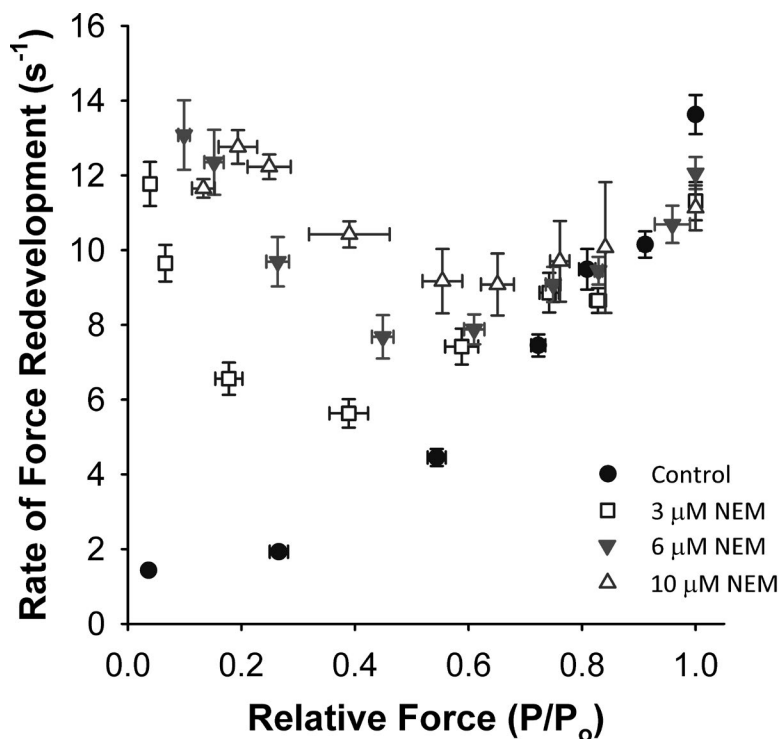


Figure 1. Effects of a strong binding derivative of myosin subfragment-1 (NEM-S1) on the rate constant of force development in rabbit permeabilized, fast-twitch, skeletal muscle fibers. See Fitzsimons et al. (2001). Control: The rate of force development increases by nearly an order of magnitude when the level of activation with Ca^{2+} is increased from near threshold ($P/P_0 = 0$) to saturating ($P/P_0 = 1.0$) for force development. NEM-S1: Infusion of a strong binding, nonforce-generating derivative of myosin subfragment-1 (*N*-ethylmaleimide-modified S1, or NEM-S1) speeds the rate of force development at submaximal concentrations of Ca^{2+} ($P/P_0 < 1.0$). The acceleration of force development is caused by increased cooperative binding of myosin to actin as a result of the binding of NEM-S1 to actin, displacement of the Tn/Tm regulatory strand, and increased cross-bridge binding to the thin filament. The acceleration of force development is greater at higher concentrations of NEM-S1 because of greater displacement of the regulatory strand. NEM-S1 has virtually no effect at maximal activation ($P/P_0 = 1.0$) because the Tn/Tm regulatory strand is already fully displaced and the thin filament maximally activated due to the saturation of Ca^{2+} binding to troponin C. Methods: The rate constant of force development was determined in permeabilized skeletal muscle fibers during isometric contractions evoked at free Ca^{2+} concentrations between threshold ($P/P_0 = 0$) and saturating ($P/P_0 = 1.0$) for force (P) development. The rate constant was estimated by curve fitting to the time course of isometric force redevelopment recorded after rapid release and restretch of the activated muscle fiber to disrupt cross-bridge binding. Data were obtained at 15°C in the absence or presence of NEM-S1. All values are means \pm SEM.

increase in myoplasmic Ca^{2+} would serve to maintain high levels of Ca^{2+} binding to troponin C, as confirmed by the authors' modeling of their results and also to sustain high levels of thin filament activation. As consequences of these events, cooperative recruitment of cross-bridges to the thin filament would be reduced or absent, and the rate of force development would be determined principally by the rate of cross-bridge cycling without the slowing effects of cooperative recruitment.

Physiological implications

The findings of the experiments reported by Bakker et al. (2017) provide greater understanding of the mechanistic basis for a physiologically relevant intervention that increases the rate of force development early in the tetanic stimulation of fast-twitch motor units. A burst of two or three higher-frequency action potentials at the beginning of a tetanus maintains myoplasmic Ca^{2+} at high concentration throughout the initial period of force development, even though the amount of Ca^{2+} released in response to the second stimulus is less than the first. The resulting sustained Ca^{2+} activation of the thin filament reduces the time taken to achieve high levels of force and power in both reflex

and voluntary activations of ballistic contractions of fast-twitch muscle fibers. Such contractions, when they occur, are powerful and fast and especially important in escape responses in both simple and complex organisms, as well as in the rare (for most humans in Western nations) need to perform maximal work against very heavy external loads.

A mechanism that increases the rate of force development in muscle is energetically expensive, but such costs are typically transient and thus a secondary design consideration for an organism under threat of injury or worse. An important consideration in this regard is that individual motor units do not undergo fused tetanic contractions of the type that comprise the subject of the current paper. Instead, the sustained development of force by a skeletal muscle comprised of several fast-twitch motor units is typically the result of asynchronous unfused tetani of individual motor units (Henneman et al., 1974). The possibility that unfused tetani would be initiated by a higher-frequency burst of action potentials is likely to decrease as tetanic stimulus frequency is reduced, thereby increasing the efficiency of force development in intact muscles working against intermediate or low loads.

Based on the present results showing that the normalized rate of force development is accelerated by an initial burst of action potentials, we proposed that the sustained increase in myoplasmic Ca^{2+} concentration at the beginning of a tetanus does more than simply increase the number of cross-bridges recruited to force generating states. Based on results in permeabilized fast-twitch skeletal muscle fibers, it is evident that Ca^{2+} concentrations that saturate Ca^{2+} binding to troponin C also saturate the cooperative recruitment of cross-bridges that is characteristic of partially activated thin filaments. Thus, at high Ca^{2+} concentrations, slowing of force development caused by the cooperative recruitment of cross-bridges to the thin filament is minimal or absent, and the rate of force development approaches the rate predicted by the fast cycling kinetics of fast or super-fast myosin isoforms.

It is important to note that cooperative recruitment of cross-bridges is also a critical determinant of the rate of force development in cardiac muscle, where such recruitment slows the rate of force development as a function of myoplasmic root-mean-squared Ca^{2+} concentration and is modulated by the phosphorylation status of thick and thin filament proteins. However, the cardiac isoforms of myosin are considerably slower than fast-twitch skeletal muscle myosins, so that contractions of cardiac muscle are not ballistic, even when myoplasmic Ca^{2+} concentrations are high. Here, nature, through selection, has arrived at a design well suited to function as ballistic or explosive contractions of cardiac muscle would be expected to compromise pump function because of the viscous properties of blood during ejection and the viscoelastic properties of the aorta and peripheral vessels. In contrast, as Ca^{2+} concentration is lowered, the progressive slowing of contraction caused by increased cooperative recruitment of cross-bridges provides an intrinsic tuning mechanism for matching the force and speed of contraction (Moss and Fitzsimons, 2010).

As ever, the wisdom of Shakespeare interpreted by Hill (1970) applies: "There are more things in heaven and earth, Horatio, . . . and even in [skeletal] muscles".

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grant R37 HL 82900.

The authors declare no competing financial interests. Eduardo Ríos served as editor.

REFERENCES

- Aboelkassem, Y., J.A. Bonilla, K.J. McCabe, and S.G. Campbell. 2015. Contributions of Ca^{2+} -independent thin filament activation to cardiac muscle function. *Biophys. J.* 109:2101–2112. <http://dx.doi.org/10.1016/j.bpj.2015.09.028>
- Bakker, A.J., T.R. Cully, C.D. Wingate, C.J. Barclay, and B.S. Launikonis. 2017. Doublet stimulation increases Ca^{2+} binding to troponin C to ensure rapid force development in skeletal muscle. *J. Gen. Physiol.* <http://dx.doi.org/10.1085/jgp.201611727>
- Biewener, A.A. 2016. Locomotion as an emergent property of muscle contractile dynamics. *J. Exp. Biol.* 219:285–294. <http://dx.doi.org/10.1242/jeb.123935>
- Binder-Macleod, S., and T. Kesar. 2005. Catchlike property of skeletal muscle: recent findings and clinical implications. *Muscle Nerve*. 31:681–693. <http://dx.doi.org/10.1002/mus.20290>
- Campbell, K. 1997. Rate constant of muscle force redevelopment reflects cooperative activation as well as cross-bridge kinetics. *Biophys. J.* 72:254–262. [http://dx.doi.org/10.1016/S0006-3495\(97\)78664-8](http://dx.doi.org/10.1016/S0006-3495(97)78664-8)
- Campbell, S.G., F.V. Lionetti, K.S. Campbell, and A.D. McCulloch. 2010. Coupling of adjacent tropomyosins enhances cross-bridge-mediated cooperative activation in a Markov model of the cardiac thin filament. *Biophys. J.* 98:2254–2264. <http://dx.doi.org/10.1016/j.bpj.2010.02.010>
- Cheng, A.J., N. Place, J.D. Bruton, H.C. Holmberg, and H. Westerblad. 2013. Doublet discharge stimulation increases sarcoplasmic reticulum Ca^{2+} release and improves performance during fatiguing contractions in mouse muscle fibres. *J. Physiol.* 591:3739–3748. <http://dx.doi.org/10.1113/jphysiol.2013.257188>
- Fitzsimons, D.P., and R.L. Moss. 2007. Cooperativity in the regulation of force and the kinetics of force development in heart and skeletal muscles: cross-bridge activation of force. *Adv. Exp. Med. Biol.* 592:177–189. http://dx.doi.org/10.1007/978-4-431-38453-3_16
- Fitzsimons, D.P., J.R. Patel, K.S. Campbell, and R.L. Moss. 2001. Cooperative mechanisms in the activation dependence of the rate of force development in rabbit skinned skeletal muscle fibers. *J. Gen. Physiol.* 117:133–148. <http://dx.doi.org/10.1085/jgp.117.2.133>
- Henneman, E., H.P. Clamann, J.D. Gillies, and R.D. Skinner. 1974. Rank order of motoneurons within a pool: law of combination. *J. Neurophysiol.* 37:1338–1349.
- Hill, A.V. 1970. First and Last Experiments in Muscle Mechanics. Cambridge University Press, Cambridge, UK. 158 pp.
- Hoh, J.F.Y. 2002. 'Superfast' or masticatory myosin and the evolution of jaw-closing muscles of vertebrates. *J. Exp. Biol.* 205:2203–2210.
- McMahon, T.A. 1984. Muscles, Reflexes, and Locomotion. Princeton University Press, Princeton, NJ. 354 pp.
- Moss, R.L., and D.P. Fitzsimons. 2010. Regulation of contraction in mammalian striated muscles—the plot thick-ens. *J. Gen. Physiol.* 136:21–27. <http://dx.doi.org/10.1085/jgp.201010471>
- Rome, L.C., and S.L. Lindstedt. 1998. The quest for speed: muscles built for high-frequency contractions. *News Physiol. Sci.* 13:261–268.