Reduced tetanic calcium in fatigue of whole skeletal muscle

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One mechanism of fatigue may be that the rise of intracellular calcium (Ca^{2+}_i) during contraction of fatigued muscle is not sufficient to fully activate the contractile proteins (1-3). Studies of calcium in single skeletal muscle fibers using Ca^{2+}-sensitive indicators suggested fatigue was associated with reduced tetanic $[Ca^{2+}]_i$ (1-3). The present study aimed to determine in a whole muscle if there is a relationship between altered contractility during fatigue and changes of cytosolic free calcium. The approach was to measure the tetanic force of whole bullfrog semitendinosus muscles while monitoring calcium with the Ca^{2+}-sensitive fluorescent indicator Indo-1. These whole muscle experiments extend previous single cell studies of fatigue and $[Ca^{2+}]_i$ (1-3) in several directions. First, while fatigue has been extensively studied in whole muscle, no studies have monitored changes of $[Ca^{2+}]_i$ and force during fatigue of whole muscle. Second, calcium measurements with Indo-1 have important advantages in minimizing artifacts due to motion of the preparation, leakage or bleaching of indicator during the experiment, or differences in indicator concentration between experiments. Indo-1 has not yet been exploited to monitor calcium in contracting whole skeletal muscle. Finally, with the high signal to noise fluorescence measurements obtainable from whole muscle, it was possible to quantitate the relaxation phase of the calcium signal. A slowing of force relaxation with fatigue is well known. However, quantitative relationships between changes in both force- and calcium relaxation with fatigue have not yet been defined.

Experimental methods

Bullfrog semitendinosus muscles were loaded with the calcium-sensitive indicator Indo-1 by arterial perfusion with an oxygenated loading solution consisting of Ringer, Indo-1-AM (5 μm), calf serum (5%), probenecid (1 mM), pluronic acid (2%) and dimethyl sulfoxide (1%), for 40 min at 4 ml/hr and 30°C. Indo-1-loaded muscles were set near the twitch-optimum length and supramaximally stimulated through parallel wire electrodes placed on either side of the muscle. Force and calcium (inferred from Indo-1 fluorescence) were measured during tetani (0.1 ms pulses at 100 Hz for 200 ms) at various levels of fatigue induced by repeated stimulation. Indo-1 fluorescence was recorded using a SLM 48000S spectrofluorometer. Indo-1-loaded muscles were illuminated at 350 nm through a fiber optic cable and the fluorescence intensity of the muscle was measured at 400 nm and 470 nm. Changes in calcium were inferred from changes in the ratio (R) of 400/470 nm intensities. During relaxation, time constants for the fall in force and R were calculated from a least squares fit to exponential expressions.

Results

Figure 1 shows that tetanic stimulation (indicated by the horizontal line) caused fluorescence to increase at 400 nm (I_{400}) and decrease at 470 nm (I_{470}). Changes of $[Ca^{2+}]_i$ were inferred from the ratio (R) of I_{400}/I_{470} (Fig. 1). Artifacts due to motion or changes of indicator content have similar relative effects at each wavelength and therefore cancel in the ratio. The rise of $[Ca^{2+}]_i$ started almost immediately after stimulation and preceded force development. During the tetanus, $[Ca^{2+}]_i$ rapidly reached a steady state and remained almost constant while force increased. The fall of $[Ca^{2+}]_i$ started soon after the end of stimulation and preceded the fall of force. Figure 2 shows the relationship between force and R during fatigue (means ± std error, n=7). Progressive decreases of R were accompanied by proportional decreases of force. Fatigue also resulted in a slowing of the rate at which both force and calcium relaxed at the

end of the stimulation. Figure 3 shows that the time constants of relaxation of force and R both increased in parallel as force decreased with fatigue.

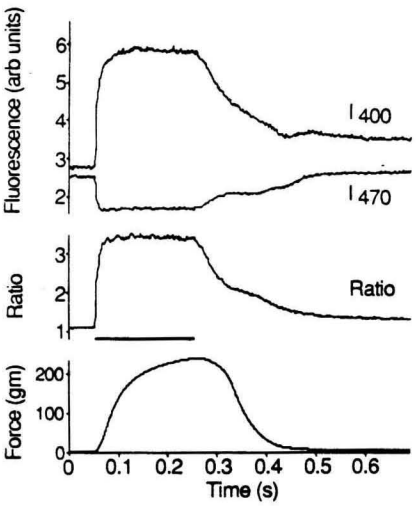

Fig. 1. Typical records of fluorescence at 400nm, 470nm, their ratio (reflecting Ca^{2+}) and force during a tetanus (horizontal bar).

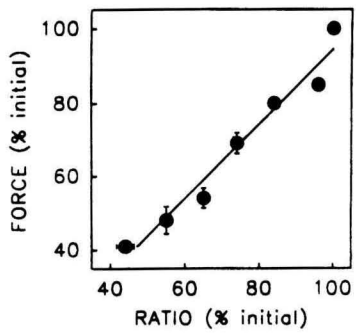

Fig. 2. Decreased force and R (reflecting Ca^{2+}) during fatigue (means ± std err n=7).

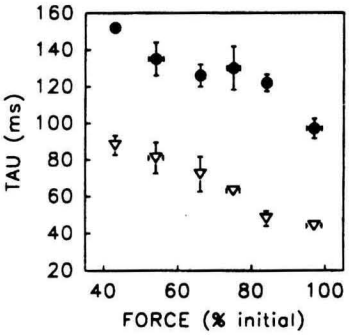

Fig. 3. (at right). Increases of relaxation time constants of force (▽) and R (•) with fatigue (means ± std err n=4).

Discussion

There were two major findings. First, fatiguing stimulation of whole skeletal muscle caused proportional decreases of force and R. This suggests that intracellular calcium was diminished with fatigue and therefore a component of the decrease in force may arise from a decrease in the tetanic level of calcium available to activate the contractile proteins. Second, fatiguing stimulation caused progressive slowing of relaxation of force and R. This suggests that relaxation of calcium was slowed with fatigue and therefore slowed force relaxation with fatigue may be mediated by slowed sequestration of calcium from the myoplasm. In conclusion, the findings of this study are consistent with measurements from single fibers and suggest that changes in calcium handling may play a significant role in the fatigue produced by intermittent tetanic stimulation.

References

1. Allen DG, JA Lee & H. Westerblad. Intracellular calcium and tension during fatigue in isolated single muscle fibers from xenopus laevis. *J. Physiol.* 415: 433-458, 1989.
2. Lee JA, H Westerblad & DG Allen. Changes in tetanic and resting $[Ca^{2+}]_i$ during fatigue and recovery of single muscle fibers from xenopus laevis. *J. Physiol.* 433: 307-326, 1991.
3. Westerblad H & DG Allen. Changes of myoplasmic calcium concentration during fatigue in single mouse muscle fibers. *J. Gen. Physiol.* 98: 615-635, 1991.