Muscle Fatigue and the Role of Transverse Tubules

Abstract. *Skeletal muscle stimulation for 300 pulses, either as single twitches or as tetani, increases the amount of calcium extracted in a Ringer solution containing strontium. The strontium is used to prevent reuptake of calcium by the transverse tubules. The accumulation of calcium in the transverse tubules during stimulation may account for muscle fatigue.*

The primary functions of the transverse tubular element in excitation-contraction coupling in fast skeletal muscle fibers are (i) to act as a conduit for the muscle action potential to the fiber interior and (ii) to serve as the site for coupling of the action potential to the release of stored calcium from the terminal cisternae. The calcium released from the terminal cisternae forms a localized high concentration in their vicinity and then diffuses to the region of overlap between thick and thin filaments. As the myoplasmic free Ca^{2+} concentration rises above $10^{-7}M$ in the vicinity of the overlap of thick and thin filaments, the inhibitory effect of tropomyosin on cross-bridge formation is overcome by the binding of Ca^{2+} to troponin C. The muscle fiber relaxes as the calcium released to the sarcoplasm is removed by the longitudinal reticulum and restored to the terminal cisternae (*1*, *2*).

Recent experiments (*3*) suggest that the transverse tubular element also has a major role in removing the calcium that is released from the terminal cisternae during excitation-contraction coupling. The increased calcium concentration in the transverse tubular element after stimulation may explain the uncoupling of the action potential from contraction during fatigue (*4*, *5*).

In experiments (*6*) in which we measured $^{45}Ca^{2+}$ influx during stimulation of the frog sartorius muscle we used the following procedure. Sartorius muscles were exposed to ^{45}Ca Ringer solution for short periods (10 minutes) and stimulated (1 Hz) during the last 5 minutes of equilibration with ^{45}Ca for 300 pulses. The muscles were removed, rinsed four times at 1-second intervals, and then washed out in Ringer solution (1.0 m*M*, Ca^{2+}) for 90 minutes. Collections were made and a desaturation curve constructed. This procedure allowed us to measure the residual ^{45}Ca in the muscle and the amount of ^{45}Ca in the slow component by the intercept of the ordinate at zero time. It also permitted measurement of an increase in calcium influx of 0.2 pmole/cm^2 per muscle twitch. The membrane area (300 cm^2/g, wet weight) in this calculation did not include the transverse tubular membrane area (2700 cm^2/g, wet weight) (*1*, *6*).

We modified this procedure to decrease any reuptake of ^{45}Ca or Ca^{2+} into the muscle fiber that might occur during the long washout period. We extracted ^{45}Ca and Ca^{2+} from sartorius muscle into a strontium Ringer solution (1.0 m*M*) for 15 minutes. Weiss (*7*) showed that strontium markedly increases ^{45}Ca washout from rabbit aortic muscle into calcium-free Ringer solution. We then measured the ^{45}Ca and Ca^{2+} in the extraction solution and the strontium uptake, and residual ^{45}Ca and Ca^{2+} in the muscle by extraction in 0.1*N* HCl. A 15-minute extraction period in strontium Ringer solution removed the ^{45}Ca and Ca^{2+} from the interstitial space and allowed measurement of the specific activity of the extracted calcium and the residual muscle calcium. Calcium and strontium were measured by atomic absorption (Perkin-Elmer model 703).

The extraction of ^{45}Ca from the muscle fibers after stimulation for 5 minutes at 1 Hz in a Ringer solution (1.0 m*M*) was compared with extraction into a strontium Ringer solution (1.0 m*M*). The composition of the Ringer solution was 111.0 m*M* NaCl, 2.5 m*M* KCl, 1.0 m*M* $CaCl_2$, 0.76 m*M* Na_2HPO_4, and 0.19 m*M* NaH_2PO_4 (*p*H 7.3). The strontium Ringer solution was prepared by replacing $CaCl_2$ with $SrCl_2$ (1.0 m*M*). The stimulus strength was 25 V and pulse duration was 5 msec. The effect of stimulation at 1 Hz for the last 5 minutes of a 10-minute exposure to ^{45}Ca Ringer solution (1 μCi/ml) is shown in Table 1.

Resting and stimulated muscles were placed in Ringer solution for 15 minutes, and the total calcium content and residual ^{45}Ca content measured. The Ringer solution was analyzed for ^{45}Ca. There was no significant difference between stimulated and resting muscle in total calcium content. A net increase of 0.035 μmole/g was observed in the residual ^{45}Ca content of the stimulated muscle and was attributed to retention of ^{45}Ca by the sarcoplasmic reticulum following influx. This additional ^{45}Ca uptake in the residual fraction amounted to 0.39 pmole/cm^2 per twitch, which is about double the value (0.20 pmole/cm^2 per twitch) measured in our earlier experiments. Total ^{45}Ca uptake was increased by 0.12 μmole/g, which amounts to an uptake of 1.3 pmole/cm^2 per twitch. Since no significant change in total calcium was observed, calcium efflux from stimulated muscle is equal to influx within the limits of experimental error.

The data in Table 2 were obtained from an experiment similar to that described for Table 1 except that a strontium Ringer solution was used. The total ^{45}Ca uptake increased 0.10 μmole/g, from 0.21 to 0.31 μmole/g; the residual and extracted ^{45}Ca were increased by 0.025 and 0.08 μmole/g. The calcium

Table 1. Effect of stimulation and ^{45}Ca influx (10 minutes) at 20°C in frog sartorius muscles. Values are mean ± standard error of the mean (*N* = five paired muscles). Abbreviation: N.S., not significant.

Calcium fraction	Concentration (μmole/g)			*P*
	Resting	Stimulated	Change	
^{45}Ca extraction	0.25 ± 0.03	0.35 ± 0.06	0.10 ± 0.05	.05
^{45}Ca residual	0.081 ± 0.002	0.116 ± 0.008	0.035 ± 0.009	<.01
Total ^{45}Ca	0.34 ± 0.03	0.46 ± 0.06	0.12 ± 0.05	<.05
Total Ca	1.88 ± 0.10	1.80 ± 0.12	−0.08 ± 0.05	N.S.

Table 2. Extraction of ^{45}Ca and Ca in strontium Ringer solution. Values are mean ± the standard error of the mean. Numbers in parentheses are the number of muscles.

Fraction	Concentration (μmole/g)		
	Resting	Stimulated*	Stimulated†
Sr uptake	0.30 ± 0.01 (12)	0.30 ± 0.03 (16)	0.28 ± 0.02 (12)
Ca extraction	0.26 ± 0.01 (16)	0.55 ± 0.05‡ (16)	0.52 ± 0.05‡ (12)
^{45}Ca extraction	0.16 ± 0.01 (24)	0.24 ± 0.02‡ (16)	0.27 ± 0.02‡ (12)
^{45}Ca residual	0.045 ± 0.002 (24)	0.070 ± 0.007‡ (16)	0.066 ± 0.004‡ (12)
Ca residual	1.52 ± 0.04 (16)	1.35 ± 0.04‡ (16)	1.10 ± 0.02‡ (12)

*One hertz (twitch) for 5 minutes totaling 300 shocks. †One hundred hertz (tetanus), three volleys of 1-second duration, 1 second apart during last 5 seconds of a 10-minute equilibration with ^{45}Ca. ‡Significantly different from control values at $P < .0025$.

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influx per twitch was equal to 1.1 pmole/cm^2 per twitch on the basis of the increase in total ^{45}Ca uptake and 0.27 pmole/cm^2 per twitch on the basis of the increase in residual ^{45}Ca uptake.

The major difference was observed in the extracted calcium; stimulation caused an additional 0.29 μmole/g of calcium to be extracted in strontium Ringer solution (0.55 μmole/g − 0.26 μmole/g). The additional loss is reflected in the residual calcium, which was lower in concentration in the stimulated muscle (1.35 μmole/g) than in the resting muscle (1.52 μmole/g).

The additional calcium extracted from the stimulated muscle was not matched by a gain of strontium, indicating that there was a net loss of calcium, and it was not replaced by strontium. The specific activity ratio of the calcium extracted from resting muscle was 0.61 and the specific activity of the calcium extracted from stimulated muscle was 0.45. The reduced specific activity was accounted for by increased extraction of calcium from an unlabeled pool. Stimulation apparently led to translocation of a fraction of the unlabeled calcium released from the terminal cisternae to the transverse tubules.

Muscles stimulated at 100 Hz for three 1-second intervals during the last 5 seconds of a 10-minute equilibration period in ^{45}Ca lost more calcium to the extraction medium than the strontium taken up; the excess was 0.24 μmole/g (Table 2). This loss of calcium is in good agreement with the loss of 0.29 μmole when the muscles were stimulated at 1 Hz for 5 minutes.

The calcium mobilized from the terminal cisternae to the transverse tubular element amounted to 0.8 nmole/g per twitch. At the end of 120 twitches, 96 nmole/g would be accumulated in the transverse tubules and by the end of 300 twitches, 240 nmole/g. If we used 0.4 percent of the fiber as an estimate of the transverse tubular volume, 80 percent of which is junctional (*1*), the transverse tubular volume would amount to 3.4 μl/g (wet weight). The calcium concentration in the transverse tubular element at the end of 120 twitches would be 28.2 m*M*, and at the end of 300 twitches, 70.6 m*M*. The marked increase in transverse tubular calcium would raise the threshold for coupling of the action potential to contraction and thus cause uncoupling of the action potential from contraction. The muscle twitch response at 1 Hz is maintained for the first 2 minutes of stimulation and then decreases to 43 percent of the initial twitch tension at the end of 5 minutes (Fig. 1). The translocation of

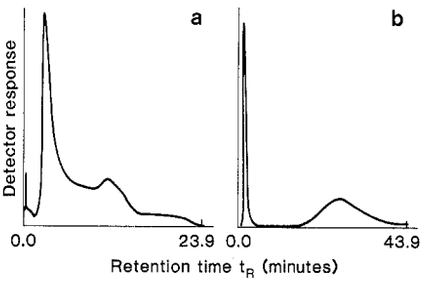

Fig. 1. The decline of twitch response of frog sartorius muscle (in vitro) when stimulated at 1 Hz for 5 minutes. Stimulation was by means of longitudinal current, pulse amplitude was 25 V, and pulse duration was 5 msec.

calcium from the terminal cisternae to the transverse tubular element is more than sufficient to account for the fatigue of muscles during repetitive stimulation at 1 Hz.

The amount of calcium released from the terminal cisternae during a twitch is approximately 91 nmole/g (*8*). The translocation of 0.8 nmole/g of calcium during the twitch to the transverse tubular element represents 0.9 percent of the calcium released from the terminal cisternae. The major portion of the released calcium must still be sequestered by the sarcoplasmic reticulum. The presence of a Ca^{2+}-dependent adenosine triphosphatase in the transverse tubular element (*9*) may provide the means for translocation of the calcium released from the terminal cisternae to the transverse tubular element. Reuptake of calcium from the transverse tubular element to the terminal cisternae could occur during the negative afterpotential, which is prolonged by increasing extracellular calcium from 1.8 to 8.0 m*M*.

The transverse tubular element serves not only as a conduit for the action potential in excitation-contraction coupling but also as a means for feedback through calcium accumulation from the terminal cisternae during the coupling process to decrease the effectiveness of the action potential in excitation-contraction coupling.

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Sedimentation Field Flow Fractionation of Liposomes

Abstract. *Quantitative analyses of the particle size distributions of liposomes were performed in 30 to 60 minutes by exponential-field sedimentation field flow fractionation. This gentle new separation method exhibits great potential for the high-resolution fractionation and the size or molecular weight analysis of a wide variety of biological macromolecules and colloidal suspensions.*

The characterization of biological polymers and colloids in the 10- to 1000-nm size range has been hampered by a lack of suitable separation techniques. The problem is particularly important in liposome research, because the increasing use of liposomes in drug delivery applications necessitates a rigorous characterization of the particle size distribution (*1*). Recently, sedimentation field flow fractionation (SFFF), a high-resolution separation technique originated by Giddings and others (*2*), has been introduced to fractionate and analyze colloids and soluble macromolecules within this size interval (*3*). We have applied SFFF to the size analysis of phospholipid vesicle dispersions, both because of inherent interest in this subject and because liposomes are ideal models for demonstrating the general applicability of SFFF to the separation of biological colloids and soluble biomacromolecules. Liposomes represent a model system since they can be prepared with size distributions in the 20- to 1000-nm range. Moreover, the ability of SFFF to handle labile materials can be tested since liposomes are sensitive to shear forces. Furthermore, the currently used electron microscopic analysis method is very time-consuming, which discourages routine particle size analysis by this approach (*4*).

The SFFF equipment and techniques used here have been described in (*3, 5*). Separations are carried out in a channel

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