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Cytochemical Study on the Intracellular Calcium Translocation during the Contraction-Relaxation Cycle in Scorpionfish Swimbladder Muscles

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Abstract: To establish the intracellular Ca translocation during the contraction-relaxation cycle in the swimbladder muscle (SBM) of a scorpionfish *(Sebastiscus marmoratus)*, a cytochemical study using a pyroantimonate (PA) method was performed. In the resting state, PA precipitates cytochemically indicating the presence of Ca were found only in the terminal cisternae (TC) of the sarcoplasmic reticulum (SR). On the other hand, during caffeine-induced contractures, the number of PA precipitates remarkably decreased in the TC, and they appeared newly in the myoplasm, distributed diffusely around myofibrils. During the recovery period, after the onset of relaxation from the caffeine-induced contracture, PA precipitates disappeared in the myoplasm, and were newly found in fenestrated collars (FC) and longitudinal tubules (LT) of the SR. Later, in the fully relaxed fibers after tetanus, PA precipitates were again found mainly at the TC. These results supported our previous view that Ca^{2+} released from the TC during contraction is first taken up mainly by the FC and the LT during the relaxation, and then gradually returns to the TC by passing through the SR lumen.
Keywords: intracellular Ca translocation, contraction-relaxation cycle, sarcoplasmic reticulum, scorpionfish swimbladder muscle, pyroantimonate method

Introduction

It is well known that the contraction-relaxation cycle of muscles is regulated by the change in the myoplasmic Ca concentration[1)]. In vertebrate skeletal muscles, the Ca concentration change is caused by its release and uptake by the sarcoplasmic reticulum (SR)[1)]. However, the process of intracellular Ca translocation during the contraction-relaxation cycle has not been investigated in detail.

Using ^{45}Ca autoradiography, Winegrad[2)] first demonstrated the change of intracellular Ca distribution during the contraction-relaxation cycle in frog skeletal muscles, and proposed that Ca ions are released at the terminal cisternae (TC) of the SR during the contraction, taken up at the longitudinal tubules (LT) of the SR during relaxation, and transported slowly to the TC during the recovery period. However, based on electron probe X-ray microanalysis of cryosections in frog muscle fibers frozen at rest, during and after tetanus, Somlyo and colleagues[3)] claimed that, during relaxation, released Ca ions are again taken up directly into the TC, but not into the LT. To clarify the above discrepancy, the authors examined the process of intracellular Ca translocation during the contraction-relaxation cycle by electron probe X-ray microanalysis of cryosections of scorpionfish swimbladder muscles (SBM), and revealed that the Ca ions released from the TC are taken up at the LT and fenestrated collars (FC) of the SR, and transported to the TC by passing through the SR lumen during the recovery period from muscle contraction[4)]. To further confirm and support the previous studies, in the present study, the authors examined

intracellular Ca translocation during the contraction-relaxation cycle of SBM fibers by using the potassium pyroantimonate (PA) method, an excellent cytochemical method to evaluate intracellular Ca translocation[5, 6].

Materials and Methods

The procedure to prepare the SBM of adult scorpionfishes, *Sebastiscus marmoratus*, was previously described in detail[7]. Briefly, the muscle bundle of 3 - 5 fibers with tendons was dissected from the posterior part of the SBM in Ringer's solution. Muscle bundles were mounted horizontally in an acrylic chamber filled with Ringer's solution; one end of the muscle bundle was clamped, and the other was connected to a strain gauge to monitor the isometric force before and during fixation. For conventional electron microscopy, the muscle bundles were fixed with a 2.5% glutaraldehyde solution and postfixed with a 2% osmium tetroxide (OsO_4) solution. For cytochemical electron microscopy, they were fixed with a 1% osmium tetroxide solution containing 2% potassium pyroantimonate (PAOs solution; pH adjusted to 7.2 by 0.01 N CH_3COOH/0.1 N KOH) at the resting state, during the caffeine contracture induced by application of Ringer's solution containing 2 mM caffeine, during relaxation, and at the fully relaxed state after the contracture. Several fiber bundles were fixed with PAOs solution just after tetanus caused by electrical stimulation using a supramaximal AC current (100 Hz).

Small pieces were cut from the middle region of SBM fibers fixed by conventional or cytochemical methods. They were then dehydrated with a graded series of ethanol, substituted with propylene oxide, and embedded in Quetol 812 epoxy resin. Ultrathin sections were cut longitudinally on ultramicrotomes (Porter- Blum MT-2 or Reichert Ultracut-N), stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (JEM2000 or JEM1230: JEOL). For elemental identification of PA precipitates, unstained sections were analyzed with an energy dispersive X-ray microanalyzer (Tracor Northern TN5450 or JEOL EX-14033JTP). X-ray emission was collected over a detection time of 200 sec.

Results and Discussion

Ultrathin sections cut longitudinally from the middle region of SBM fibers fixed with glutaraldehyde and OsO_4 were observed by electron microscope. A single SBM fiber was previously reported to contain two types of triadic contacts, the AI-type triad in the fiber middle and the Z-type triad in both fiber ends[7, 8]. Therefore, in the present study, the fibers treated with conventional fixatives exhibited only AI-type triads (Fig. 1). Triadic contacts composed of TC and a transverse tubule (T tubule) were located around the level of the boundary between the A- and I-bands of sarcomeres. Between triadic contacts, FC of the SR were observed around the level of the M-band and Z-band. Furthermore, the LT of the SR were clearly observed around the level of the overlap zone of A-bands, although it was difficult to confirm the LT around the I-band due to the short length between two TC in that SR unit.

Structural features of T-SR systems constructed with T tubules and the SR were naturally held in the SBM fibers fixed cytochemically with PAOs solution, although membranes and myofilaments were unclear because glutaraldehyde as a protein fixative was not used and conventional electron staining was not performed. In the fibers fixed at the resting state, electron-opaque PA precipitates were found in the SR, especially at the TC (arrows in Fig. 2A), and at the N-line of the sarcomeres (Fig. 2A). Similar localization of PA precipitates at the N-line was reported previously in the resting fibers of frog skeletal muscle[9-11]. In contrast to the resting state, in the SBM fibers fixed cytochemically during the caffeine-infduced contracture, the number of PA precipitates conspicuously decreased in the TC, but were diffusely distributed in the A-band region (Fig. 2B). These results suggest that Ca ions stored in the TC are released into the myoplasm to activate the contractile elements of sarcomeres during mechanical activity of SBM fibers. On the other hand, at the N-line of sarcomeres, there was no change in the distribution of PA precipitates, suggesting no correlation with the contraction-relaxation cycles. In the SBM fibers fixed during relaxation after the caffeine-induced contracture, PA precipitates were found not only in the TC, but also in the FC (arrow in Fig. 2C) and LT (Fig. 2C), reflecting Ca re-uptake into the SR. In the SBM fibers fixed at the fully relaxed state after

Fig. 1. Conventional electron micrograph of a longitudinal section obtained from the middle region of a SBM fiber, showing sarcomeres and T-SR system including AI-type triads. Arrows indicate T-tubules. Scale bar, 1 μm.

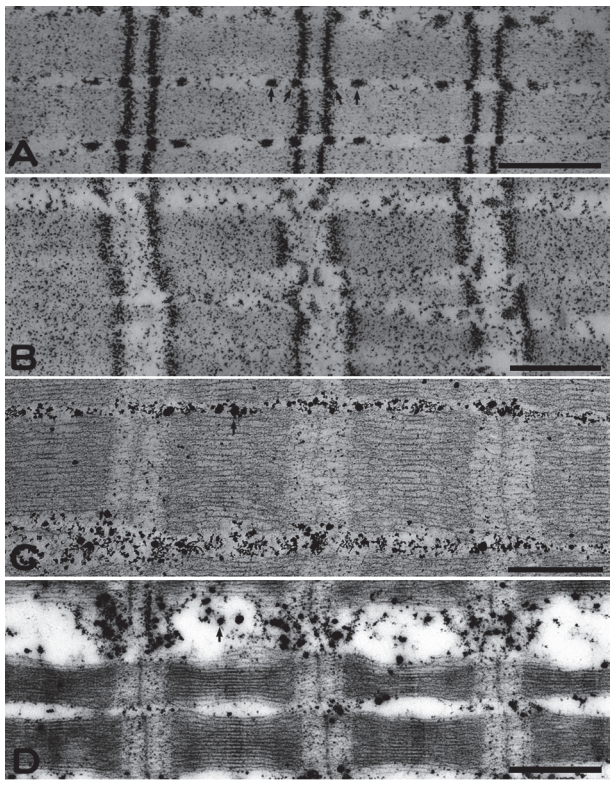

Fig. 2. Intracellular Ca translocation during the contraction-relaxation cycle of scorpionfish SBM fibers, demonstrated by the cytochemical PA method. A. Resting fibers, showing the localization of PA precipitates mainly in the TC of the SR (arrows). B. Fibers fixed during caffeine-induced contracture, showing that PA precipitates disappeared from the TC, and appeared around the sarcomeres. C. Fibers fixed during relaxation from caffeine-induced contracture, showing that PA precipitates disappeared around the sarcomeres, and appeared in the FC (arrow), LT, and TC of the SR. D. Fibers fixed at the fully relaxed state after the contraction induced by electrical stimulation, showing the localization of PA precipitates mainly in the TC. Scale bars, 1 μm.

isometric tetanus induced by electrical stimulation, PA precipitates were found exclusively around the TC, but not around the FC or LT (Fig. 2D). This

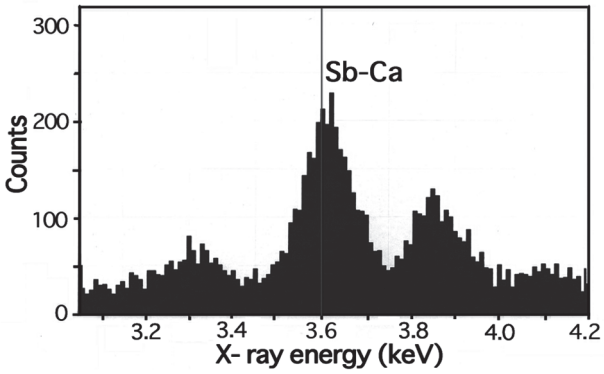

Fig. 3. A typical example of X-ray spectra from PA precipitates found in the SR of resting SBM fibers. The vertical gray line indicates the position of Sb-Lα emission at 3,600 eV. Note the distinct peak at 3,620 eV (Sb-Ca combination peak). Peaks of Sb-Lβ1 (3,840 eV) and SB-Lβ2 (4,100 eV) are also shown. The ordinate indicates the number of X-ray events, and the abscissa shows the X-ray energy in keV (range 3.05 ~ 4.20 keV).

suggest that, during the course of relaxation, Ca ions pumped up to the regions of the FC and LT are transferred to the TC through the SR lumen. During and after relaxation, no PA precipitates were distributed around the N-line (Fig. 2C and D).

X-ray microanalysis was performed to demonstrate that PA precipitates are composed of PA and Ca, and closely reflect the Ca distribution. The X-ray spectra obtained from PA precipitates in the SBM fibers fixed at different states of the contraction-relaxation cycle exhibited a distinct spectral peak at 3,620 eV (Fig. 3), a combination peak of antimonite and calcium (Sb-Ca)[5-7]. Based on the X-ray spectra, relative concentration ratios of elements were calculated (Table 1), confirming the presence of Ca in PA precipitates.

It is difficult to explain the PA precipitate distribution around the N-line. Although PA precipitates were reported to disappear during contraction of frog skeletal muscles in previous studies[9, 10], such a distribution change of PA precipitates was not observed in the present study. Furthermore, the localization of PA precipitates around the N-line was not noted during muscle relaxation. It is therefore unlikely that PA precipitates observed around the N-line are correlated with intracellular Ca translocation during the contraction-relaxation cycle in SBM muscles.

In conclusion, the present study supports the previous view[4] that Ca ions released into the myoplasm from the TC during contraction are taken up mainly by the FC and the LT, and then transported to the TC during and after relaxation, passing through the SR lumen.

Table 1. Elemental concentration ratios of pyoroantimonate precipitates found in the fibers at different physiological states during the contraction-relaxation cycle of scorpionfish swimbladder muscles

Physiological state	Structure**	Elemental concentration ratio*				
		Sb-Lα	Ca-Kα	K-Kα	Mg-Kα	Na-Kα
Resting	SR-TC	1.00	0.27 ± 0.04	0.34 ± 0.08	0.05 ± 0.03	0.08 ± 0.05
	A-band	1.00	0.25 ± 0.10	0.33 ± 0.04	0.07 ± 0.03	0.57 ± 0.27
Caffeine contracture	SR-TC	1.00	0.18 ± 0.11	0.42 ± 0.08	0.03 ± 0.01	0.06 ± 0.05
	A-band	1.00	0.11 ± 0.07	0.45 ± 0.10	0.10 ± 0.09	0.13 ± 0.12
Post caffeine contracture	SR-TC	1.00	0.39 ± 0.10	0.20 ± 0.06	0.06 ± 0.02	0.09 ± 0.02
	SR-FC	1.00	0.24 ± 0.09	0.25 ± 0.05	0.07 ± 0.06	0.06 ± 0.03
Post tetanus	SR-FC	1.00	0.18 ± 0.13	0.27 ± 0.10	0.01 ± 0.02	0.21 ± 0.04

* mean ± S.D., n=10.

**TC-SR: terminal cisternae of the sarcoplasmic reticulum (SR), SR-FC: fenestrated collars of the SR.

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