Ultrastructural Studies of Calcium Location during the "Catch" Contraction of Clam Smooth Adductor Muscle Cells

Authors: Tanaka, Tomonori, Ishida, Hideki, Ohtsu, Kohzoh, and Matsuno, Akira

Source: Zoological Science, 15(6) : 855-859

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.15.855

ZOOLOGICAL SCIENCE **15**: 855–859 (1998)

Ultrastructural Studies of Calcium Location during the "Catch" Contraction of Clam Smooth Adductor Muscle Cells

Tomonori Tanaka, Hideki Ishida, Kohzoh Ohtsu and Akira Matsuno*

Department of Biological Science, Faculty of Life and Environmental Science, Shimane University, Matsue 690, Japan

ABSTRACT—Contraction of molluscan adductors has been classified into three states; 1) resting state, 2) contracted state, 3) prolonged "catch" state. Among these, the "catch" state is considered a peculiar state, which requires little expenditure of energy, but in which contraction can be maintained for long periods. It is not yet known whether "catch" muscle contraction is regulated by Ca, or where Ca translocates during resting state through "catch" state, if the muscle contraction is regulated by Ca. We attempted to observe Ca translocation in muscle cells during contraction by the K-pyroantimonate method. We fixed "catch" muscle cells for electron microscopy with fixative including K-pyroantimonate, and observed where electron-dense precipitates, in which Ca is concentrated, were located in the muscle cells in the three states of contraction. At the resting state, precipitate was located at cell peripheries, in positions such as at the inner surface of cell membranes and in sarcoplasmic reticular systems (SRs). In the contracted state, they were located within the cytoplasm. At the "catch" state, they were found in both the cytoplasm and at peripheries, although the number of precipitates in peripheral areas was small. Thus, we show that calcium translocates in the cells during resting-contraction-catch cycles of "catch" muscle contraction.

INTRODUCTION

Molluscan adductors and retractors can maintain tension for prolonged periods with little energy expenditure. These sustained contractions are called the "catch" contraction. In particular, retractor muscles and opaque portions in adductors of bivalves, which are composed of smooth muscle cells, show "catch" contraction (Lowy and Hanson, 1962; Lowy *et al*., 1964). The "catch" contraction is characterized by three states of contraction; 1) resting state, 2) contracted state similar to that in general smooth muscle, 3) prolonged "catch" state.

Many structural and physiological studies have used molluscan "catch" muscles to study this particular contraction (Pfitzer and Ruegg, 1982; Ishii *et al*., 1989a; Matsuno and Kuga, 1989; Matsuno *et al*., 1993). Biochemical studies of "catch" muscles have focused mainly on paramyosin, because it is a major and characteristic protein in "catch" muscles (Szent-Gyorgyi *et al*., 1971; Elliott and Bennett, 1984; Castellani *et al*., 1983; Watabe *et al*., 1990; Matsuno *et al*., 1996). However, it is not yet clear in detail how "catch" muscle maintain prolonged contraction, and how the "catch" contraction is released.

Contraction of smooth muscle cell is regulated by calcium, as it is in cross striated muscle cells. Calcium in cross striated muscle cells is stored in sarcoplasmic reticula (SR) when the cells are at resting state. When the cells are excited, calcium is dispersed from the SRs to the cytoplasm, and the cells come into contracted state. Similar calcium regulation has been recognized in contraction of smooth muscle cells (Twarog and Muneoka, 1972; Debbas *et al*., 1975; McGuffee *et al*., 1976; Suzuki, 1982). Several reports observed ultrastructural translocation of calcium during relaxation and contraction of smooth muscle cells, using conventional pyroantimonate methods (Suzuki and Sugi, 1978; Sugi *et al*., 1982; Suzuki, 1982; Aguas, 1983). As the method is capable of detecting calcium in living cells, several studies have reported Ca-translocation in "catch" muscle (Ishii *et al*., 1989a, b). A few reports have also examined Ca-translocation during the "catch" contraction (Atsumi *et al*., 1974; Atsumi and Sugi, 1976). These reports suggested that calcium in the cytoplasm decreased during the "catch" contraction, and discussed the effects of decreasing calcium in cytoplasm. Quantitative Ca-translocation during contraction-catch-resting cycle of the "catch" contractions, however, remains obscure.

It is difficult to estimate calcium effects on "catch" state, because contraction of smooth muscle cells is generally attended by calcium influx through cell membranes from cell exteriors. Sugi and Daimon (1977) reported calcium translocation in smooth muscle using the pyroantimonate method. They fixed muscle cells with osmium fixative containing 2.5% potassium pyroantimonate, and could detect electron-dense

* Corresponding author: Tel. +81-852-32-6430; FAX. +81-852-32-6449.

precipitate of intracellular cations. It may thus be possible to determine if calcium translocates during catch contraction using this method. We attempted to identify calcium translocation in "catch" muscle cells during a contraction-catch-resting muscle contraction cycle.

MATERIALS AND METHODS

The materials used for this study were posterior adductors of giant clams (*Meretrix lusoria*), collected near the Oki Marine Biological Station of Shimane University. Posterior adductors were removed with a diamond cutter, together with shell at both ends. A cross-striated portion was then carefully removed from the adductor preparation, and the remaining shell attached at both ends was cut into small squares. These preparations were used for physiological experiments and electron microscopy.

Physiological experiment

One end of the prepared adductor muscle was connected to a transducer arm, and the other end hooked to the bottom of a small chamber in which experimental solutions could be exchanged. The chamber was filled with artificial sea water to wash the muscle. The adductor muscle came into resting state during the 30 min washing period. The muscle was then washed twice with Ca-free artificial sea water, and submerged into in Ca-free artificial sea water containing 10^{-2} M acetylcholine. The muscle began to contract in the acetylcholine solution within 30 sec, and reached full contraction 3 min after contraction began (contracted state). After full contraction, the muscle was washed with Ca-free artificial sea water, and left for 5-10 min (catch state). The muscle was then washed again with Ca-free artificial sea water and submerged in 10^{-2} M 5-hydroxytryptamine (5HT). The muscle began to relax within 2 min, and reached full resting state after 5 min (resting state). Thus, we obtained preparations at three states of contraction.

Electron microscopy

Adductors at the three states described above were immediately fixed for electron microscopic observation. Adductors at each state were fixed with 1% osmium-tetroxide and 2% potassium-pyroantimonate in Ca-free artificial sea water, by exchanging the solution in the chamber after the physiological experiment. After 90 min fixation, the adductors were cut into small pieces, and fixed again with osmium-tetroxide/potassium-pyroantimonate solution for 30 min. Fixative without potassium-pyroantimonate was also applied to control specimens at each state. The specimens were next dehydrated by a series of ethanol solutions (70, 80, 90, 100%), and embedded in Epoxy resin, followed by emerging agent QY-1. Embedded and hardened specimens were cut into thin sections and examined in an electron microscope. Some sections were observed after staining with aqueous uranyl acetate, followed by a lead nitrate solution to clarify the cell components.

RESULTS

Bivalve adductors are generally not suitable for physiological experiments of "catch" mechanism, because of difficulties in preparation. However, our preparation technique was successful, and we could prepare adductors which showed typical "catch" contraction similar to that of anterior byssus retractor muscle of mussels (ABRM). Thus, our preparations show the three states of muscle contraction; contracted, "catch" and resting states (Fig. 1).

The sensitivity of Potassium-pyroantimonate in detecting calcium ion between 0.1 mM to 0.8 mM of concentration was first tested (Fig. 2). The results show it to be sensitive at a range of calcium concentration, producing insoluble and opaque calcium precipitates between 0.1 mM to 0.8 mM concentration in 540 nm of wavelength light. As it is well known that muscle cells come into contracted state at 10 mM calcium concentration, calcium in SRs at resting state and in cytoplasm at contracted state would be detected by our experiment.

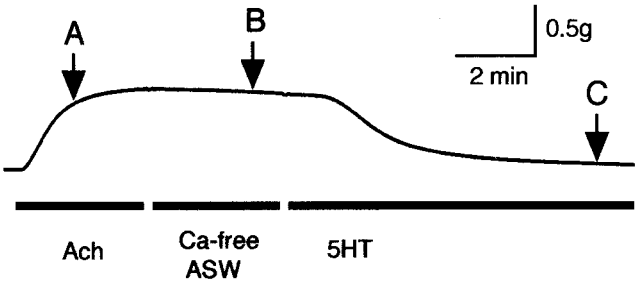

Fig. 1. Time-dependent tension of a "catch" muscle during the contraction-catch-resting cycle. Points of fixation: A, contracted state; B, catch; C, resting.

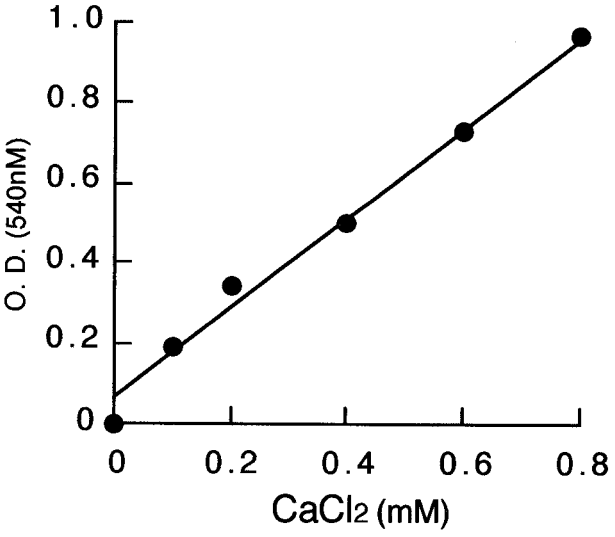

Fig. 2. Optical density of Ca-precipitate by pyroantimonate. This shows pyroantimonate can detect calcium down to 0.1 mM. Optical density (OD) was measured through a 540 nm wavelength filter.

Ultrastructure

General view of muscle cell

Clam adductor "Catch" muscle cells at resting state are oval in cross section, and are loosely connected to neighboring cells (Fig. 3). Cells bearing many myofilaments ran parallel to each other (Fig. 3). Enlarged views of cells revealed more details: small vesicles were observed at the periphery of the cells, and these vesicles were usually attached with cell membranes (Fig. 4). However, no ultrastructural differences among the cells at the three state were observed with respect

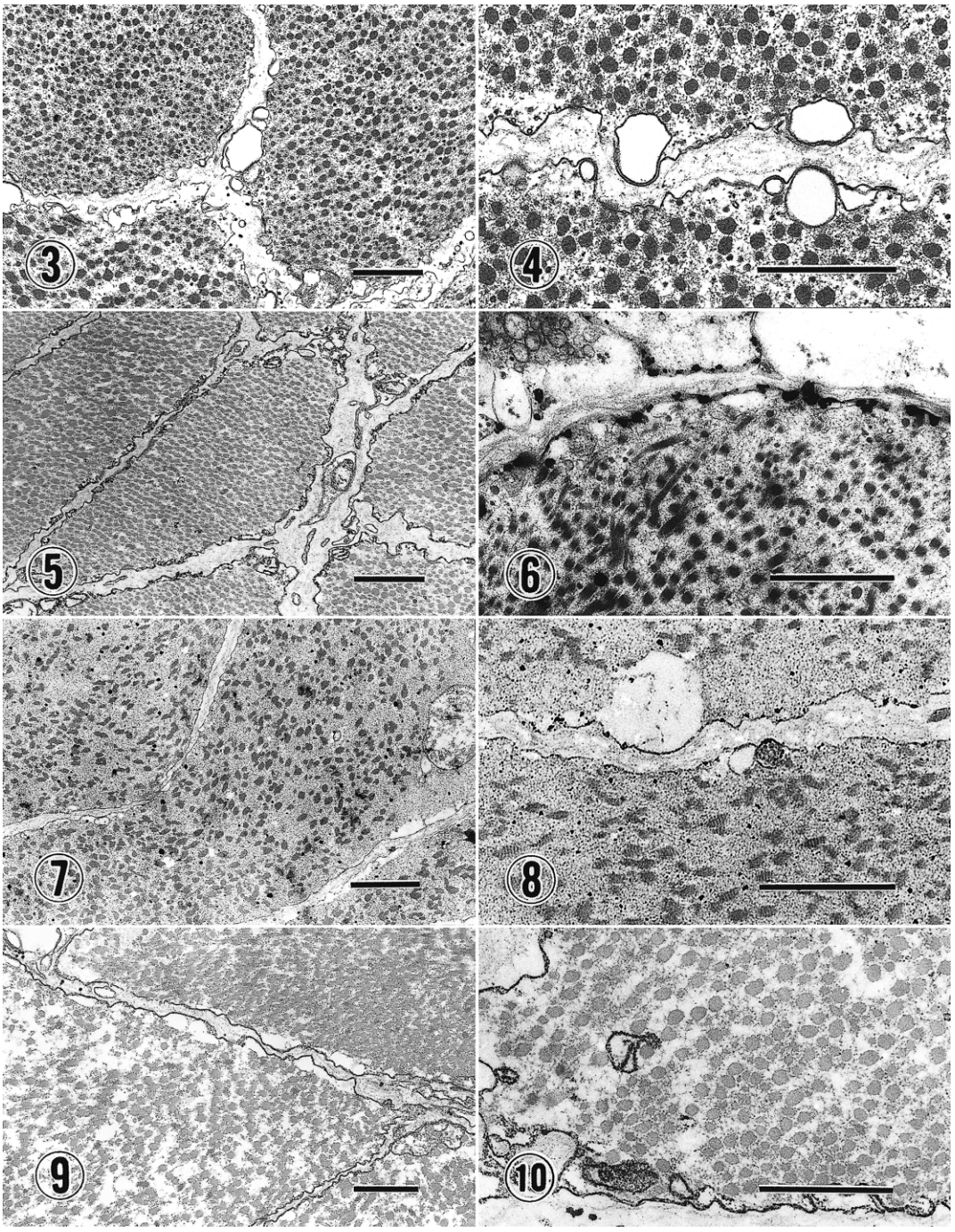

Fig. 3. Electron micrograph of cells in a control cross section at resting state, using fixative without K-pyroantimonate. Cells in the muscle are various diameters, and are connected to each other with thin connective tissue. × 6,100. Scale bar, 2 μm.

Fig. 4. Enlarged views of muscle cells prepared as in Fig. 3. Myofilaments are evenly distributed and SRs are situated at cell peripheries. × 10,200. Scale bar, 2 μm.

Fig. 5. Electron micrograph of muscle cells in a cross section at resting state. Cells were fixed with osmium and pyroantimonate. Precipitates occur at cell surfaces. × 9,600. Scale bar, 2 μm.

Fig. 6. Enlarged views of resting state cells prepared as in Fig.5. Precipitates are located at SRs and cell membranes. × 12,300. Scale bar, 2 μm.

Fig. 7. Electron micrograph of muscle cells at contracted state. Precipitates are translocated throughout the cytoplasm, and precipitates at cell membranes have disappeared. × 11,500. Scale bar, 2 μm.

Fig. 8. Enlarged view of muscle cells at contracted state. No precipitates occur at SRs and cell membranes. × 15,600. Scale bar, 1 μm.

Fig. 9. Electron micrograph of muscle cells at “catch” state. Precipitates in the cytoplasm are decreased, but have not disappeared. × 8,700. Scale bar, 2 μm.

Fig. 10. Enlarged views of cells at “catch” state. Precipitates at SRs and cell membranes are more abundant than in contracted state, but are fewer than in resting state. × 30,400. Scale bar, 0.5 μm.

to diameter. The differences in diameter do not represent the degree of contraction in each state, but simply shows that the cells are large or small.

Resting state: The pyroantimonate precipitates at resting state of "catch" muscle cells were mainly located at cell peripheries (Fig. 5). Enlarged views showed the precipitates occurred mainly at the inner surface of the cell membrane and in the inner spaces of vesicles (SRs) associated with the cell membrane (Fig. 6). They were occasionally observed on the membranes of mitochondria (Fig. 5). The precipitates varied from about 20 nm to 150 nm in diameter. Larger precipitates were often concentrated in SRs or near the cell membranes (Fig. 6).

Contracted state: Pyroantimonate precipitates are dispersed evenly throughout the cytoplasm at contracted state, and are smaller than those in the resting state (Fig. 7). Enlarged views also showed that precipitates at SRs had disappeared, and those at cell membranes decreased in abundance (Fig. 8).

Catch state: Pyroantimonate precipitates were recognized at cell membranes, SRs and adjacent to mitochondria (Fig. 9), but those in cytoplasm had disappeared (Fig. 9). Beside these contrasts in precipitate location between the catch and contracted state, a clear difference can also be seen in precipitate size. Precipitates in the contracted state are larger than those of the "catch" state (Figs. 8 and 10).

Counting of Ca-precipitates

To confirm quantitative differences in translocation of precipitates in cells of each state, precipitates (50-130 nm in diameter) were counted from micrographs. We classified the locations of precipitates into three regions: cytoplasm, SRs and cell membranes. The results show pyroantimonate precipitates are located in characteristic areas in the cells at each state (Fig. 11). At resting state, approximately 80% of the precipitates were located at cell membranes, with the rest evenly divided between SRs and cytoplasm. In contrast, at contracted state 93% were dispersed in the cytoplasm. At catch state, the percentage in the cytoplasm falls to 47%, with 42% at cell membranes, and 11% at SRs.

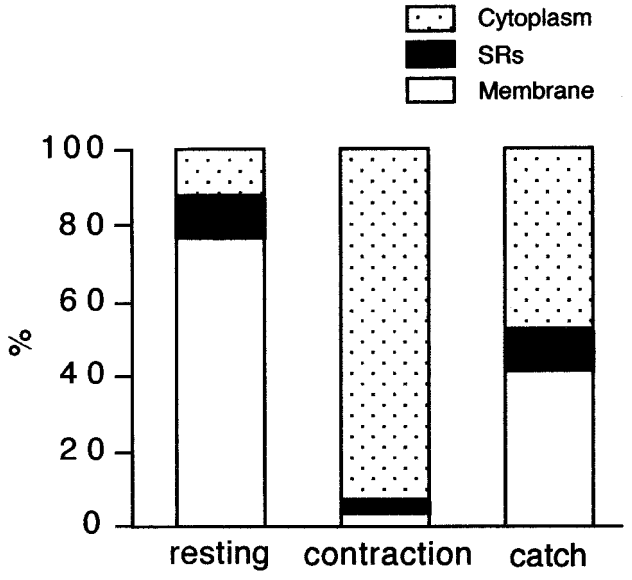

Fig. 11. Precipitate distribution in the cell at each state of contraction.

DISCUSSION

Hauck and Achazi (1987) considered that a major cause of "catch" state was that thick filaments in the cell were attached to each other by fused paramyosin molecules during "catch" contraction. However, it is more probable that the contraction is based on cross-bridges between thick myosin and thin actin filaments (Bennett and Elliott, 1989), and the cycle of cross-bridges is delayed by phosphorylation of myosin molecules (Achazi, 1979; Cohen and Castellani, 1988).

On the other hand, it is important to remember that muscle contraction is regulated by calcium: that is, muscle contracts with activation of cross-bridges triggered by calcium, and come into resting state through the reverse process. At the resting state, calcium is restored to the SRs. Ishii *et al.* (1989a) measured free calcium concentration in cytoplasm at resting and contracted states of "catch" muscle using fluorescent calcium indicator fura-2. They concluded that high calcium concentration is not required for maintenance of "catch" tension, and that free calcium concentration is close to resting values during the "catch" state (Ishii *et al.*, 1989b).

The condition (free or bound) in which calcium occurs in cytoplasm during "catch" state is not yet clear. It is very difficult to measure calcium concentrations in muscle cells, because living cells change their physiological conditions dynamically. Accordingly, we measured calcium translocation using the K-pyroantimonate method. The method used was modified from that reported by Sugi and Daimon (1977). As shown in Fig. 1, 2% K-pyroantimonate can detect calcium as low concentration as 10^{-5} M. These results agree well with a previous report on K-pyroantimonate sensitivity (Wick and Hepler, 1982), and the method we used gives a reliable indication of where Ca is located in the cells. We did not analyze the electron-dense precipitates to verify that they do contain Ca, as this has already been established by previous x-ray microanalysis of similar precipitates (Sugi and Daimon, 1977; Sugi *et al.*, 1982).

Studies similar to ours have been reported from many kinds of muscle cells. This work suggests that Ca-pyroantimonate precipitates are located around the cytoplasm when the cells are at contracted state, and in the SRs when at resting state (Sugi and Daimon, 1977; Suzuki and Sugi, 1978; Sugi *et al.*, 1982; Suzuki, 1982). Some studies also report numbers of "catch" muscle cells using such methods (Atsumi *et al.*, 1974; Atsumi and Sugi, 1976), but few identify quantitative translocation of precipitates during "catch" contraction.

Our examination of the location of precipitates at resting and contracted states show similar translocation to that reported previously. However, our results show about half the

precipitates remain in the cytoplasm during “catch” contraction. Atsumi and Sugi (1976) observed decrease of myoplasmic free calcium during the “catch” state, and concluded that the “catch” contraction may be maintained under much lower myoplasmic free Ca ion concentration than active contraction. Our observations that less than 50% of precipitates remain in the cytoplasm during “catch” contraction differ from physiological reports using fluorescent fura-2, which apparently showed that free calcium in cytoplasm was close to resting values during the “catch” state (Ishii *et al*., 1989a). This difference arises because Ishii *et al*. (1989a) measured only free calcium in the cytoplasm, and did not detect calcium bound with Ca-binding proteins such as myosin and calmoduline,whereas our measurements include both free and bound calcium. We suspect that bound calcium is more important than free calcium in maintaining the “catch” contraction, but it is not yet clear how and why the calcium functions during the “catch” contraction.

ACKNOWLEDGMENT

This study was supported by a Grant-in-Aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

Achazi RK (1979) Phosphorylation of molluscan paramyosin. Pflugers Arch 379: 197–201

Aguas AP, Nickerson PA (1983) Increased Ca^{2+} in the sarcoplasm of aortic smooth muscle cells from rats made hypertensive with DOC. J Submicrosc Cytol 15: 425–431

Atsumi S, Sugi H, Aikawa M (1974) The role of calcium ions in the regulation of active and catch contractions in the anterior byssal retractor muscle of *Mytilus edulis*. Proc Japan Acad 50: 775–778

Atsumi S, Sugi H (1976) Localization of calcium-accumulating structures in the anterior byssal retractor muscle of *Mytilus edulis* and their role in the regulation of active and catch contractions. J Physiol 257: 549–560

Bennett PM, Elliott A (1989) The ‘catch’ mechanism in molluscan muscle: an electron microscopy study of freeze-substituted anterior byssus retractor muscle of *Mytilus edulis*. J Mus Res Cell Motil 10: 297–311

Castellani L, Vibert P, Cohen C (1983) Structure of myosin/paramyosin filaments from a molluscan smooth muscle. J Mol Boil 167: 853–872

Cohen C, Castellani L (1988) New perspectives on catch. Comp Biochem Physiol 91C: 31–33

Debbas G, Hoffman L, London EJ, Hurwitz L (1975) Electron microscopic localization of calcium in vascular smooth muscle. Anat Rec 182: 447–472

Elliott A, Bennett PM (1984) Molecular organization of paramyosin in the core of molluscan thick filaments. J Mol Biol 76: 477–493

Hauck R, Achazi RK (1987) The ultrastructure of a molluscan catch muscle during a contraction-catch-relaxation cycle. Eur J Cell Biol 45: 30–35

Hauck R, Achazi RK (1991) In situ phosphorylation of contractile proteins of a molluscan (*Mytilus edulis*) catch muscle in different functional states. Comp Biochem Physiol 100B: 237–242

Ishii N, Simpson AWM, Ashley CC (1989a) Free calcium at rest during “catch” in single smooth muscle cells. Science 243: 1367–1368

Ishii N, Simpson AWM, Ashley CC (1989b) Effects of 5-hydroxytryptamine (serotonin) and forskolin on intracellular free calcium in isolated and fura-2 loaded smooth-muscle cells from the anterior byssus retractor (catch) muscle of *Mytilus edulis*. Pflugers Arch 414: 162–170

Lowy J, Hanson J (1962) Ultrastructure of invertebrate smooth muscles. Physiol Rev 42: 34–47

Lowy J, Millman BM, Hanson J (1964) Structure and function in smooth tonic muscles of lamellibranch mollusks. Proc Roy Soc Lond B 160: 525–536

Matsuno A, Kuga H (1989) Ultrastructure of muscle cells in the adductor of the boring clam *Tridacna crocea*. J Morphology 200: 247–253

Matsuno A, Ishida H, Hori H (1993) Two kinds of thick filament in smooth muscle cells in the adductor of a clam, *Chlamys nobilis*. Tissue & Cell 25: 325–332

Matsuno A, Kannda M, Okuda M (1996) Ultrastructural studies on paramyosin core filaments from native thick filaments in catch muscles.Tissue & Cell 28: 501–505

McGuffee LJ, Bagby RM (1976) Ultrastructure, calcium accumulation, and contractile response in smooth muscle. Am J Physiol 230: 1217–1224

Pfitzer G, Ruegg JC (1982) Molluscan catch muscle: Regulation and mechanics in living and skinned anterior byssus retractor muscle of *Mytilus edulis*. J Comp Physiol 147: 137–142

Sugi H, Daimon T (1977) Translocation of intracellularly stored calcium during the contraction-relaxation cycle in guinea pig taenia coli. Nature 269: 436–438

Sugi H, Suzuki S, Daimon T (1982) Intracellular calcium translocation during contraction in vertebrate and invertebrate smooth muscles as studied by the pyroantimonate method. Can J Physiol Pharmacol 60: 576–587

Suzuki S (1982) Physiological and cytochemical studies on activator calcium in contraction by smooth muscle of a sea cucumber, *Isostichopus badionotus*. Cell Tissue Res 222: 11–24

Suzuki S, Sugi H (1978) Ultrastructural and physiological studies on the longitudinal body wall muscle of *Dolabella auricularia*. J Cell Biol 79: 467–478

Szent-Gyorgyi AG, Cohen C, Kendrick-Jones J (1971) Paramyosin and the filaments of molluscan “catch” muscles. J Mol Biol 56: 239–258

Twarog BM, Muneoka Y (1972) Calcium and the control of contraction and relaxation in a molluscan catch muscle. Cold Spring Harbor Symp Quant Biol 37: 489–504

Watabe S, Kantha SS, Hashimoto K, Kagawa H (1990) Phosphorylation and immunological cross-reactivity of paramyosin: A comparative study. Comp Biochem Physiol 96B: 81–88

Wick SM, Hepler PK (1982) Selective localization of intracellular Ca^{2+} with potassium antimonate. J Histochem Cytochem 30: 1190–1204

(Received June 29, 1998 / Accepted October 1, 1998)