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SHORT COMMUNICATION

NON-CATCH CONTRACTION IN PARAMYOSIN-CONTAINING MUSCLE IN AN ECHINOTHURIID SEA URCHIN *ASTHENOSOMA IJIMAI*

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It has long been known that certain molluscan smooth muscles become stretch resistant and can hold the shell closed for extended periods without fatigue and at low rates of oxygen consumption. This prolonged state of contraction is called catch, and the maintenance of catch force is at least 10 times more economical than the contraction that precedes catch. This mechanism has been extensively studied in the anterior byssus retractor muscle (ABRM) of *Mytilus edulis* (Rüegg, 1971, 1986).

Molluscan catch muscle is structurally characterized by the presence of very long filaments (over 30 μm), which are much thicker (over 40 nm) than those of skeletal muscle (15 nm). The extra thickness of catch muscle filaments is caused by the presence of paramyosin, which constitutes the core of the thick filaments (Hanson *et al.* 1957). Based on morphological evidence, it has been suggested that paramyosin is responsible for catch. However, Lowy *et al.* (1964) proposed a hypothesis in which contractile linkages form between thick and thin filaments

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during the contraction preceding catch and these are then locked in the attached state. Despite a long controversy concerning the mechanism of catch, it is still difficult to decide between these hypotheses.

In the present experiments, the radial muscle of an echinothuriid sea urchin, *Asthenosoma ijimai* (Tsuchiya and Amemiya, 1977), was studied mechanically, biochemically and histologically in an attempt to ascertain whether the presence of paramyosin was related to catch. As is shown below, this muscle does not show any catch phenomena, although the technique of monoclonal immunoblot assay clearly demonstrates the presence of paramyosin in the muscle.

The test of this sea urchin is flexible because the plates of which it is made are loosely connected. The animal is able to change its height appreciably by contracting or relaxing the radial muscle, whose distal ends are attached to the plates. All the bundles come together at their proximal ends, forming 10 sheets of a fan-like structure (Tsuchiya and Amemiya, 1977). A small bundle of the radial muscle (0.3–1.0 mm in diameter and 6–8 mm in length) was dissected out together with a piece of test plate at the distal end and connective tissue at the proximal end. One end of the muscle preparation was connected to a force transducer (Aksjeselskapet Mikro-Electronikk AE801) and the other end to a length step generator (General Scanning G100PD). The preparation was bathed in artificial sea water with a composition of (in $mmol\,l^{-1}$): NaCl, 485; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 50; pH adjusted to 8.2 with $NaHCO_3$. The muscle was stimulated in three ways: by application of acetylcholine chloride (ACh, $5\times10^{-5}\,mmol\,l^{-1}$), by repetitive electrical stimulation (4–10 Hz) or by direct current stimulation. During the resulting contraction the muscle was quickly released by 0.8–1.2 % of its resting length and any redevelopment of force after the release was monitored (Fig. 1). To determine resting length a slack resting muscle was slowly stretched with a micromanipulator. The resting force stayed at zero while the slack remained and started to increase as the muscle tightened with further stretch. The resting length was taken as the length at which the resting force could be recognized on the force record. The time of a length step was varied between 5 and 100 ms and no differences in force responses were seen except for the initial quick length change resulting from elasticity.

It is known that, in the molluscan catch muscle, force does not redevelop if it is released during catch (Rüegg, 1971). When the radial muscle was stimulated by ACh, force developed immediately and was maintained throughout the application of ACh (Fig. 1A). After washing, force decreased quickly. When the radial muscle was released during an ACh-induced contracture, force could be redeveloped both early and later during the contracture. In the ABRM, typical of catch muscle, repetitive electrical stimulation induces phasic contraction, whereas direct current stimulation induces catch contractions. In the radial muscle, a fused phasic contraction could be induced by an appropriate frequency of electrical stimulation (4–6 Hz); after cessation of stimulation, force decreased rapidly (Fig. 1B). Force redeveloped quickly after it had been released by applying shortening length steps during the period of repetitive stimulation. The radial

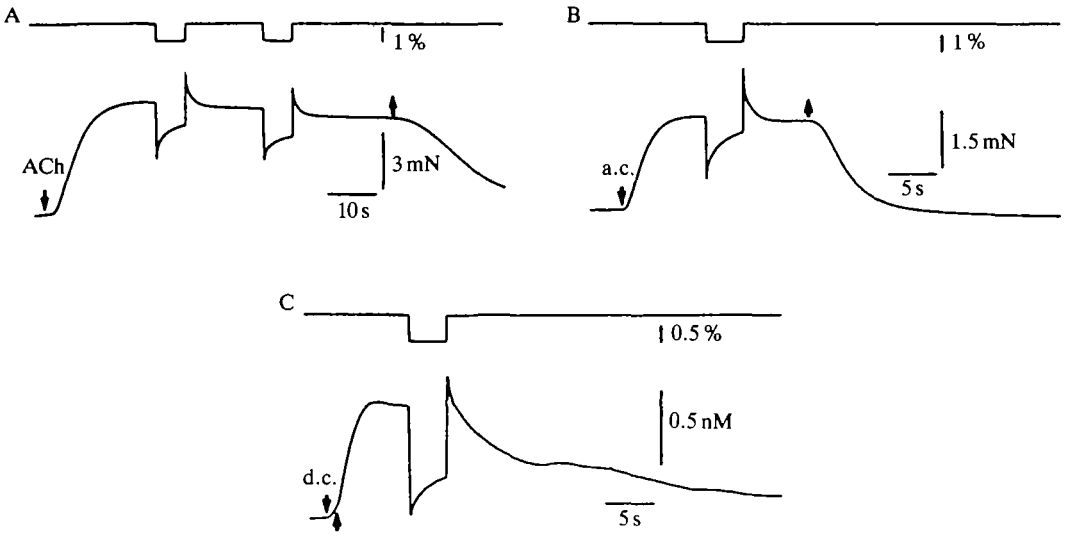

Fig. 1. Typical examples of force redevelopment in *Asthenosoma ijimai* radial muscle after a shortening length step during contraction. (A) Contraction induced by acetylcholine (ACh) (5×10^{-5} mol l^{-1}). (B) Contraction induced by a repetitive electrical stimulation at 4 Hz (a.c.). (C) Contraction induced by direct current stimulation for 1 s (d.c.). Note that, in all cases, force redeveloped clearly after the release. Arrows mark the beginning and end of stimulation.

muscle responded to brief (0.3–1.0 s) direct current stimulation with a contraction of 15–30 s, and when the muscle was released at the maximum force, the force redeveloped (Fig. 1C). The results of each of the quick-release experiments were confirmed in more than six preparations and the total number of animals used was 11. The mechanical responses of the radial muscle to drugs and to the change in the concentration of extracellular calcium ions have already been examined and no indications of catch have been found (Tsuchiya and Amemiya, 1977).

The radial muscle of *Asthenosoma ijimai* and, for comparison, the lantern muscles of *Asthenosoma ijimai* and *Pseudocentrotus depressus* were studied by immunoblot analysis to test for the existence of paramyosin. Muscle proteins were extracted with a sodium dodecyl sulphate (SDS) buffer solution containing 2% SDS, 2% mercaptoethanol, 40 mmol l^{-1} sodium phosphate and 1 mmol l^{-1} phenylmethylsulphonyl fluoride (PMSF), pH 7.0, and were stored in 50% glycerol containing 1 mmol l^{-1} PMSF, 50 mmol l^{-1} KCl, 2 mmol l^{-1} $MgCl_2$, 10 mmol l^{-1} sodium phosphate and 0.05% NaN_3, pH 7.0 at 20°C. Electrophoresis was carried out using a 10% acrylamide gel in a discontinuous Tris–glycine buffer system. The gel was stained with Coomassie Brilliant Blue. A monoclonal antibody specific for sea urchin paramyosin, SPM-2, was prepared using paramyosin purified from the lantern muscle of *Pseudocentrotus depressus* (Obinata *et al.* 1975) as an immunogen (Satoh and Obinata, 1988). Proteins were electrophoretically transferred from SDS–polyacrylamide gels to a nitrocellulose membrane. These were treated with

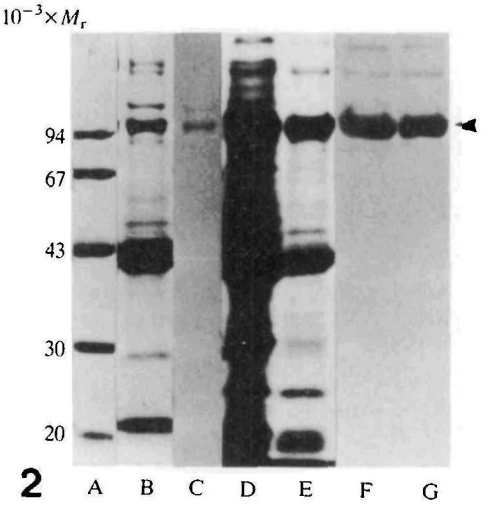

Fig. 2. SDS–acrylamide gel electrophoresis and immunoblot analysis of sea urchin paramyosin. (A) Protein markers; (B,C) the lantern muscle of *Pseudocentrotus depressus*; (D,F) the lantern muscle of *Asthenosoma ijimai*; (E,G) the radial muscle of *Asthenosoma ijimai*. The proteins in C, F and G were transferred to a nitrocellulose membrane and reacted with anti-paramyosin antibody (SPM-2). Ten times more of the sample material was applied in lanes D and F, because of the lower content of muscle proteins in the radial muscle. The paramyosin bands are marked with an arrowhead.

3% gelatin and then incubated for 1 h with the monoclonal antibody to paramyosin. Peroxidase-labelled goat anti-mouse IgG bound to the paper was detected as the diaminobenzidine reaction product with nickel and cobalt ions.

Fig. 2B,D,E shows a prominent band at approximately $94 \times 10^3 M_r$, which represents subunits of the sea urchin paramyosin of $120 \times 10^3 M_r$ (Obinata *et al.* 1975) and suggests that paramyosin is contained not only in the radial muscle of *Asthenosoma ijimai* but also in the lantern muscle of *Asthenosoma ijimai* and *Pseudocentrotus depressus*. These results were confirmed by immunoblot analysis, which shows the clear reactivity of the paramyosin-like protein from all three specimens with the antibody prepared against paramyosin (Fig. 2C,F,G).

In this study electron microscopy of transverse sections through the radial muscle shows smooth muscle cells containing many thick and thin filaments, with the thick filaments, 20–50 nm in diameter and 2–5 μm in length, surrounded by many thin filaments, 5–6 nm in diameter. Paramyosin has been found in the muscles of lower vertebrates such as molluscs, arthropods and insects (Levine *et al.* 1976). In molluscan smooth muscles, catch properties seem to be correlated with the size of the thick filaments and the abundance of paramyosin (Margulis *et al.* 1979; Levine *et al.* 1976). Levine *et al.* (1976) classified the paramyosin-containing muscles into three groups according to their paramyosin content, the length of their thick filaments and the force developed by them. In the radial muscle of *A. ijimai*, the length of the thick filaments is 2–5 μm, the diameter

20–50 nm and the active force produced by ACh is 0.3–0.5 kg cm^{-2}, suggesting that this muscle may belong to class II of their classification, although the molecular ratio of paramyosin to myosin was not measured in this muscle. In the ABRM, a class III muscle, the length of the thick filaments is about 25 μm and the diameter 65–70 μm. In reviews Rüegg (1971, 1986) has suggested two mechanisms to account for catch; one possibility is that the catch force is maintained by paramyosin–paramyosin interactions, the other is that catch is induced by actin/myosin cross-linkages that persist after the end of active contraction (Lowy *et al.* 1964; Tameyasu and Sugi, 1976). The present results using an anti-paramyosin monoclonal antibody clearly show that paramyosin is contained in the radial muscle of *A. ijimai*. The mechanical experiments measuring force recovery after quick release show no sign of catch in this muscle and it is postulated that the presence of paramyosin in this muscle is not related to catch.

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