Stretch-Induced Force Development in *Mytilus* Smooth Muscle during Submaximal Activation

Suechika SUZUKI, Kazunari NOGUCHI, and Haruo SUGI

Department of Physiology, School of Medicine, Teikyo University,
Itabashi-ku, Tokyo, 173 Japan

Summary When the anterior byssal retractor muscle of *Mytilus edulis* is stretched by 5–20% during submaximal activation with 100 mM K^+ or 10^{-5} M acetylcholine, it exhibited a delayed force development after the completion of stretch. The effect of ions and drugs on the stretch-induced activation suggests the stretch-induced release of Ca^{2+} from the inner surface of the plasma membrane.

Key words: stretch-induced activation, *Mytilus* smooth muscle.

It has long been known that, in various types of vertebrate and invertebrate smooth muscles, a rapid stretch applied to a resting muscle produces a delayed force development after completion of stretch. In rabbit cerebral smooth muscle, the stretch-induced force development is resistant to Ca antagonists and removal of $[Ca^{2+}]_o$, suggesting that the force development is associated with Ca^{2+} release from some intracellular structures [1]. Nakayama *et al.* [2] studied the intracellular translocation of Ca^{2+} in rabbit cerebral artery smooth muscle during the stretch-induced force development by the pyroantimonate method, which has been effective in studying the intracellular Ca^{2+} translocation in various types of smooth muscle [3–6], and demonstrated the stretch-induced Ca^{2+} release from the inner surface of the plasma membrane into the myoplasm to activate the contractile system.

On the other hand, Atsumi and Sugi [3] have shown by the pyroantimonate method that, in the anterior byssal retractor muscle (ABRM) of a common mussel *Mytilus edulis*, the regulation of active and catch contractions is associated with the intracellular translocation of Ca^{2+} between the inner surface of the plasma membrane and the myoplasm. The present work was undertaken to examine the possibility of the stretch-induced Ca^{2+} translocation from the inner surface of the plasma membrane into the myoplasm by recording the force response of *Mytilus* ABRM to stretch.

The experiments were performed with small ABRM fiber bundles (diameter, 0.3–0.5 mm) with a piece of shell attached to one end and the byssal organ left at

---

Received on August 13, 1992; Accepted on October 14, 1992

the other. The preparation was mounted horizontally at its slack length in an experimental chamber (3 ml) filled with artificial sea water (497 mM NaCl, 10 mM KCl, 20 mM CaCl₂, 52 mM MgCl₂, pH 7.2 by NaHCO₃); one end was clamped to a force transducer (Shinko, U-gage) while the other end was connected to a servo-motor (General Scanning, PD-100). The preparation was made to contract isometrically with high $[K^+]_o$ (100–400 mM, substituted for Na⁺) or acetylcholine (ACh, 10⁻⁵–10⁻³ M) [7], and then stretched rapidly (by 5–20% of the initial slack length in 5–10 ms) with the servo-motor. The fiber length changes were recorded with a light source-photodiode system. The force and length changes of the preparation were recorded on an ink-writing oscillograph. Since the ABRM fibers relax slowly after simple removal of high $[K^+]_o$ or ACh, the contracted preparation was made to relax with 10⁻⁶ M 5-hydroxytryptamine (5-HT) which causes rapid and complete relaxation of the ABRM [7]. In some experiments, the preparation was mounted in another chamber consisting of three compartments filled with isotonic KCl, paraffin oil, and the experimental solution respectively, to record changes in demarcation potential between the KCl-depolarized and the experimental solution segments of the fibers in response to stretch [7]. All experiments were made at room temperature (20–23°C).

When the ABRM fibers were stretched by 5–20% at the plateau of the maximum isometric force produced by the supramaximal concentration of high $[K^+]_o$ (400 mM) or ACh (10⁻³ M), the force rose to a peak at the completion of

Fig. 1. Force changes in response to stretch in the fully activated ABRM fibers. The fibers were activated with 400 mM K⁺ in A, and with 10⁻³ M ACh in B, and stretched by 10% after the maximum isometric force was attained. Top trace shows time course of stretch. In this and subsequent figures, the initial force development in response to high $[K^+]_o$ or ACh was recorded on a time base about 5 times slower than that during stretch. Records A and B were obtained from the same preparation.

Fig. 2. Force changes in response to stretch in the submaximally activated and the resting ABRM fibers. The fibers were activated with 100mM K^+ in A, and with 10^{-5} M ACh in B, and then stretched by 10%. Note distinct delayed force development after the completion of stretch. The resting fibers were stretched by 10% in C. Records A, B, and C were obtained from the same preparation.

stretch, and then only decayed exponentially with time (Fig. 1) [8]. Similar results were obtained when the fibers were stretched during the slow decay of force after the removal of 10^{-3} M ACh (catch state). If, on the other hand, the fibers were stretched during submaximal activation with 100mM K^+ or 10^{-5} M ACh, the initial rapid rise and the subsequent rapid decay of force was followed by a distinct force development as shown in Fig. 2, A and B, even when the initial force in response to 100mM K^+ or 10^{-5} M ACh was almost zero or only a small fraction ($< 10\%$) of the maximum K^+ - or ACh-induced isometric force. The stretch-induced delayed force development in the submaximally activated ABRM fibers was at least several times reproducible. The delayed force development was not observed by stretching resting fibers (Fig. 2C) either at the slack length or at a slightly stretched length with resting forces of 1–2 g. The extent of the delayed force development differed from preparation to preparation. The delayed force development became less conspicuous with decreasing amount of stretch from 10 to 5%, and with increasing amount of stretch from 10 to 20%. These results indicate that, in contrast with rabbit cerebral artery smooth muscle [1,2] in which the contractile system is activated by stretching the resting fibers, the contractile system in the ABRM fibers can only be further activated by stretching the submaximally activated fibers.

The stretch-induced force development in the submaximally activated ABRM fibers was still observed in the absence of external Ca^{2+} or in the presence of

verapamil (10^{-6} M) and Mn^{2+} (10 mM), which are known to inhibit Ca^{2+} -influx across the plasma membrane. The experiments were also made in which the ABRM fibers were mounted in the three compartment chambers to detect membrane potential changes when the fiber segment, submaximally activated with 100 mM K^{+} or 10^{-5} ACh in the experimental solution compartment, was stretched. No appreciable stretch-induced electrical changes were observed in the stretched fiber segment, indicating that stretch per se does not cause repetitive firing of fiber membrane or membrane depolarization. These results may preclude the possibility that the stretch-induced activation of the submaximally activated ABRM fibers is associated with Ca^{2+} -influx.

Based on the present results, the stretch-induced delayed force development in the submaximally activated ABRM fibers may be explained as being due to the release of Ca^{2+} from the inner surface of the plasma membrane into the myoplasm to further activate the contractile system. The absence of the stretch-induced activation in the resting fibers (Fig. 2C) and the fibers in the catch state may be taken to indicate that the Ca^{2+} -binding sites at the inner surface of the plasma membrane do not readily release Ca^{2+} by stretch, unless depolarization-induced or ACh-induced Ca^{2+} -releasing process is partially going on. On the other hand, the absence of stretch-induced activation in the fully activated fibers (Fig. 1) might be simply due to that the Ca^{2+} -binding sites at the inner surface of the plasma membrane no longer retain Ca^{2+} at the time of stretch.

REFERENCES

1. Nakayama K: Calcium-dependent contractile activation of artery produced by quick stretch. *Am J Physiol* **242**: H760-768, 1982
2. Nakayama K, Suzuki S, and Sugi H: Physiological and ultrastructural studies on the mechanism of stretch-induced contractile activation in rabbit cerebral artery smooth muscle. *Jpn J Physiol* **36**: 745-760, 1986
3. Atsumi S and Sugi H: 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 (Lond)* **257**: 549-560, 1976
4. Sugi H and Daimon T: Translocation of intracellularly stored calcium during the contraction-relaxation cycle in guinea-pig taenia coli. *Nature* **269**: 436-438, 1977
5. Suzuki S and Sugi H: Ultrastructural and physiological studies on the longitudinal body wall muscle of *Dolabella auricularia* II. Localization of intracellular calcium and its translocation during mechanical activity. *J Cell Biol* **79**: 467-478, 1978
6. Suzuki S and Sugi H: Mechanism of intracellular calcium translocation in smooth muscle. In: *The Role of Calcium in Biological Systems*, ed. Anghileri LJ and Tuffet-Anghileri AM, CRC Press, Boca Raton, Florida, pp 201-217, 1982
7. Sugi H and Yamaguchi T: Activation of the contractile mechanism in the anterior byssal retractor muscle of *Mytilus edulis*. *J Physiol (Lond)* **257**: 531-547, 1976
8. Lowy J and Millman BM: The contractile mechanism of the anterior byssal retractor muscle of *Mytilus edulis*. *Philos Trans R Soc B* **246**: 105-148, 1963