Anti-troponin-T monoclonal antibody crossreacts with all muscle types

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Summary

Monoclonal antibodies to troponin-T were produced by the hybridoma technique. Culture supernatants were initially screened using an enzyme-linked immunoabsorbent assay (ELISA). Positive clones were subcloned twice and further characterized. One of these, 7/H3:C9:D10, produced antibodies against troponin-T; immunoblotting experiments indicated its specificity for only troponin-T when challenged with a variety of striated muscle myofibrillar proteins. Indirect immunofluorescence staining with the antibody shows specific I-band staining in both adult and embryonic skeletal and cardiac muscle of various vertebrate species. In addition, intense but diffuse cytoplasmic staining was seen in chicken gizzard smooth muscle. Our results suggest that troponin-T contains an antigenic determinant that is common to both striated and smooth muscle.

Introduction

In vertebrate striated muscle, the calcium-dependent interactions between actin and myosin that lead to tension development and contraction are regulated by the tropomyosin–troponin complex (Ebashi & Endo, 1968; Ebashi et al., 1968). Troponin itself is a complex of three subunits, Tn-I, Tn-C and Tn-T. Without the troponin complex, the contractile interaction involving myosin, actin and tropomyosin is activated regardless of calcium concentration. Tn-I causes inhibition of this contractile response and the addition of Tn-C removes the inhibition of Tn-I at all Ca\(^{2+}\) concentrations (Greaser & Gergely, 1971; Ebashi, 1974a,b). In the presence of Tn-C, Tn-I and Tn-T, the contractile system becomes sensitive to calcium ions. In this respect, Tn-T, which does not have any significant function directly related to calcium ions, represents a truly regulatory component of the troponin complex (Ohtsuki, 1980; Ohtsuki & Nagaro, 1982).

The regulation of smooth muscle contraction is not as well defined. Much evidence has accumulated indicating the presence of multiple regulatory mechanisms which can be associated with either the thick filament (Bremel, 1974; Adelstein et al., 1977;
Hartshorne et al., 1977; Small & Sobieszek, 1977; Sobieszek, 1977; Adelstein, 1978) or thin filaments (Bloomquist & Yaney, 1979; Litten et al., 1979; Marston et al., 1980). With the recent development of a procedure for the isolation of Ca\(^{2+}\)-sensitive thin filaments from vascular smooth muscle (Marston et al., 1980; Walters & Marston, 1981), it appears that smooth muscle thin filaments may be Ca\(^{2+}\) regulated. Two mechanisms have been proposed for this regulation. Thin filaments prepared by the method of Marston et al. (1980) contain regulatory factors which behave in the same way as the troponin–tropomyosin system of skeletal muscles. This evidence is not entirely new as some of the earliest work on smooth muscle regulation suggested the presence of factors resembling troponin which could confer Ca\(^{2+}\) sensitivity to smooth or skeletal muscle actomyosin (Ebashi et al., 1966; Carsten, 1971; Sparrow & Van Bockxmeer, 1972; Ito & Hotta, 1976; Grand et al., 1977). However, this earlier work was criticized on the basis that the ‘native tropomyosin’ fractions used as a source of regulatory factors may contain significant amounts of myosin light chain kinase and calmodulin. A second mechanism of smooth muscle regulation associated with the thin filament is the ‘Leiotonin’ system proposed by Ebashi and his colleagues (Ebashi et al., 1977; Ebashi, 1980). These investigations suggest that Leiotonin, an 80 000 mol. wt actin-binding protein together with a Ca\(^{2+}\)-binding subunit activate a normally inactive smooth muscle actin filament in the presence of Ca\(^{2+}\), and that myosin regulation plays no role in modulating smooth muscle contractility.

In this paper, we describe a mouse monoclonal antibody raised against chicken skeletal muscle troponin-T that reacts with all three muscle types in immunofluorescence assays. Biochemical assays combining SDS gel electrophoresis and immunoblotting techniques have directly demonstrated that the antibody binds to only troponin-T in both skeletal and cardiac muscle homogenates and myofibril preparations. Indirect immunofluorescence findings confirm the I-band specificity of troponin-T localization in striated muscle and at the same time, suggest the presence of a troponin-T-like molecule in smooth muscle. Immunoblots of smooth muscle homogenate showed two reactive bands with comparable mobility to skeletal muscle troponin-T in SDS–polyacrylamide gels. These results again raise the possibility of thin filament regulation of smooth muscle contractility via a troponin-like molecule.

Methods

Preparation of tropomyosin and troponin
Tropomyosin and troponin were extracted from breast muscle or from the leg muscle of adult chicken as described by Greaser & Gergely (1971). The troponin complex was chromatographed on DEAE cellulose (Van Eerd & Kawasaki, 1973) and troponin-T was isolated and purified (Greaser & Gergely, 1971).

Immunization
Female BALB/c mice were injected intraperitoneally with 500 \(\mu\)g of the tropomyosin–troponin mixture, which was suspended in an equal volume of Freund’s complete adjuvant. After 2½ weeks, the mice were boosted intraperitoneally with 200 \(\mu\)g of the tropomyosin–troponin extract.