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LETTER TO THE EDITOR

Smooth muscle mysosin filament controversy, once again?

The Irish statesman and philosopher, Edmund Burke, aptly stated, 'Those who don't know history are destined to repeat it.' While this also applies to scientific research, it is common and often worthwhile to repeat published experiments in one's own laboratory. However, dismissing the earlier studies (history) in order to accentuate one's own findings does not lead to advancement in the field. Such is the case in a recent publication (Liu *et al.* 2013). The difference in the Seow and colleagues' (Liu *et al.* 2013) conclusions and our data (Ashton *et al.* 1975) centres on differences in the length, variability in lengths, stability of myosin filaments and the architecture of the contractile apparatus in smooth muscle tissues. Why does this matter? Because these properties contribute to the fundamental contractile phenotype characteristic of smooth muscle (SM), such as the sliding filament basis of the length–tension curve and the ability of smooth muscles to develop equivalent amounts of force (2.2×10^6 dyn cm^{-2}) compared with vertebrate striated muscle fibres in spite of the approximately fivefold lower concentration of myosin in SM.

In our earlier study (Ashton *et al.* 1975), the length of the thick filaments in rabbit portal mesenteric vein was measured in longitudinal sections (Fig. 1) using stereo views to establish that the filaments were completely within the plain of the section and that overlapping portions of two filaments did not introduce an error (see Plates II, III and IV in Ashton *et al.* 1975). To allow clear visualization of filaments, a section thickness of 0.16–0.18 μm was chosen because this thickness was expected to contain just two to three filaments at different levels based on the 60–80 nm thick filament lattice measured in this muscle (Rice *et al.* 1971). As discussed below, intermediate high voltage microscopy was used to allow precise visualization of myosin filaments in sections of this thickness. The length of such 'complete' filaments in longitudinal sections, not transverse sections as stated by Dr Seow, was 2.2 ± 0.14 μm (mean ± SD) (n = 20; mode 2.2–2.4 μm). Shorter filaments were incomplete as they were always seen to exit the plane of the section. These results were supported by serial reconstructions of myosin filaments from 0.47 μm-thick transverse sections, although precision of the measurements is less than in the longitudinal sections as the extent of the protrusion of the tapered filament ends into the 'terminal' sections could not be accurately measured. However, of the 255 filaments followed, they all were present in 4–6 sections, with the majority complete in 5 sections, consistent with the longitudinal filament length measurements.

Seow *et al.* addressed the question of myosin filament length using thinner 50–60 nm-thick transverse sections of sheep trachealis, sheep pulmonary artery and rabbit carotid artery smooth muscle cells. A distribution of filament lengths was measured in all cases, with 24% of

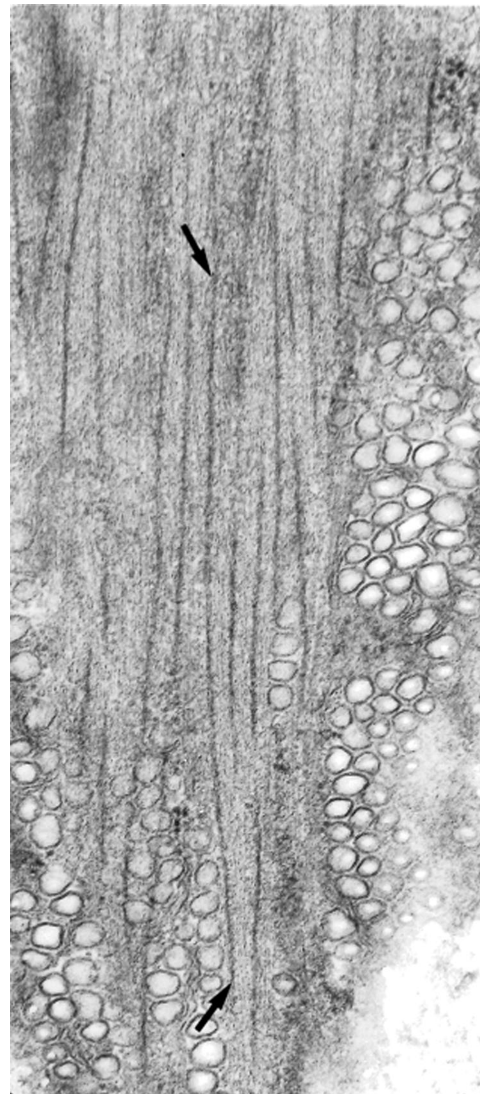

Figure 1. Myosin filaments in portal vein are 2.2 ± 0.14 μm long
Single view from a stereo pair of electron micrographs of 160 nm-thick longitudinal section cut near the surface of a smooth muscle cell in the rabbit portal mesenteric vein. A 2.3 μm-long myosin filament (ends marked by arrows) is completely included in the section when viewed in stereo. Magnification ×50,000.

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filaments being 50 nm or less in length in the sheep trachea that increased to 38% upon stimulation with acetylcholine. In sheep pulmonary artery, 40% of filaments were 100 nm or less in length. Distribution of lengths fell rapidly, with the majority of filaments being <500 nm. The authors interpret their findings as being due to a dynamic equilibrium of linear myosin polymerization and depolymerization with myosin dimers as substrates for filament formation (Liu *et al.* 2013). Myosin monomers, dimers, tetramers and hexamers cannot be imaged in these sections. Lengths measured in longitudinal sections of trachealis were similar but as these are made on thin 50 nm-thick sections in the absence of stereo tilt views, it is highly unlikely that complete myosin filaments would be captured. This fundamental difference from our study is dismissed by Dr Seow and colleagues as reflecting the reduced resolution in our 0.47 μm-thick transverse sections suggesting that in our study we could not resolve gaps between longitudinally aligned short filaments. I would like to correct that misinterpretation. The whole point of using intermediate (200 kV) high voltage imaging is the greater penetration of electrons allowing the use of thicker sections than conventional electron microscopy with no loss of resolution. Thicker sections increase the sample depth through the smooth muscle cells. Furthermore, the goniometer stage on this instrument allowed tilting of the specimen to obtain stereo views, such that in one view the transverse filaments are seen in cross-section and the other tilted view shows the side of the filament revealing any gaps or discontinuities (see Plates VI and VII in Ashton *et al.* 1975). Thus, this argument does not account for the differences in the two studies. Interestingly, Dr Seow and colleagues have also reported the longer form of myosin (1.8–2.2 μm) in pig trachealis (Herrera *et al.* 2005). If so, one must conclude that in the trachealis differences in myosin filament lengths and distribution of lengths must occur between species. The special mechanical properties of tracheal smooth muscle has been attributed to plasticity of the filaments, as suggested by Dr Seow. If true, then one would expect to observe these differences in the sheep, but not pig, trachealis, a possible direction for new studies. As pointed out in our Discussion, 'It should also be emphasized that the myosin filaments of vertebrate smooth muscle other than portal-mesenteric vein may have significantly different lengths from that found in this study.' Indeed there are small variations in myosin filament length in some striated muscles (Franzini-Armstrong, 1970) but nothing within the extreme range of mini-filament lengths as reported by Seow *et al.*

The regulation of non-muscle and smooth muscle myosin monomeric and polymeric states has been studied extensively *in vitro* and depends on salt composition, ionic strength, ATP and phosphorylation of the regulatory myosin light chain (RLC20). Bipolar, side polar, short and long filaments can be formed and it has been difficult to translate these *in vitro* experiments to the *in situ* myosin filament structure. This is compounded by the well-known difficulties of achieving good fixation of smooth muscle myosin especially in tissues with a great deal of connective tissue such as arteries. On the other hand, polymerization and depolymerization of myosin filaments readily occurs and is necessary in cultured cells, cells undergoing mitosis, proliferation and migration such as during development, angiogenesis and tissue remodelling. Phosphorylation of RLC20 promotes filament assembly as originally shown in thymus cells (Scholey *et al.* 1980) and since reported in many non-muscle cells (Tan *et al.* 1992). Thus, in terms of smooth muscle it is important to discriminate between cultured cells and tissues. This is illustrated in a recent study demonstrating a large population of total myosin existing in the folded 10S monomeric state in cultured airway smooth muscle cells, using antibodies specific for the 10S form (Milton *et al.* 2011). In contrast, earlier studies found no evidence for any 10S molecules in smooth muscle cells in chicken gizzard tissue, in either the relaxed or contracted state, also using antibodies to probe for the 10S species (Horowitz *et al.* 1994). Altogether it would seem that myosin filament conformation in the sheep trachealis, sheep pulmonary artery and rabbit carotid artery smooth muscle cells, reported by Dr Seow, resemble non-muscle, proliferating or cultured smooth muscle cells.

Overall, the intent of this commentary is to clarify the erroneous interpretation of our data on myosin filament length in the rabbit portal mesenteric vein and to add a cautionary note concerning the complexity of determination of the smooth muscle myosin filament characteristics. While it is difficult to envision short filaments, including myosin dimers and multimers of eight molecules, positioning themselves in an orderly fashion between oppositely polarized long actin filaments in such a way that they can account for the well-established mechanical properties of smooth muscle, this model raised by Dr Seow and colleagues remains open for rigorous testing. No information is provided for the kinetics of myosin turnover in filaments. If the proposed increased turnover of myosin with acetylcholine stimulation resulting in longer filaments contributes to the acetylcholine-induced contraction of the sheep trachealis, the kinetics must be sufficiently rapid to account for the rapid rise in tension and stiffness (latencies of 500 ms) upon electrical stimulation of bovine trachea (Kamm & Stull, 1986).

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Additional information

Competing interests

None declared.

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