Variations in myosin expression along the length of orbital fibres in the rabbit extraocular muscle

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Extraocular muscles (EOM) have two layers of muscle fibres with different functions: orbital fibres that control the position of the recently discovered soft tissue pulleys (Demer *et al*., 2000), and global fibres that rotate the globe. Pulleys make the axes of action of EOMs depend on eye orientation, and this is thought to provide a simple mechanism for implementing Listing's law governing eye rotations. Both layers have SIFs (singly innervated fibres) and MIFs (multiply innervated fibres), with ultrastructural features resembling amphibian fast twitch and slow-tonic fibres, respectively. EOM fibres express 9 myosin heavy chain (MyHC) isoforms, comprising those in developing and adult limb and cardiac muscles, and 2 EOM-specific isoforms, EO-MyHC and slow-tonic MyHC. Orbital fibres display a systematic variation of MyHCs along their length, correlated with ultrastructural features, but earlier studies were unable to specify the precise MyHC isoforms involved. We use here a battery of monoclonal antibodies capable of unambiguously identifying each of the 9 MyHCs to study MyHC changes in serial sections of rabbit superior rectus muscle by immunohistochemistry.

According to ultrastructural criteria (Davidowitz *et al*., 1977), there are three major orbital fibre types: the oSIF, the coMIFs (orbital MIF of constant diameter); and the voMIFs, which vary in diameter from 5μm along the middle portion of their length to around 15 μm in their ends. The oSIFs and coMIFs are short, whilst the voMIFs are the longest. Orbital MIFs have an 'en plaque' neuromuscular junction in addition to distributed 'en grappe' endplates in global MIFs.

We show that variations in MyHC expression in orbital fibres closely parallel structural variation along the length. These changes occur at three sites: (1) At the EPZ, the oSIFs express EO-MyHC, the fastest MyHC, associated with high sarcoplasmic reticulum (SR) and mitochondrial volume. On either side of the EPZ, these fibres express the slower 2A and/or embryonic MyHCs, with decreased SR and mitochondrial volume. (2) The coMIFs and voMIFs at the EPZ express α-cardiac MyHC, the fastest of the slow MyHCs, where the ultrastructure is fast twitch. They continue to show twitch ultrastructure on either side of the EPZ, where they coexpress α-cardiac and embryonic MyHC. (3) In the distal quarter of the orbital layer, the oSIFs and coMIFs end, presumably by inserting onto the pulley, the orbital layer is entirely made up of voMIFs. Here the fibres mainly co-express slow-tonic and embryonic MyHC and show ultrastructural features of amphibian slow-tonic fibres. In the far proximal end of the muscle, oMIF mainly express embryonic MyHC with a small proportion of fibres co-expressing slow-tonic MyHC.

We propose that only the oSIFs and coMIFs insert into the pulleys and actively translocate them during saccades. Forward translocation of pulleys is achieved by passive stretching due to contraction of the antagonist, the presence of the very fast EPZ region permitting sudden collapse of tension necessary for rapid repositioning of the pulleys. The voMIFs insert onto the globe. The slow-tonic MyHC may provide ripple-free tension to hold the eyeball steady during a gaze, and the faster narrow segment may be a specialisation to allow for rapid relaxation and fibre lengthening during a change of gaze involving contraction of an antagonist.

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