

Fibre types in rat laryngeal muscles and their transformations following denervation and reinnervation

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Intrinsic laryngeal muscles cricothyroid (CT) and thyroarythenoid (TA) differ in myosin expression. CT expresses limb myosin heavy chains (MyHCs) while TA expresses a MyHC found in extraocular (EO) muscles (Lucas *et al.*, 1995), in addition to limb isoforms. A definitive classification system for laryngeal muscle fibre types does not exist at present. Earlier studies on the effects of denervation (Shiotani & Flint, 1998) and reinnervation (Shiotani *et al.*, 2001) on the MyHC profiles of whole laryngeal muscles are suggestive of neural influence on MyHC expression, but fibre type transformation at the cellular level has not been shown.

Immunohistochemical analyses with highly specific monoclonal antibodies (mAbs) against various MyHCs were used to study muscle fibre types in rat CT and TA, and to investigate whether nerves to laryngeal muscles control MyHC expression. CT was found to have the full complement of limb fibre types. TA had three major fibre types based on MyHC composition: 2b/eo, coexpressing 2B and EO MyHCs, 2x/2b, coexpressing 2X and 2B MyHCs, and 2x, expressing 2X MyHC. Type 2a and slow fibres were absent. TA consisted of two divisions: the external division (TA-X), which is homogeneously 2b/eo, and the vocalis division (TA-V), composed principally of 2x and 2b/eo fibres, with a minority of 2x/2b fibres. The use of these mAbs has established the feasibility of classifying laryngeal muscle fibre types by their MyHC composition in spite of the extensive occurrence of hybrid fibres containing multiple isoforms.

The recurrent laryngeal nerve (RLN) which innervates both divisions of the TA as well as other laryngeal muscles except the CT were cut and allowed to reinnervate these muscles in 16 rats. The left RLN transection was performed on sixteen 10-week old Sprague Dawley rats. The animals were anaesthetised by intraperitoneal injection of ketamine hydrochloride (35mg/kg) and xylazine hydrochloride (5mg/kg). The TA from 4 animals were examined immunohistochemically at 2, 4, 6 and 12 weeks postoperatively. Commencing four weeks after cutting and re-uniting the RLN, numerous 2b/eo fibres in TA-X began to express 2X MyHC, while EO and 2B MyHC expression in these fibres progressively declined. By 12 weeks, $16.5 \pm 2.5(\text{SE})\%$ of fibres in the TA-X were of type 2x. These findings suggest that nerve fibres originally innervating 2x fibres in TA-V and other muscles had randomly cross-innervated 2b/eo fibres in the TA-X and converted them into 2x fibres. We conclude that MyHCs in laryngeal muscle fibres are subject to neural regulation, in common with limb and jaw muscles.

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