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RAPID COMMUNICATION

Unusual Intrafusal Fibres in Human Muscle Spindles

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Summary

We have studied the morphology and pattern of expression of myosin heavy chain (MHC) isoforms of intrafusal fibres in a human first lumbrical muscle. Each intrafusal fibre type, namely nuclear bag_1, nuclear bag_2 and nuclear chain fibres, had a distinct MHC composition and distribution of different MHC isoforms along the whole length of intrafusal fibres. However, most muscle spindles analyzed also contained one or several intrafusal fibres exhibiting an extrafusal or mixed pattern of immunoreactivity which did not correspond to any of the described intrafusal fibre types. We conclude that the latter fibres do not represent new intrafusal fibre types, but their morphology and expression of MHC merely reflects the differences in their innervation owing to their unusual localization at the edge or outside the axial bundle of intrafusal fibres.

Key words

Human lumbrical muscles • Muscle spindles • Intrafusal fibre types • Myosin expression • Immunocytochemistry • Neuronal influences

Introduction

In mammalian species studied so far, the existence of three intrafusal fibre types, namely the nuclear bag_1, the nuclear bag_2 and the nuclear chain fibres (for review see Boyd 1980, Barker and Banks 1994, Zelená 1994), has been corroborated by specific monoclonal antibodies recognizing different myosin heavy chain isoforms (MHCs) (for review see Soukup *et al.* 1995, Walro and Kucera 1999). The morphological complexity of muscle spindles, however, increases from the rat and cat to the human spindles (Cooper and Daniel 1963, Kucera and Dorovini-Zis 1979, Eriksson *et al.* 1994, Pedrosa-Domellöf and Thornell 1994). In the present study, we have analyzed the MHC expression in intrafusal fibres of human muscle spindles in the first lumbrical muscle excised during hand surgery from a 35-year-old patient with his informed consent. Serial cross-sections were stained with monoclonal antibodies recognizing slow twitch, fast twitch, slow twitch and fast IIA, fast IIA, fast IIB, fast IIA and IIB, embryonic, fetal/neonatal and slow tonic MHC. In addition, a mAb recognizing the myofibrillar M band protein (M_r 160 000) staining positively all fibres except intrafusal nuclear bag_1 fibres was used (Fig. 1d). The PAP immunocytochemistry (Sternberger 1979) was performed as described earlier (e.g. Pedrosa-Domellöf and Thornell 1994).

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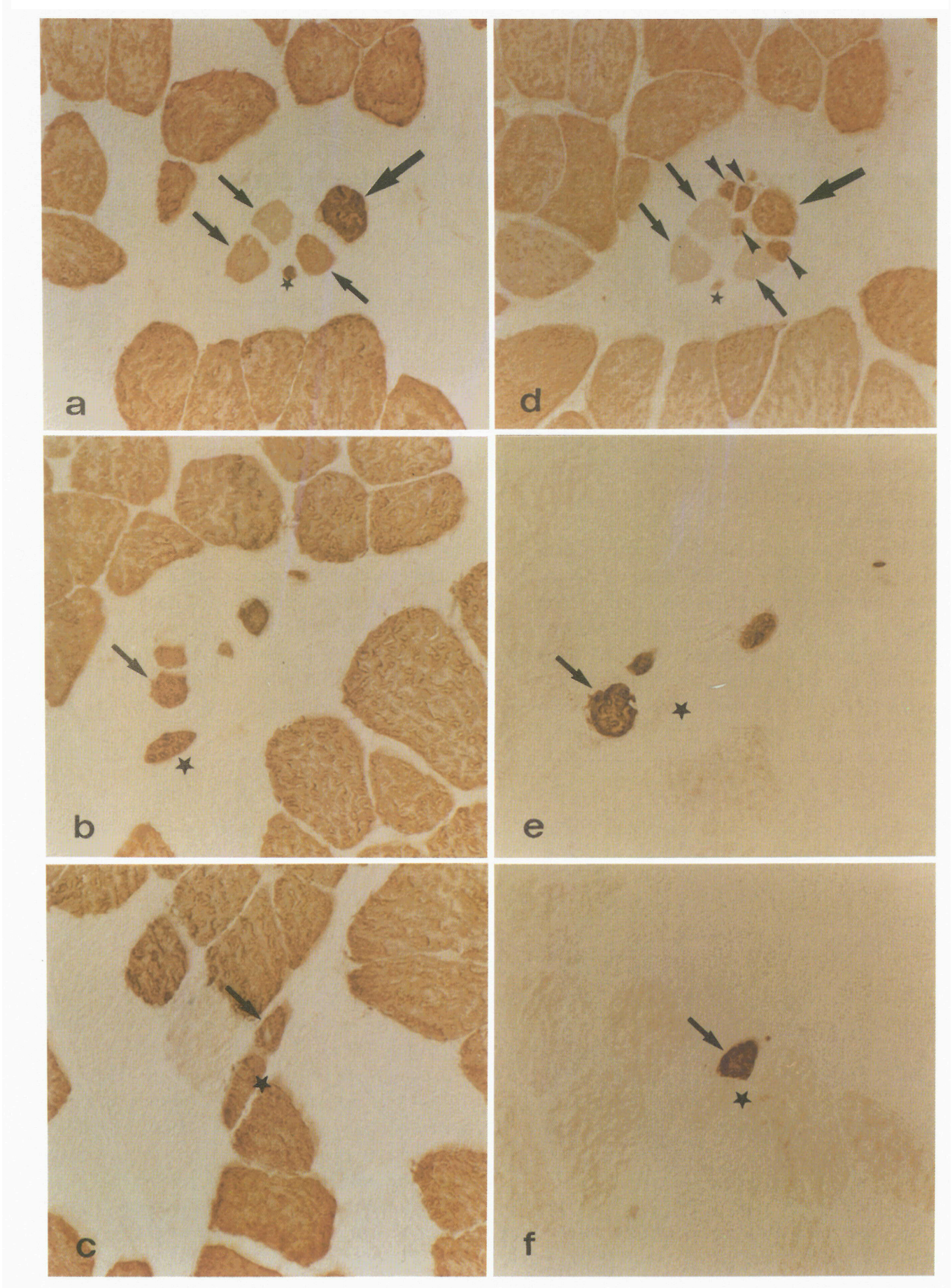
a
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We have analyzed 11 spindles throughout most of their length (3-5 mm), containing 125 intrafusal fibres (x = 11.4, range 8-15). From these, 31 fibres were classified as nuclear bag_1 fibres, characterized by the presence of slow tonic and, in the polar zone, also slow twitch MHC; 23 fibres were classified as nuclear bag_2 fibres reacting with monoclonal antibodies against slow tonic, slow twitch, fast twitch and fetal/neonatal MHC; 53 fibres were recognized as nuclear chain fibres containing fast twitch, embryonic and fetal/neonatal MHC (Fig. 1a-f). The remaining 18 intrafusal fibres did not fit into any of these intrafusal fibre types. These latter fibres could be divided according to their MHC content into the following groups: 1) mixed chain/type I-like intrafusal fibres, 2) small-diameter intrafusal fibres corresponding to extrafusal type I or type II fibres, and 3) large-diameter intrafusal fibres corresponding to extrafusal type I fibres. Intrafusal fibres in these three groups differed in their origin and position in the spindle.

1) The first group, comprising four fibres in two spindles, contained chain-like fibres, which spanned throughout the intracapsular part of the spindle. They exhibited MHC expression typical for the nuclear chain fibres at one pole, but their immunocytochemical reactions at the other pole resembled those of extrafusal type I fibres characterized by the presence of slow twitch MHC.

2) The second group consisted of 10 fibres found in five spindles, which usually originated within the inner B zone, sometimes separated from the rest of the intrafusal fibres by one or more capsule layers. However, they usually joined other intrafusal fibres in the axial bundle along their course to the spindle polar region and terminated with other intrafusal fibres. Their immunocytochemical reactions corresponded either to extrafusal type I fibres (8 fibres in four spindles) expressing exclusively slow twitch MHC (Fig. 1a-f) or to type II fibres (two fibres in one spindle) characterized by the presence of fast twitch MHC.

3) The third group, found in one spindle, consisted of four fibres, confined to one half of the spindle only, completely separated during their intracapsular course from the rest of intrafusal fibres by several layers of the spindle capsule. They originated in the outer B zone, spread towards the near spindle pole and continued beyond the spindle end as extrafusal fibres. They eventually lost their capsular ensheathment towards the spindle pole and gradually attained diameters comparable to extrafusal fibres. They expressed slow twitch MHC and on the whole corresponded to extrafusal type I fibres.

Do these three groups represent new intrafusal fibre type(s) or does their staining result from the specific localization and/or aberrant innervation within the spindle?

1) The chain/type I fibres most likely represent nuclear chain fibres, which lack (or have reduced) sensory and/or gamma innervation at one pole, where they correspond to extrafusal type I fibres. Chain fibres, which lack motor innervation at one pole, were described in the cat (Kucera 1981, Banks, personal communication). On the other hand, the latter fibres could be innervated at this pole by extrafusal motor axons of the " slow type" as suggested by their content of slow twitch MHC.

2) The small diameter "type I or II fibres" most likely represent encapsulated "intrafusal" fibres that are not innervated by sensory (primary) axons, probably because they do not extend over the central sensory region and are separated from intrafusal fibres in the axial bundle. As these fibres lack sensory innervation, they do not express spindle-specific MHCs (slow tonic, embryonic and fetal/neonatal isoforms). They are probably innervated by extrafusal motor axons since they expressed slow or fast twitch MHC. Corresponding nuclear chain-like fibres, which entirely lacked sensory (both primary and secondary) endings, were found in a muscle spindle in human biceps brachii muscle (Kucera 1986), or were enclosed in the muscle spindle capsule in the human lumbrical muscle (Cooper and Daniel 1963). A similar short small-diameter fibre (700 μm in length) was found at the polar zone in a cat tenuisimus muscle spindle (Sutherland, personal communication). In a guinea-pig lumbrical muscle, one spindle was found

Fig. 1a-f. *Serial cross sections through the outer A zone (a,d), the outer B zone (b,e) and C region (c,f) of a spindle from the first human lumbrical muscle stained with monoclonal antibodies against slow twitch (a-c), slow tonic (e,f) MHCs and an M protein (d). The spindle contains one nuclear bag_2 fibre (thick arrow), three nuclear bag_1 fibres (thin arrows), four nuclear chain fibres (arrowheads) and one small-diameter fibre (asterisk), which originates in the outer A zone (a,d) and that is stained with mAb against slow twitch (a-c), but not slow tonic (e,f) MHC. Note that the spindle capsule is not stained by the used monoclonal antibodies. x 500.*

which, in addition to the usual intrafusal fibres, had two fibres identified as "extrafusal" (Banks, Ph.D. Thesis, personal communication). They were extracapsular at both poles and intracapsular in the equatorial region, lying freely in the periaxial space. These two fibres had no sensory innervation, but each of them had a motor endplate inside the spindle capsule.

3) The large-diameter extrafusal-like fibres in the third group were completely separated along their entire course through the spindle by several layers of the capsule and were not in contact with either sensory innervation or even with the internal spindle milieu. These fibres are apparently innervated by extrafusal motor axons, as their characteristics resemble extrafusal type I fibres. It can be supposed that "intrafusal" fibres corresponding to fast type II fibres would also be found in a larger sample.

It has recently been shown that number of fast and slow "intrafusal" fibres in regenerated muscle spindles of muscle isografts reflects the quantitative relation between "fast" and "slow" motor axons in the ingrowing nerve and that it corresponds to the number of fast and slow extrafusal fibres (Jirmanová and Soukup 1995, Soukup and Thornell 1997, Mráčková *et al.* 1999). Furthermore, it was confirmed by an ultrastructural study (Soukup and Novotová 2000) that regenerated spindle fibres in heterochronous isografts are not innervated by sensory axons, but only by extrafusal motor axons. These findings suggest that intrafusal satellite cells remain plastic and that regenerating "intrafusal" myotubes, if deprived of sensory innervation, permit motor axons to determine their phenotype within the adaptation range of a given fibre type. It is a matter of speculation, why the "intrafusal" fibres in the first two groups of intrafusal fibres in the first human lumbrical muscle do not grow more in girth, if they were innervated by extrafusal motor axons. One possible explanation is that these fibres are short as they terminate within the spindle capsule. It is possible that short muscle fibres cannot grow in diameter above a certain limit. This speculation is supported by the existence of large-diameter fibres of the third group that continued extracapsularly as extrafusal fibres. However, the influence of factors operating inside the spindle also cannot be excluded: the internal spindle milieu within a limited space inside the capsule could contribute to the restriction of growth of intrafusal fibres in girth. Such an effect was excluded in the third group of large-diameter fibres, as these fibres were completely separated by several capsular layers from the spindle interior.

The above cases suggest a special relation between some "intrafusal" fibres and the muscle spindle. Extrafusal fibres anchored in the separate compartments among capsular layers apparently run in parallel with the sensory terminals inside the spindle. It is a matter of speculation whether such an arrangement is of any physiological significance. The existence of corresponding collagen bundles running "in parallel" with sensory terminals was described in tendon organs of the rat (Zelená and Soukup 1983) and the cat (Jami 1992). The contraction of "in parallel" localized muscle fibres could then lead to spindle discharges by stretching the opposite pole of the spindle, while muscle contraction usually has an "unloading" effect upon spindle activity (Matthews 1972). It was suggested that if the capsules of human spindles enclose neighboring extrafusal fibres, this could further increase the responsiveness of human spindle afferents to contractions of individual motor units (Hulliger 1984). Whether we can consider these "in parallel fibres" as the fourth physiological fibre type system, besides the three – the "dynamic nuclear bag_1" and the "static nuclear bag_2 and nuclear chain" systems – described previously (Boyd 1980, Gladden 1985), however, remains still uncertain.

We thus conclude that the three "unusual" fibre groups described in human lumbrical muscles do not represent new intrafusal fibre types, but their expression of MHCs merely reflects the shortcomings in their innervation due to their particular localization, usually outside the intrafusal axial bundle. Providing that mononucleated precursors of these "intrafusal" fibres are the same as for the intrafusal fibres in the axial bundle, this would indicate that intrafusal myoblasts, similarly as intrafusal satellite cells, are also plastic and that they can develop their phenotype according to their innervation within their intrinsic adaptation range. On the other hand, if we consider the possibility that muscle fibres arise as the consequence of a common developmental program and that the major divergence between the intrafusal and extrafusal fibres is due to the presence of sensory innervation on the intrafusal fibres, the pseudo-intrafusal fibres may simply be due to accident of development. The increased complexity and variability of muscle spindles in human muscles in comparison with those of other mammals, should alert us to realize that a similar complexity has been revealed by electrophysiological investigations of the human stretch reflex (Matthews 1991) in comparison to classical neurophysiological experiments in the decerebrate cat.

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