Expression of Myosin Heavy Chain (MyHC) Isoforms in Rat Intrafusal Muscle Fibres after Neonatal Deefferentation and Subsequent Denervation

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Abstract. The analysis of developing intrafusal fibres is not feasible in the absence of primary sensory axons, as neonatal denervation leads to the disintegration of muscle spindles On the other hand, neonatal deefferentation does not arrest their differentiation and, moreover, it leads to the neomyogenesis of supernumerary intrafusal profiles If the sciatic nerve was sectioned in 4-week-old rats deefferented at the birth, muscle spindles survived, the neomyogenesis proceeded and the denervated intrafusal fibres expressed the spindle specific slow tonic (STO) MyHC The expression of MyHC pattern in individual fibres and the differentiation of the fibre type characteristics were, however, less obvious compared to the control or deefferented spindles The newly formed intrafusal profiles (which differentiated from satellite cells in the absence of innervation) expressed the STO MyHC particularly when they developed in a spatial relation to nuclear bag fibres

Key words: Myosin heavy chain — Intrafusal fibre types — Neuronal induction — Muscle development — Muscle spindles

Development and maturation of muscle spindles in the rat hind limbs proceeds from FD17-18 to the fourth PN week (for review see Zelená 1994) By the immunocytochemical reactions on fresh frozen cryosections, nascent nuclear bag fibres can be identified already at this stage by strong staining with mAbs against slow tonic (STO) MyHC, the nuclear bag_2 myotubes present from FD17 also contain slow twitch/beta cardiac (STW) MyHC and to some degree neonatal MyHC, the nuclear bag_1 myotubes, present from fetal day (FD) 19 contain neonatal MyHC and by FD21 STO and STW MyHCs, nuclear chain precursors present from FD21 and PND3 contain neonatal MyHC (Kucera *et al* 1988, Pedrosa and Thornell 1990, Pedrosa-Domellof *et al* 1991) In each spindle, all intrafusal fibres are contacted by the same Ia axon, present from the onset of spindle differentiation, whereas motor axons contact intrafusal fibres shortly before or after birth (for review see Zelená 1994) From the birth, regional staining variations are present along the length of nuclear bag myotubes, as staining of the nuclear bag_2 fibres with mAbs against STO MyHC decreases from the equatorial to the polar region and the nuclear bag_1 fibres cease to express STW MyHC progressively from the equator towards the poles Seven days

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after the birth, muscle spindles appear rather mature, with a well developed capsule, a small periaxial space, typical pattern of mATPase activity and an almost mature staining pattern with mAbs against MyHCs (Pedrosa and Thornell 1990, for review see Zelena 1994, Soukup *et al* 1995)

The expression of muscle spindle specific MyHCs and their nonuniform distribution along the distance of intrafusal fibres raise questions regarding the regulation of this complex MyHC pattern It was proposed (Zelená 1957) that the development and maintenance of muscle spindles depend on the morphogenetic and trophic influence of sensory innervation In confirmation, muscle spindles do not form in muscles denervated during fetal development and immature muscle spindles present at the birth rapidly disintegrate after neonatal denervation or deafferentation (Kucera and Walro 1987, Pedrosa *et al* 1990, for review see Zelená 1994, Soukup *et al* 1995) Mature rat muscle spindles do not degenerate and survive denervation although the intrafusal fibres atrophy and increase in number after prolonged denervation (Gutmann and Zelená 1962, Zelená 1957) On the other hand, neonatal deefferentation performed by removal of the lumbosacral spinal cord 48 h after birth does not arrest differentiation of the full complement of four intrafusal fibres, but it even stimulates the neomyogenesis of new supernumerary intrafusal profiles (Soukup *et al* 1993, Zelená and Soukup 1993) After deefferentation, however, the typical restrictive pattern of expression of MyHC isoforms along the length of intrafusal fibres fails to develop and STO and STW MyHC isoforms are expressed over most of the length of nuclear bag_2 and bag_1 fibres (Soukup *et al* 1990) As a result, both deefferented nuclear bag fibres become similar to each other and the regional differences, typical for control nuclear bag fibres are not developed Motor innervation thus contributes to the development and maintenance of regional differences in the expression of MyHC isoforms along the length of intrafusal fibres (for review see Soukup *et al* 1995)

Similarly as in deefferented rats, muscle spindles in hind limbs deefferented at birth and denervated 3–4 weeks later by exairesis of the sciatic nerve survive and the neomyogenesis proceeds in the absence of sensory innervation (Novotová and Soukup 1995) Although various types of intrafusal fibres could be distinguished, showing their spindle specific MyHC expression (Fig 1), many denervated fibres exhibited mixed staining patterns and variations in mATPase and MyHC expression along their length not observed in control rats Typical regional differences between nuclear bag_1 and bag_2 were missing and many denervated chain fibres reexpressed MyHCs not present in adult control spindles including the STO MyHC The newly formed intrafusal profiles expressed the spindle specific STO MyHC, in particular if they developed in a spatial relation to nuclear bag fibres The STO positivity observed in the central zone of some nuclear chain fibres in control and deefferented spindles (Pedrosa-Domellof *et al* 1991), was frequently observed along the substantial part of denervated chain fibres Although some supernumerary profiles exhibited mATPase activity and MyHC immunoreactivity similar to the parent nuclear bag_1, bag_2 or chain fibres, many profiles displayed atypical staining patterns (cf Soukup *et al* 1993) The corresponding MyHC expression was also achieved after prolonged denervation performed in adult rats (Soukup *et al* 1995) The expression of MyHC isoforms and the differentiation of fibre type characteristics were thus less obvious than in control or even in deefferented spindles and these changes were apparently due to the arrest or even loss of "specialization" in the regional expression of MyHCs under the control of sensory and motor innervation

Our findings support the suggestion that intrafusal satellite cells are of two types, those derived from nuclear bag and those derived from nuclear chain fibres and that intrafusal fibres (at least of the nuclear bag type) originate from a special cell lineage

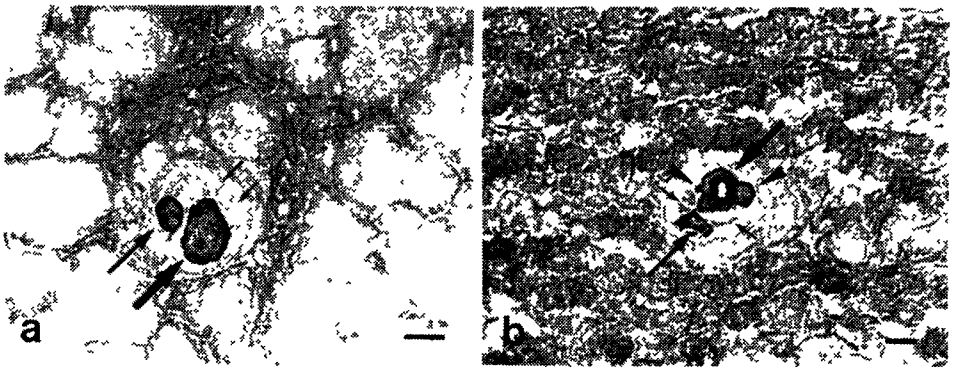

Figure 1. Transverse sections of muscle spindles from EDL muscles of a 2-month-old control rat **(a)** and a 2-month-old rat deefferented 2 days after birth and denervated 24 days later **(b)** stained with mAbs against slow tonic MyHC isoform Note the presence of unstained nuclear chain fibres (small arrows) and of strongly stained nuclear bag_2 (thick arrows) and nuclear bag_1 (thin arrows) fibres in both **a** and **b** and of supernumerary profiles found in apposition to nuclear bag_2 fibre (arrowheads) in **b** Bars indicate 10 μm a $\times$500, b $\times$400

different from extrafusal muscle fibre type precursors (Pedrosa and Thornell 1990) They are also in agreement with the proposal that sensory terminals play a key role in inducing and supporting the differentiation of intrafusal fibres (Zelená 1957) and that fusimotor innervation contributes to the expression of phenotype differences among intrafusal fibres (for review see Zelená 1994, Soukup *et al* 1995)

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