Morphogenesis of rat muscle spindles after nerve lesion during early postnatal development

S. SCHIAFFINO and S. PIEROBON BORMIOLI

National Research Council Unit for Muscle Biology and Physiopathology, Institute of General Pathology, University of Padova, Italy

Received 23 October 1975; revised and accepted 12 December 1975

Summary

The influence of innervation on muscle spindle morphogenesis has been investigated in rat hind-limb muscles by sectioning the sciatic nerve, with suture of the stumps, at various postnatal stages. After nerve section at 4 or 7 days of age a proportion of spindles survived during the denervation phase and developed, during the subsequent reinnervation phase, into atypical structures. The reinnervated spindles were recognized by the presence of a limiting capsule but lacked the characteristic distinction of equatorial and polar regions. The intrafusal fibres were fewer than normal and were indistinguishable in size and fine structure from extrafusal fibres; they had a single motor endplate and lacked sensory nerve terminals. In reinnervated muscles of animals operated at 13 and 22 days of age there was a progressive tendency towards a restoration of normal spindle structure and innervation. These findings indicate that muscle spindle morphogenesis is profoundly altered by nerve lesion at early developmental stages, apparently as a result of inadequate sensory reinnervation. This study also shows that the differentiation of intrafusal fibres is dictated by their specific pattern of innervation and is not intrinsically predetermined.

Introduction

Muscle spindle development in the rat takes place in the late foetal stages and in the first three weeks of extrauterine life (Zelená, 1957; Marchand and Eldred, 1969). The morphogenetic changes during this developmental period have been recently better defined by electron microscope investigation (Landon, 1972; Milburn, 1973). At birth the rat spindle has two presumptive nuclear-bag intrafusal fibres together with myotubes and myoblasts, and a primary sensory ending. The motor innervation begins to arrive by birth. By the fourth postnatal day all the four intrafusal muscle fibres, two nuclear-bag and two nuclear-chain fibres are present, but do not display ultrastructural variations; the sensory terminals are well developed and motor terminals are also encountered. By the twelfth day, intrafusal fibres are clearly differentiated and fusimotor innervation is fully developed. The initial enlargement of the periaxial space takes place at this time.
The early development of muscle spindles appears to be entirely dependent upon innervation. After nerve crushing at birth, reinnervated rat muscles were found to contain no spindles at all or a few atypical small spindles (Zelená, 1957; Hník and Zelená, 1961). Atypical spindles with intrafusal fibres apparently continuous with extrafusal fibres have been described recently by Werner (1973a) after nerve crushing in newborn rats. By contrast, after nerve lesion at later stages of development, mammalian muscle spindles do not disintegrate and seem to recover a normal structure and function with reinnervation (Tower, 1932; Zelená, 1964; Homma, 1969; Yellin, 1970; De Santis et al., 1972; Werner, 1973b; Schröder, 1974a and b). There is apparently a rather abrupt transition in the sensitivity of muscle spindles to denervation during the early postnatal period. In order to obtain a better understanding of the morphogenetic influence of innervation in this critical phase of development we have compared the effect of denervation performed at 4, 7, 13 and 22 days after birth. The procedure used was nerve section followed by suture of the stumps in order to permit subsequent reinnervation. Histochemical and ultrastructural techniques were used to characterize the reinnervated spindles. Our findings confirm and extend the results of a previous report (Yellin, 1970) showing that the differentiation of intrafusal fibres is profoundly modified by alteration of the nerve supply before the maturation of the spindle is completed.

Materials and methods

The experiments were performed on Wistar rats at 4, 7, 13 and 22-days postnatal. The animals were anaesthetized by cooling. The sciatic nerve was cut at the level of the sciatic notch and the stumps were sutured. After a period of 6–12 months the rats were killed and the extensor digitorum longus (EDL), soleus and plantaris muscles were removed from the operated and contralateral sides. An additional group of animals operated at 7 days were killed 1 to 3 weeks later in order to monitor early changes induced by denervation in developing spindles. Experimental and control muscles were usually mounted as a single block, immersed in liquid nitrogen and cross-sectioned at about 10 μm in a cryostat. A limited number of serial sections cut through the mid-belly of the muscles were generally used but almost complete series of transverse sections were also made in a number of muscles. The sections were stained with haematoxylin-eosin, or processed for the histochemical demonstration of myosin adenosin-triphosphatase (ATPase) activity (Padykula and Herman, 1955), using preincubation at pH 10.4 and 4.35 (Guth and Samaha, 1969), succinate dehydrogenase (SDH) (Nachlas et al., 1957) and mitochondrial α-glycerophosphate dehydrogenase (α-GPDHase) (Hess and Pearse, 1961). Serial frozen cross-sections were also incubated for acetylcholinesterase activity in a modified Koelle medium (Koelle and Friedenwald, 1949).

The number of spindles and the number of intrafusal fibres per spindle was determined in randomly selected transverse sections cut through the mid-belly of the muscles. The total number of extrafusal fibres was also counted in the same sections. Fibre cross-sectional areas were measured planimetrically.

Operated and control muscles were also processed for electron microscopy by a procedure which allows the rapid identification of muscle spindles (Pierobon Bormioli and Schiaffino, 1975). In brief the method consists in processing for electron microscopy 30–50 μm transverse cryostat sections of glutaraldehyde-fixed muscles. Flat embedded sections can be directly examined under the light microscope and spindles are easily recognized. Consecutive cryostat sections were used for serial study of single spindles.