Brief Reports

INTRAFUSAL MUSCLE FIBER HISTOCHEMISTRY FOLLOWING ITS MOTOR REINNERVATION

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Most intrafusal muscle fibers in muscle spindles of the rat soleus muscle reinnervated for 3-12 months appeared unchanged histochemically. Some fibers, however, were morphologically and histochemically abnormal and the abnormalities often concerned only focal fiber regions, perhaps in relationship to spindle reinnervation by inappropriate motor axons.

Materials and Methods

In eight young (3-4 weeks old) and three adult (150 g) Wistar rats the ventral roots L4, L5, L6 on the right side were crushed under Nembutal anesthesia (60 mg/kg) to accomplish transient motor denervation followed by reinnervation of m. soleus. Following the surgery the right foot remained paralyzed at the ankle for 4-6 weeks. Most animals regained their ability to move the ankle by the 8th postoperative week; this was taken to indicate the reestablishment of functional connections between α motor fibers and the extrafusal muscle. Following the surgery the right foot remained paralyzed at the ankle for 4-6 weeks. Most animals regained their ability to move the ankle by the 8th postoperative week; this was taken to indicate the reestablishment of functional connections between α motor fibers and the extrafusal muscle. No similar indication of functional muscle spindle reinnervation was available, however, it may be noted that fusimotor spindle reinnervation occurs readily following nerve crush (2) and in Schröder’s experiments (9) all spindles in rat lumbrical muscles were morphologically reinnervated within 2 months after high sciatic nerve crush.

The animals were killed 3 months (two rats), 6 months (four rats) and 12 months (2 rats) after the surgery. The experimental muscle and the contralateral control muscle were removed and quenched in isopentane cooled to -160°C with liquid nitrogen. The frozen specimens were sectioned serially on a cryostat at 12 μm and incubated for “myofibrillar” adenosine triphosphatase (ATPase) at pH 9.4 following alkali (pH 10.4) or acid (pH 4.3) preincubation of alternate sections according to Dubowitz and Brooke (3).

In one rat 1 week after the surgery the ventral roots were removed distal to the crush lesion, fixed in gluteraldehyde and semi-thin (1 μm) cross-sections were stained with methylene blue and examined. All axons were undergoing Wallerian degeneration.

Results

Normal soleus muscles stained with ATPase reaction displayed regular intermixing of light (type I) and dark (type II) extrafusal muscle fibers. Three types of intrafusal muscle fibers were identified in every muscle spindle encountered, as described by James (5) and Banks et al. (1). Fibers with low alkaline, acid-labile ATPase activity were recognized as nuclear bag; those with high alkaline, acid-stable ATPase activity were nuclear bag fibers. The nuclear chain fibers represented the third fiber type and they had low alkaline, acid-labile ATPase activity (Fig. 1A). Only minor differences of ATPase staining intensity were noticed along the intrafusal fibers except for the extreme polar regions of the spindle where the nuclear bag fibers had altered their staining properties and stained in a manner...
Fig. 1. Sections of spindles stained for ATPase after alkali preincubation taken from control m. soleus (A) and m. soleus reinnervated for 6 months (B, E–H) and 12 months (C, D). All ×900. A, intrafusal muscle fiber complement in a typical control spindle. b₁—nuclear bag fiber, b₂—nuclear bag fiber, nc—nuclear chain fiber. B, a spindle from a reinnervated muscle with several nuclear bag fibers (b₁). C and D, sections of a spindle showing fibers of the usual ATPase stain at one spindle pole (C) but abnormally pale nuclear bag fiber (b₂) throughout the opposite pole (D). E–H periodic sections of a spindle showing regional staining abnormalities affecting all fibers. E, nuclear bag fiber (b₁), bag₂ fiber (b₂) and two nuclear chain fibers (nc) of normal ATPase staining properties. F and G, sections through the adjoining region spanning 200 μm with nuclear bag₂ and nuclear chain fibers of abnormal ATPase staining. H, a section further towards the spindle pole shows again fibers stained in the usual way.

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FIG. 2. Periodic sections spanning 500 \( \mu \text{m} \) of a spindle from m. soleus reinnervated for 6 months stained for ATPase following alkali (A-D, F) or acid (E) preincubation. The fibers are followed through their capsular and extracapsular regions towards the spindle pole. All \( \times 900 \). A, nuclear bag, fiber \( (b_1) \), bag, fiber \( (b_2) \) and two nuclear chain fibres \( (n_{c_a}, n_{c_b}) \) of normal staining. B, adjoining region with enlargement of a nuclear chain fiber \( (n_{c_a}) \). C, nuclear chain fiber \( (n_{c_b}) \) is about to terminate. D, the enlarged fiber \( (n_{c_a}) \) stretches for 200 \( \mu \text{m} \) beyond the point of termination of nuclear chain \( (n_{c_b}) \) fiber. E, Fibers stained with ATPase after acid preincubation. F, The enlarged fiber \( (n_{c_b}) \) had terminated abruptly and the appearance of the remaining two nuclear bag fibers is normal for the spindle extreme polar region.

Similar to Type I extrafusal fibers with these two ATPase reactions (8).

Muscles studied 3 to 12 months following crushing of their motor supply exhibited grouping of extrafusal fibers of the same enzymatic type, attesting to successful muscle denervation and reinnervation (6, 10). The majority of muscle spindles in the reinnervated soleus muscle contained intrafusal muscle fibers of the usual histochemical profile, although some fibers (mostly nuclear bag,) appeared to have split, and infrequently new fibers were present within the spindle capsule (Fig. 1B). Intrafusal fiber splitting is known to occur in response to spindle denervation (7, 9) and it can be assumed that fusimotor nerves to spindles with multiple bag, fibers such as shown in Figure 1B had indeed been crushed. Minority of reinnervated spindles exhibited two types of histochemical or morphologic abnormalities of nuclear bag or nuclear chain fibers followed in serial sections. A circumscribed region of variable length of an otherwise unremarkable fiber would possess changed or reversed ATPase staining properties anywhere along its intracapsular course although the fiber retained its usual diameter and length; all the fibers in a spindle could be similarly altered in a given region (Fig. 1).
In other instances an otherwise intact intrafusal fiber showed considerable enlargement of its diameter in a fiber region of variable length with or without concomitant alteration of the ATPase staining (Fig. 2). The abnormal nuclear chain fiber shown in Figure 2 seems to have enlarged in its polar region to such a degree that it resembles a type II extrafusal muscle fiber. Moreover, it appears that the fiber has enlarged in length also since it terminates much farther toward the spindle pole than the other nuclear chain fiber present, a situation that is not encountered in normal rat spindles where nuclear fibers are of about equal length. The nuclear equatorial regions of altered fibers seemed unchanged.

DISCUSSION

The occurrence of grouping of extrafusal fibers of the same ATPase staining in the reinnervated muscle indicates a change of the former enzymatic pattern and it demonstrates that the enzymatically altered extrafusal fibers were reinnervated by axons different from the original ones (6, 10). It is unclear whether the majority of intrafusal fibers in reinnervated muscle spindles retain their histochemical properties because of resistance to metabolic conversion despite a random reinnervation or are reinnervated by fusimotor axons with preference for terminating on their original fiber types. If the later mechanism is operating, and the physiologic studies of reinnervated muscle spindles suggest that it may (2), the infrequent occurrence of regional intrafusal fiber abnormalities may be due to "erroneous" fiber reinnervation by inappropriate fusimotor axons, or perhaps by an extrafusal motor axon in situations such as depicted in Figure 2. It remains an open question whether the intrafusal fiber abnormalities noticed represent a permanent alteration of fiber properties or a transient phase of spindle reinnervation with either the erroneous or the more appropriate motor innervation asserting itself fully at a later time. There is some evidence that crush lesions such as done in our study lead to more complete spindle recovery than nerve section (2).

Muscle fiber histochemical properties are dependent on fiber innervation (3). Our experiments indicate that whatever the nature of factors mediating this influence of motor axons upon their muscle fibers, they should be able to operate locally in order to account for regional histochemical differences seen along normal as well as reinnervated intrafusal muscle fibers.

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LITERATURE CITED