Histochemical Properties of Intrafusal Fibers in the Soleus Muscle of the Aged Rat

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Summary ATPase reaction profiles of intrafusal fibers in the muscle spindle of the soleus muscle of 135-week-old rats were examined. Nuclear bag₁ fibers contained an acid- and alkaline-labile form of the enzyme or an acid-labile and alkaline-stable form, nuclear bag₂ fibers contained an acid- and alkaline-stable form, and nuclear chain fibers contained an acid-labile and alkaline-stable form. These results indicate that the enzyme histochemical heterogeneity of intrafusal fibers is well-preserved during ageing.

Key words: intrafusal muscle fiber, histochemistry, ageing.

Mammalian muscle spindles, which are known to be sensory stretch receptors, contain three types of encapsulated intrafusal muscle fibers, i.e., nuclear bag₁, nuclear bag₂, and nuclear chain fibers (BANKS *et al.*, 1977). These fibers can also be distinguished histochemically on the basis of the myosin ATPase reaction: nuclear bag₁ fibers contain an acid-stable and base-labile form of the enzyme, nuclear bag₂ fibers contain an acid- and base-stable form, and nuclear chain fibers contain an acid-labile and base-stable form (OVALLE and SMITH, 1972).

Despite a number of studies on age-related changes in histochemical properties of extrafusal muscle fibers (CACCIA *et al.*, 1979; LARSSON and EDSTRÖM, 1986; ALNAQEEB and GOLDSPIK, 1987; EDSTRÖM and LARSSON, 1987), little is known about the histochemical characteristics of intrafusal muscle fibers in old age. Therefore, this study investigated ATPase reaction in intrafusal muscle fibers of the soleus muscle of aged rats.

Five 135-week-old Wistar female albino rats were used. Under ether anesthesia, the soleus muscle was removed. The tissue was mounted on chucks with compound and quickly frozen in liquid nitrogen. Fresh frozen serial sections of 8 μ m thickness were cut transversely on a cryostat-microtome at -20°C . Sections were stained for ATPase alternately after acid and alkaline preincubation (GUTH

Fig. 1. Intra- and extrafusal fibers in the soleus muscles of 2-week-old (A) and 10-week-old rats (B). The sections were stained for ATPase activity after alkaline preincubation. Muscle fibers are distinguished by the enzyme activity. Bar = 40 μ m. ms, muscle spindle.

and SAMAHA, 1969, 1970). As shown in Fig. 1, intra- and extrafusal fibers in the soleus muscles of young and adult animals were clearly distinguished by low or high enzyme activity. ATPase activity is known to directly correlate with the rate of muscle contraction (BÁRÁNY, 1967).

Of the 27 soleus muscle spindles observed, 25 contained four intrafusal muscle fibers. These fibers could be divided into three different morphological and histochemical types. Two large-diameter fibers in the muscle spindle, which were nuclear bag fibers, consisted of nuclear bag₁ fibers containing an acid- and alkaline-labile form of ATPase or an acid-labile and alkaline-stabile form, and nuclear bag₂ fibers containing an acid- and alkaline-stabile form. Two small-diameter fibers, which were nuclear chain fibers, contained an acid-labile and alkaline-stabile form of the enzyme. The other two spindles formed two nuclear bag and three nuclear chain fibers (Table 1).

On the other hand, although two types of extrafusal muscle fibers were observed in adulthood (ARIANO *et al.*, 1973; ARMSTRONG and PHELPS, 1984), all of

Fig. 2. Serial transverse sections of two muscle spindles in the soleus muscle in a 135-week-old rat. The sections were stained for ATPase activity after preincubation in acid (A) and alkaline (B). Nuclear bag₁ fibers contain an acid- and alkaline-labile form of the enzyme (spindle 1) or an acid-labile and alkaline-stable form (spindle 2), nuclear bag₂ fibers contain an acid- and alkaline-stable form, and nuclear chain fibers contain an acid-labile and alkaline-stable form. Extrafusal muscle fibers are unreactive after either acid and alkaline preincubation. Bar = 20 μ m. nb₁, nuclear bag₁ fiber; nb₂, nuclear bag₂ fiber; nc, nuclear chain fiber; ef, extrafusal fiber; sc, spindle capsule.

the extrafusal muscle fibers in the soleus muscle of aged rats showed a de-differentiation pattern of ATPase reaction, namely the enzyme was both acid- and alkaline-labile (Fig. 2).

In this study, intrafusal fibers of the soleus muscle in old age could be divided into three types according to the differences in ATPase activity. This indicates differences of the enzyme activity in the ageing process between intrafusal and extrafusal muscle fibers. Various enzyme activities in extrafusal muscle fibers markedly decrease with ageing (BASS *et al.*, 1975; HANSFORD and CASTRO, 1982). However, the enzyme activities of intrafusal muscle fibers were well-preserved during ageing. In light of the neuromuscular innervation pattern, these findings

Table 1. Numbers and ATPase staining reactions of intrafusal fibers in 27 soleus muscle spindles of 135-week-old rats.

Number of spindles	Number of fibers per spindle			ATPase reaction		
	Nb ₁	Nb ₂	Nc		Acid	Alkaline
22	1	1	2	Nb ₁ :	labile	labile
				Nb ₂ :	stabile	stabile
				Nc :	labile	stabile
3	1	1	2	Nb ₁ :	labile	stabile
				Nb ₂ :	stabile	stabile
				Nc :	labile	stabile
2	1	1	3	Nb ₁ :	labile	labile
				Nb ₂ :	stabile	stabile
				Nc :	labile	stabile

Nb₁, nuclear bag₁ fiber; Nb₂, nuclear bag₂ fiber; Nc, nuclear chain fiber.

support the observations that alpha-motoneurons, which innervate extrafusal muscle fibers, tend to show lower enzyme activity with ageing, whereas gamma-motoneurons innervating intrafusal muscle fibers maintain normal enzyme activity in old age (ISHIHARA *et al.*, 1987; ISHIHARA and ARAKI, 1988).

Nuclear bag₁ fibers of the rat exhibited a different pattern in ATPase activity from those of other species (OVALLE and SMITH, 1972; JOHNSON and OVALLE, 1986), whereas this study showed that nuclear bag₁ fibers are an acid- and alkaline-labile form of the enzyme or an acid-labile and alkaline-stabile form. It seems reasonable to assume that these differences are related to the differences in motor innervation and regulation. However, further investigations are needed to elucidate species-specificity in the enzyme activity of intrafusal muscle fibers.

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