Myosin Heavy Chains in Striated Muscle after Immobilization

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Abstract

The total content of myosin heavy chains (MHC) and their isoform pattern were studied by biochemical methods in the slow-twitch (soleus) and fast-twitch (gastrocnemius) rat muscles immobilized for 4 days in a neutral and in a shortened position.

The immobilized slow muscle atrophied more than the immobilized fast muscle of the same rat leg. The total MHC content decreased more in the slow than in the fast muscle. In the immobilized slow muscle the ratio of MHC-1 to MHC-2A(2S) remained almost unchanged, showing that similar diminution of both isoforms occurs. In the immobilized-shortened fast muscle the MHC-2A/MHC-2B ratio decreased, showing the loss of MHC-2A; the MHC-2B content remained almost unchanged. The results show that: 1) MHC-1, MHC-2A and MHC-2S, isoforms characteristic of fatigue-resistant fibres, are very sensitive to immobilization: 2) the MHC-2B is insensitive to immobilization; 3) the fast MHC isoforms differentiate in sensitivity to immobilization.

The results obtained from the immobilized muscles are compared with those obtained from the tenotomized and denervated muscles. The influence is discussed of inactivity and of lack of a neuromuscular junction on the muscle content of the particular MHC isoforms.

Key words: atrophy, muscle adaptation, muscle immobilization, myosin heavy chains, striated muscle.

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Striated muscle is known to be a very plastic and adaptable tissue. Alterations can occur after changes in innervation or function, following hormone influence and aging. Myosin, the major muscle-specific protein, is very adaptable to functional demands [25, 29]. Myosin is composed of two heavy chains (MHC) and four light chains. Myosin subunits have many isoforms, specific for different muscles or fibre types. Maximum velocity and power of muscle contraction is correlated with the MHC type [2, 7]. Mechanisms regulating expression of the individual myosin isoforms in mature skeletal muscle are still obscure [9, 11]. Also regulation of the protein degradation in skeletal muscle is far from being elucidated [28].

As observed previously in the rat leg muscles atrophying after denervation and after tenotomy, the particular MHC isoforms react selectively and variously to changes in muscle innervation and function. Namely, the MHC-1 and MHC-2B are susceptible to lack of innervation, whereas the MHC2A and MHC-2S are relatively unsusceptible [16]. On the other hand, the isoforms characteristic for fatigue-resistant fibres, i.e. the MHC-1, MHC-2A and MHC-2S are very susceptible to tenotomy. Contrary to that, the MHC-2B is relatively unsusceptible to tenotomy [19]. Both the denervated and tenotomized muscle atrophied in comparable degree [14, 19], in spite of differences between the two experimental models. The denervated muscle is inactive and devoid of a connection with the nervous system, whereas in the tenotomized one a muscle nerve junction remains intact, but activity of the motoneuron decreases following decreased activity of the afferent system [13, 20, 33]. Additionally, the tenotomized muscle is shortened and unloaded. Both shortening and unloading cause muscle atrophy [8, 26]. In the present work investigation of immobilized muscles was undertaken in order to complement information concerning regulation of the MHCs content. The muscles were immobilized in a neutral position or in shortening. Comparison between them allows to show the difference between the effects of the inactivity with a normal and with a lowered function of the afferent system. The latter model is similar to tenotomy in many aspects. These two models of inactivity may assume muscle hypokinesia or hypodynamia, respectively. In par-

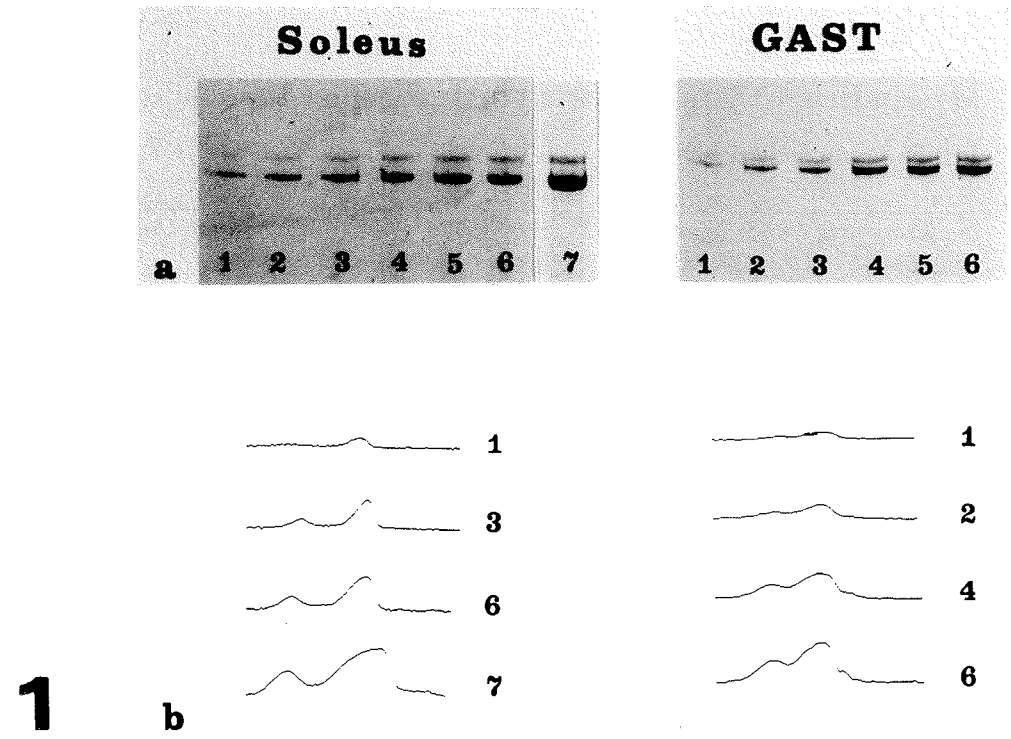

Figure 1. Correlation between the amount of protein subjected to electrophoresis and the intensity of staining of individual MHC-bands. a) SDS/PAGE of myofibrils from control soleus and GAST muscles was performed as described in the Materials and Methods. Amount of myofibrillar protein subjected: lane 1, 0.5 µg; lane 2, 1 µg; lane 3, 1.5 µg; lane 4, 2 µg; lane 5, 2.5 µg; lane 6, 3 µg; lane 7, 9 µg. Electrophoretograms were scanned as described in the Materials and Methods. The sum of all MHCs in the sample was take as 100%. Percentual distribution of the MHCs in the soleus was for the MHC-1 and MHC-2S, respectively: lanes 1-6, 76% and 24%; lane 7, 75% and 25%. Percentual distribution of the MHCs in the GAST was for the MHC-1, MHC-2A and MHC-2B, respectively: lane 1, 3%, 29% and 68%; lane 2, 5%, 29% and 66%; lane 3, 4%, 29% and 67%; lane 4, 4%, 30% and 66%; lane 5, 4%, 31% and 65%; lane 6, 6%, 31% and 63%. b) Scannings of some electrophoretograms shown in (a).

Table 1. Comparison among experimental models applied

	Inactivity	Shortening	"Unloading"	Lack of muscle nerve connection
Denervation	+			+
Tenotomy	+	+	+	
Immobilization in shortening	+	+	±	
Immobilization	+			

Effects of experimental conditions are indicated by "+".

allel with the immobilized muscles, some denervated and tenotomized ones were examined for comparison. The differences among the experimental models applied are summarized in Table 1. The early response of MHCs to the used experimental procedures was examined.

Materials and Methods

Experiments on rats

Female albino Wistar rats (2.5-3 months old, 150-190 g) were used. They were fed a regular diet of laboratory chow and water ad lib. The slow-twitch muscle - soleus and fast-twitch muscle - gastrocnemius (GAST) of the same animals were studied. The muscles were immobilized in a neutral or in a shortened position by immobilization of the ankle joint between aluminium rods at an angle of 130-160°. Depending on the changed length after immobilization, the experimental soleus muscles were arbitrarily pooled into three groups: those shortened on the average of up to 80% of the contralateral length (referred to as 20%-shortened), those shortened to 84% (16%-shortened) and those with the length as in the contralateral muscle. In some cases the results from all immobilized-shortened soleus muscles were pooled. The GAST muscles were taken from the same animals as the soleus ones, and they were correspondingly pooled into the groups. Four groups of muscles served as controls: (1) the muscles from the contralateral leg; (2) the muscles from nonexperimental animals from the same population; (3) the muscles tenotomized by cutting the Achilles tendon; (4) the muscles denervated by cutting the sciatic nerve. All procedures: joint immobilization, tenotomy and denervation were done under ether anaesthesia. Muscles from 2-4 animals were used in each experiment. Results from some experiments were pooled.

Preparation of myofibrils

At 4 days (in some experiments on the tenotomized and denervated muscles at 12-16 days) after the operation animals were killed by decapitation and the muscles were immediately removed, measured, weighed and transferred to ice. A fraction of crude myofibrils was separated as described in detail [15, 19]. Myofibrils from both experimental and contralateral legs were obtained under exactly the same conditions, with a carefully controlled and monitored solvent volume during procedure, and were studied in parallel.

The total content of protein in myofibrils was estimated by the method of Lowry et al. [23].

Estimation of the total MHC content

The total MHC content in the muscle was evaluated by estimating the total MHC in myofibrils as follows. Myofibrillar proteins were separated by SDS/PAGE as described by Weber & Osborn [30]; samples (20-40 μg of protein) were subjected to 6-8%-polyacrylamide gels. After separation and staining with Coomassie Blue, individual bands were cut out and eluted from gels; the amount of eluted protein was estimated colorimetrically. The procedure was described previously in detail [19]. The data from each experiment was derived from analysis of at least 12 gels from at least 8 electrophoresis runs.

Analysis of MHC isoforms

MHC isoforms were separated from isolated myofibrils by SDS/PAGE (6% gel) with high glycerol concentration (37%) as described by Carraro & Catani [3], as modified by Danieli Betto et al. [6]. Individual isoforms of MHC: 1, 2A, 2B and 2S were identified according to their electrophoretic mobility [4, 27] as previously [19]. Relative amounts of MHC isoforms were determined by comparing

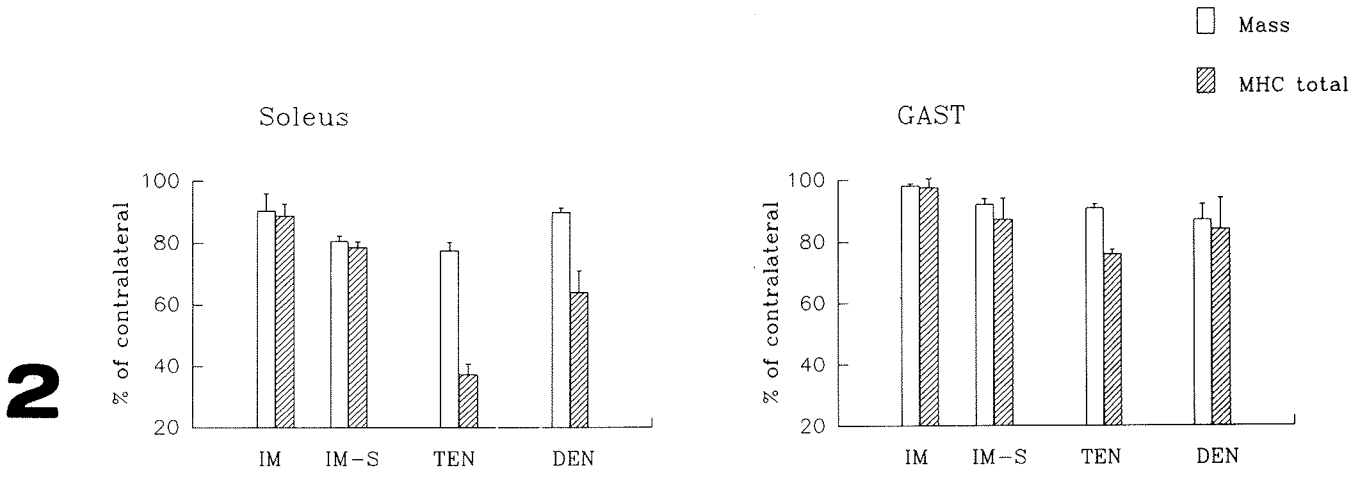

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Figure 2. Changes in mass and total MHC content in the experimental muscles. The muscle content of the total MHC were evaluated in the 4-days experimental and contralateral muscles, as described in the Materials and Methods. IM, immobilization in neutral position; IM-S, immobilization in shortening (all shortened muscles were pooled); TEN, tenotomy; DEN, denervation. Values are means ± S.E.M.

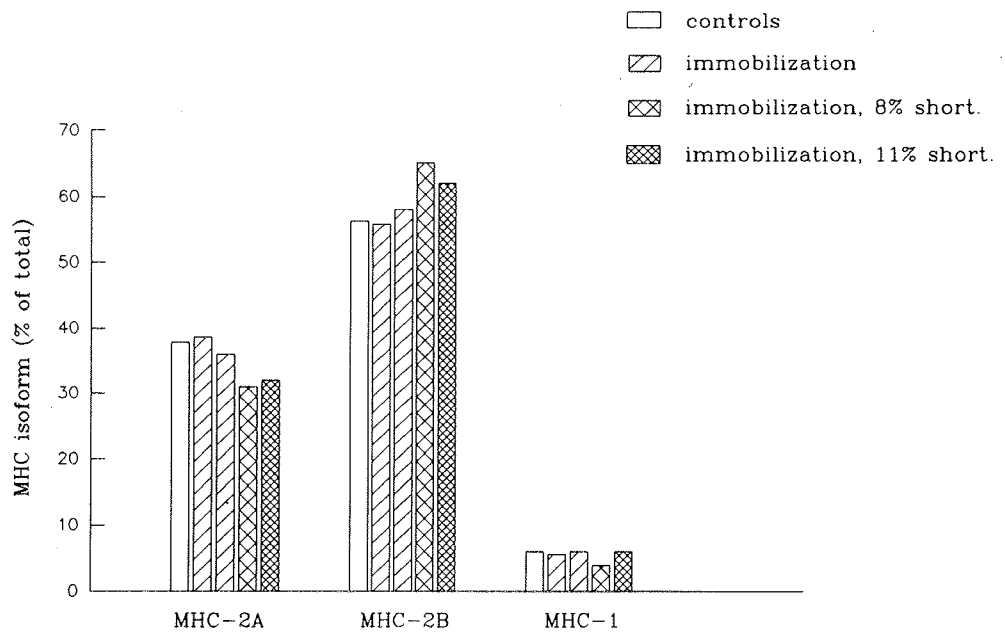

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Figure 3. Changes in the MHC isoform pattern in the immobilized GAST muscles. Electrophoretograms of the MHC isoforms were performed and scanned as described in the Materials and Methods and in Fig. 1. The sum of all MHCs in the sample was taken as 100%. Each type of bar represents another group of animals. All groups were studied in the same experiment; results of one electrophoresis run are shown. Two groups of muscles immobilized in a neutral position were taken.

the degree of intensity of staining with Coomassie Blue of the electrophoretic bands by using a Panasonic CCD Video Camera and digitized using an IBM 386 computer equipped with Visionetics frame graber card. The image was then scanned using densitometry software written by Dr Pawel Pomorski. A linear response for the individual MHC bands was obtained when 0.5 μg to 9 μg of myofibrillar protein was analysed (Fig. 1). The data from each experiment derived from at least 4 independent electrophoresis runs.

The results were evaluated statistically by the sign test [5].

Results

Muscle length, mass and total MHC content

Length of the control muscle was 18 ± 0.2 mm and 23 ± 0.3 mm (mean values ± SEM) for the soleus and GAST, respectively. Weight of the control muscles was 74 ± 1.5 mg and 972 ± 11 mg (mean ± SEM) for the soleus and GAST, respectively.

After immobilization for 4 days in a neutral position, the mass decreased by about 10% in the soleus; and it remained almost unchanged in the GAST (Fig. 2). After immobilization for 4 days in a shortened position, the diminution of the length and mass were not of the same degree for the soleus and GAST muscles of the same leg. In the soleus both the length and mass decreased by about 16-20% whereas in the GAST by about 8-11% only (Fig. 2, Table 2). In all immobilized muscles the percentual decrease in the total MHC content was very similar to that in the muscle mass (Fig. 2). Contrary to that, in the 4-days tenotomized soleus and GAST muscles and in the 4-days denervated soleus, the decrease of the total MHC content was much higher than the decrease of the mass (Fig. 2). Such disproportion extended alongside the atrophy progress (results not shown). The data concerning the tenotomized and denervated muscles are comparable to the previous data [14, 16, 19].

MHC isoforms

The MHC isoforms were separated by SDS/PAGE with high glycerol concentration as described in the Materials and Methods. The MHC of the control soleus muscle contained about 23% of type 2S isoform and about 77% of type 1. This proportion remained practically unaltered in the soleus muscle immobilized both in a shortened and in a neutral position (Table 2).

The MHC of the control GAST contained about 32% of isoform 2A, 62% of 2B and 6% of isoform 1. In the muscle immobilized in a neutral position this proportion did not alter. Contrary to that, in the GAST immobilized in a shortened position the proportion of MHC isoforms changed significantly within 4 days: the MHC-2A and MHC-1 decreased, whereas the MHC-2B increased (Fig. 3 and Table 2).

Proportions of the MHCs in the immobilized-shortened muscles changed similarly as those in the tenotomized muscles, but quite differently than in the denervated muscles (for comparison Tables 2 and 3). Proportions of the

*Table 2. Changes in the MHC isoform pattern in the immobilized muscles. Electrophoretograms of the MHC isoforms were performed and scanned as described in the Materials and Methods and in Fig. 1. The amounts of individual MHC isoforms were estimated. The sum of all MHCs in the sample was taken as 100%. Results from some experiments and from several independent estimates are pooled. Values are means ± S.E.M. The individual MHC isoform was compared with corresponding MHC-isoform of controls running in the same electrophoresis run. Results from at least 12 pairs from each group were taken and compared by the sign test [5]. Differences statistically significant: *, $p < 0.05$; ***, $p < 0.001$.*

Group	Muscle	Length of muscle, % of contralateral	MHC total, % of contralateral	Amount of MHC isoforms 1	2A/S/	2B
1.	Soleus	100 ± 1.5	89 ± 4	80 ± 0.6	20 ± 0.6	
		Contralat.		80 ± 0.5	20± 0.5	
	GAST	100 ± 1.5	98 ± 4	6 ± 0.2	33 ± 0.4	61 ± 0.5
		Contralat.		6 ± 0.4	33 ± 1.0	61 ± 1.3
2.	Soleus	84 ± 2.0	82 ± 5	78 ± 0.6*	22 ± 0.6*	
		Contralat.		79 ± 0.6	21 ± 0.6	
	GAST	91 ± 1.5	85 ± 10	5 ± 0.3	33 ± 0.5*	62 ± 0.6*
		Contralat.		6 ± 0.3	36 ± 0.7	58 ± 0.9
3.	Soleus	80 ± 1.5	81 ± 2	78 ± 0.5	22 ± 0.5	
		Contralat.		78 ± 0.3	22 ± 0.3	
	GAST	89 ± 4.0	92 ± 6	5 ± 0.2	28 ± 0.3***	67 ± 0.3***
		Contralat.		6 ± 0.3	29 ± 0.6	65 ± 0.6

Table 3. Changes in the MHC pattern in the tenotomized and denervated muscles.

Type of experiment	Muscle	Time after operation	Amount of MHC isoforms 1	2A/S/	2B
Tenotomy	Soleus	4 days	77 ± 0.5	23 ± 0.5	
		Contralat.	76 ± 1.0	24 ± 0.5	
		12 days	75	25	
		Contralat.	76 ± 0.5	24 ± 0.5	
	GAST	4 days	5 ± 0.4	30 ± 1.1	65 ± 1.0
		Contralat.	7 ± 0.3	29 ± 0.3	64 ± 0.4
		12 days	3 ± 0.5	29 ± 2.0	68 ± 1.5
		Contralat.	6 ± 0.7	33 ± 0.8	60 ± 0.6
Denervation	Soleus	4 days	75 ± 0.5	25 ± 0.6	
		Contralat.	76 ± 0.4	24 ± 0.4	
		16 days	54 ± 0.5	46 ± 0.5	
		Contralat.	77 ± 0.4	23 ± 0.4	
	GAST	4 days	5 ± 0.6	31 ± 2.5	64 ± 3.0
		Contralat.	5 ± 0.6	31 ± 1.2	64 ± 1.7
		16 days	6 ± 0.7	42 ± 0.8	52 ± 0.9
		Contralat.	7 ± 1.2	32 ± 0.6	61 ± 0.6

See legend in Table 2.

Table 4. Sensitivity of the particular MHC isoforms to immobilization

Muscle	Length of muscle % of contralateral	Total MHC		MHC1		MHC2A/S/		MHC2B	
		Content /mg/	Loss /%/	Content /mg/	Loss /%/	Content /mg/	Loss /%/	Content /mg/	Loss /%/
Soleus	Contralat.	100		80		20			
	100	89	11	71.2	11	17.8	11		
GAST	Contralat.	100		6		33		61	
	100	98	2	5.9	2	32.4	2	60	2
Soleus	Contralat.	100		79		21			
	84	82	18	64	19	18	14		
GAST	Contralat.	100		6		36		58	
	91	89	11	4.5	25	29.4	18	55	5
Soleus	Contralat.	100		78		22			
	80	80	20	62	20	17	20		
GAST	Contralat.	100		6		29		65	
	89	92	8	4.6	23	25.5	12	62	5

Data presented in Table 2 were re-calculated and expressed relative to 100 mg of the total MHC muscle content.

Table 5. Susceptibility of the particular MHC isoforms to experimental conditions applied. Analysis of the results from Table 2-4 and of the previous ones [1, 2] allows for evaluation of susceptibility of the individual MHC isoforms to the experimental conditions applied.

	MHC isoforms		
	1	2A/S/	2B
Denervation	+		+
Tenotomy	+	+	
Immobilization in shortening	+	+	
Immobilization	+	+	

Susceptibility is indicated by "+".

MHCs in the tenotomized and in the denervated muscles (Table 3) were comparable to those described previously [16, 19].

Discussion

Experimental models

In the present work, the content of MHCs was examined in the muscles immobilized in a neutral and in a shortened position, and for comparison, in the denervated and in the tenotomized muscles. As mentioned in the "Introduction", application of such experimental models helps in differentiating among several factors, possibly influencing the content of particular MHCs. Namely, all experimental muscles were inactive; additionally, the denervated muscle was devoid of connection with the nervous system, whereas the tenotomized and the immobilized-shortened muscles were shortened and unloaded (Table 1).

The well-known fact must be stressed here, that long-lasting muscle inactivity causes disuse atrophy in the muscle of every type. We applied a short-term immobilization to examine the early response to inactivity of the slow and fast muscles. On the other hand, the shortened position of the muscle brings about elimination of part of the contractile apparatus and its reorganization with a decrease in sarcomere number [17, 31]. Thus, some loss of the MHCs could be expected. Nevertheless, when the shortened muscle is inactive, such processes take some weeks [31]. After 4 days of immobilization they are still at an initial stage [17, 18] and their contribution to the loss of MHCs seems to be not very essential.

The soleus and GAST muscles from the same legs were examined. In spite of that, both the decrease of length and atrophy were greater in the soleus than in the GAST muscle (Fig. 2, Table 2). It was perhaps due to the anatomical insertions of the muscles the relatively larger shortening of the soleus. This phenomenon was discussed also previously [19]. However, after immobilization in a neutral position (when the length of muscles remained unchanged), the soleus atrophied, whereas the GAST still remained in the range of the contralateral. Such difference is resulted by

the greater sensitivity to immobilization of the slow muscle than the fast one [10, 12, 34].

Identification of the MHC isoforms

Under the electrophoretic conditions used, the MHC-2S and the MHC-2A isoform have very similar mobility [4, 27]. These two isoforms are also called 2A and 2X [22] or IId and IIa [1]. Thus the MHC-2A band can also contain the MHC-2S isoform. In the soleus muscle this band contain exclusively MHC-2S, which is the only fast component of the rat soleus muscle myosin [4].

The MHC-1 and MHC-2B isoforms are known to be present in very low amount in rat GAST and soleus muscle, respectively. Some per cent of the MHC-1, was detected in gels of GAST; the MHC-2B was not detect in gels of soleus muscle, with exception of some overloaded gels (results not shown).

Differences between MHC isoforms in reaction to immobilization

To explain the reaction of the particular MHC isoforms to immobilization, changes in amount of total MHC and in proportions of MHC isoforms (presented in Table 2) were analysed. The results of such analysis show that in the soleus, immobilized in both a neutral and a shortened position, the content of MHC-1 and MHC-2S decreased similarly (Table 4). It means that muscle inactivity, with both the preserved and the lowered function of the afferent system, cuts down the content of the MHC-1 and MHC-2S.

In the GAST muscle immobilized in a neutral position, the content of MHCs remained practically unchanged (Table 4). In the GAST immobilized in shortening, the loss of individual MHC isoforms was various and the differences were significant (Table 2). Namely, the content of isoforms characteristic for fatigue-resistant fibres decreased considerably, that of the MHC-1 more than the MHC-2A. The content of MHC-2B isoform decreased only negligibly (Table 4).

The results allow the conclusions: (1) immobilization affects MHC-1, MHC-2A and MHC-2S, i.e. isoforms characteristic of fatigue-resistant fibres; (2) reaction on immobilization of the MHC-1 seems to be greater than that of the MHC-2A(S); (3) the MHC-2B is almost insensitive to immobilization. The conclusions are in correspondence with the previous ones from study on the tenotomized muscle [19].

Comparison of MHC reactions to immobilization. tenotomy and denervation.

Changes in the content of the particular MHC isoforms, described in this paper and in the previous ones on the denervated and tenotomized muscles [16, 19], are analyzed. Such analysis allows for some speculation and conclusions concerning susceptibility of the individual MHCs to the various conditions of function and innervation (Table 5).

The MHC-1 was found to be sensitive to all experimental conditions used. It seems that its content is dependent on muscle activity mainly (for comparison Tables 1 and 5). The muscle-nerve connection *per se* seems to be less important, since electrical stimulation can induce an expression of this isoform in the denervated fast muscle [32]. Thus, it seems that the MHC-1 content is regulated by the muscle activity mainly; the presence of innervation does not seem to be essential.

The MHC-2A and 2S are sensitive to immobilization and to tenotomy, but they are insensitive to lack of innervation. On the contrary, lack of innervation allows for their expression [16]. Thus, it seems that content of the MHC-2A(S) is regulated by muscle activity, but only in the innervated muscle.

The MHC-2B is insensitive to immobilization and relatively intensitive to tenotomy; the content of this isoform is suppressed by the increased muscle activity [16, 21]. On the other hand, the MHC-2B is sensitive to denervation [16], but stimulation of the denervated fast muscle (at a rate mimicking a motoneuron firing pattern of the fast-twitch muscle) is unable to substitute for the lost neuronal influence of the nerve [24]. Thus, it seems that the MHC-2B is insensitive to lack of function, on the contrary, it is suppressed by enlargement of muscle activity; the preserved muscle nerve junction seems to be necessary for expression of this isoform.

The results point out that both muscle activity and a trophic nerve influence are the factors regulating the content of MHC isoforms. These two factors seem to affect independently and variously the particular MHCs, and perhaps the corresponding fibre types. The action of these two factors seems to have some contrary influence. How they do act, is still an open question; it is therefore apparent that multiple mechanisms are bound to operate.

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