Selective Effect of Sensitization on the Phenotype of Slow and Fast Skeletal Muscle of Guinea Pig

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The use of monoclonal antibodies to the heavy chains of fast myosin shows that twice-repeated sensitization (ovalbumin + aluminum hydroxide gel) elicits different effects in the muscles examined, raising the content of the fast muscle fibers in *m. frenicus* and of the slow muscle fibers in *m. plantaris*. Changes in the immunohistochemical profile do not entirely correlate with changes in the contractile characteristics.

Key Words: *fast and slow muscle; sensitization; immunohistochemistry; myosins; contractile characteristics*

Humoral regulation of the function of skeletal muscle remains one of the least studied problems. Whereas the effect of some hormones on the morphofunctional characteristics of skeletal muscle has been thoroughly investigated [4,13], the role of other humoral factors in the regulation of skeletal muscle phenotypes has been neglected. It is not yet certain what effect sensitization has on different parameters of skeletal muscle [3,9], although the skeletal muscle tissue could hardly remain insensitive to allergic processes in the organism. The importance of studies of allergic reactions in skeletal muscle is primarily determined by clinical demands. Diverse disturbances of the external respiration, which may derive from altered functions of the diaphragmal and intercostal muscles, due to allergic reaction, are among the factors underlying the pathogenesis of bronchial asthma [5]. The slow and fast muscles of mammals have been shown to differ with respect to their plasticity and may respond differently to the same factors [2,10, 11]. We previously demonstrated, using histochemical methods, that sensitization does not alter the qualitative composition of myosins in the muscle fibers (MF) of the slow soleus muscle of guinea pig [3].

The aim of the present study was to investigate the qualitative composition of myosin and the contractile characteristics of the slow and fast skeletal muscle of guinea pig under conditions of protein-induced sensitization.

MATERIALS AND METHODS

The experiments were carried out on the fast (*m. plantaris*) and slow (*m. frenicus*) muscles of adult male guinea pigs weighing 350-400 g. Twice-repeated sensitization was performed by subcutaneous injections in the thigh of a solution containing 10 μg ovalbumin and 1 mg dry aluminum hydroxide gel in 1 ml of physiological saline per animal. The second injection was performed 14 days after the first. The level of sensitization was monitored by the methods of passive skin anaphylaxis [7] and thin-layer immune assay [8]; on day 21 the antibody (AB) titer constituted 1/256-1/1024 and 1/25-1/512, respectively. The muscles were examined 3 weeks after the start of experiments. Animals were decapitated under deep ether anesthesia, the muscles were excised, and cryostate sections 8 μ

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thick were indirectly immunohistochemically stained (PAP method) with monoclonal AB to the heavy chains of fast myosin (Sigma) [12]. Each series comprised 6 animals. The relative content of MF of different types was counted on photographic prints; the results were statistically processed using Student's t test. The data were regarded as reliable at $p=0.05$. The contractile characteristics were studied in preparations of the iliac and diaphragmal muscles with the aid of a photoelectric transducer [1]. We determined the following contractile characteristics: the latency, the time of development of the maximum tension, the force of contraction, the rate of contraction, the half-time of relaxation, and the plateau duration. A single muscle contraction was caused *in vitro* by the addition of carbocholine in a final concentration of 2×10^{-4} M to the preparations of the left part of the diaphragm (5 mm wide) and to the whole left iliac muscle incubated in a Tyrode-type solution under isometric conditions (preliminary stretching with a force of 2 g over 20 min) (Table 1). The composition of the physiological saline, prepared after Alonso-de Florida *et al.* [6], was as follows (mM/liter): Na^+ 142.90, K^+ 5.88, Ca^{2+} 1.26, Mg^{2+} 1.18, Cl^- 125.22, HCO^-_3 24.90, SO^{2+}_4 1.18, $H_2PO^-_4$ 1.18.

RESULTS

Immunohistochemical study demonstrated the presence of both fast (AB-responsive) and slow (not staining with AB) MF (Fig. 1) in the two muscles examined, but whereas fast MF prevailed in *m. plantaris*, slow MF prevailed in *m. frenicus*.

Comparison of the contractile characteristics of slow and fast muscles revealed marked differences between them, except for the force of a single contraction (Table 2).

Sensitization affected the immunohistochemical characteristics differently in the fast and slow muscles: the relative content of slow MF increased in *m. plantaris*, whereas in *m. frenicus* we observed an increase in the content of fast MF (Table 1).

The contractile characteristics of *m. plantaris* were virtually unchanged in sensitized animals, with the exception of the latency, which increased markedly. In *m. frenicus* sensitization led to changes in the majority of the contractile characteristics. For example, we recorded a marked

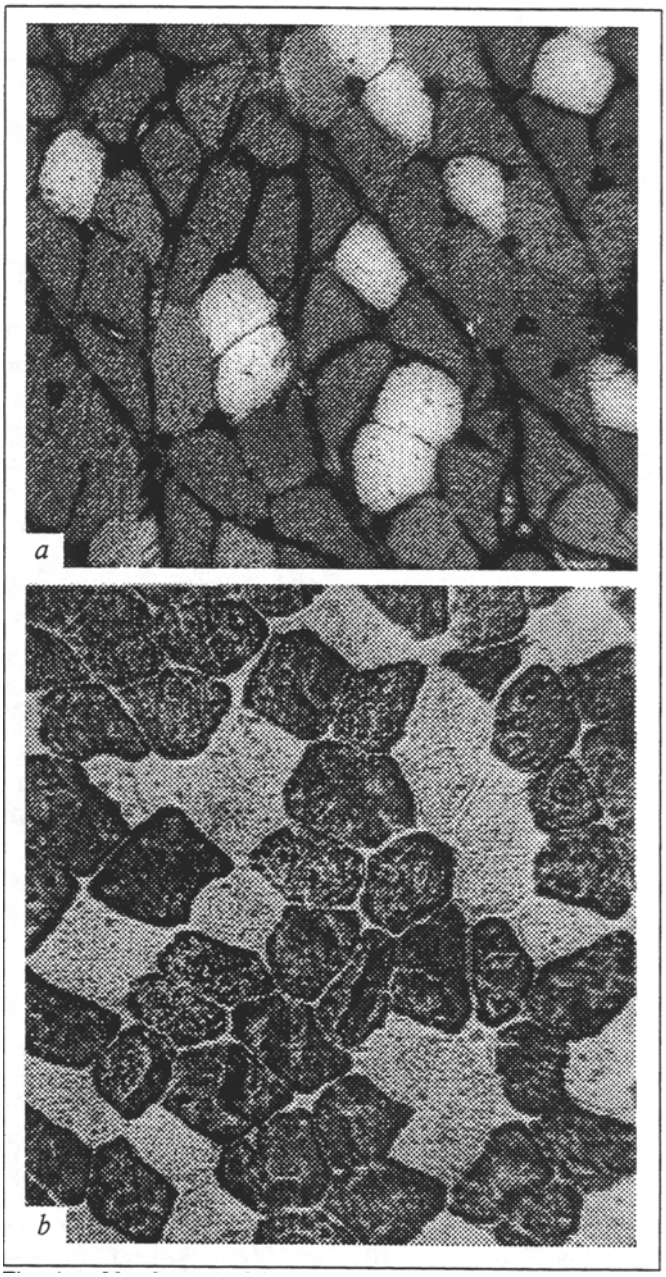

Fig. 1. *M. plantaris* (*a*) and *m. frenicus* (*b*). Immunohistochemical staining (PAP method) with monoclonal AB to heavy chains of fast myosin. Light MF: slow muscles; dark MF: fast muscles.

TABLE 1. Relative Content (%) of MF of Different Types in the Fast and Slow Muscles of Guinea Pig ($X\pm Sx$)

Experimental conditions	*M. plantaris*		*M. frenicus*	
	fast MF	slow MF	fast MF	slow MF
Intact	83.84±1.32	16.13±1.32	68.51±1.83	31.48±1.84
Sensitization	77.80±2.74*	22.20±2.74*	77.40±2.40*	22.60±2.40*

Note. An asterisk denotes reliable differences vs. the control.

TABLE 2. Contractile Characteristics of Isolated Preparations of Guinea Pig Muscles after the Addition of Carbocholine

Experimental conditions	Latent period, sec	Time of development of maximum tension, sec	Duration of plateau, sec	Half-time of relaxation, sec	Force of contraction, mg	Rate of contraction, mg/sec
Intact animals (16):						
M. plantaris	9.89±1.51	61.22±7.18	32.56±6.49	63.59±8.50	184.53±30.92	3.18±0.43
M. frenicus	4.03±0.40	13.60±0.61	5.33±0.83	15.55±0.73	147.33±15.4	10.82±0.95
Sensitized animals (11):						
M. plantaris	16.29±2.45*	66.00±14.24	27.43±7.98	64.44±8.75	172.15±48.29	2.90±0.62
M. frenicus	1.82±0.15**	21.00±1.20**	5.36±0.73	17.73±1.01	426.00±37.9**	20.23±1.46**

Note. One and two asterisks denote reliability vs. control values for $p<0.05$ and $p<0.001$, respectively. Number of animals shown in parentheses.

increase of the time, force, and rate of contraction along with a reduction of the latency (Table 2).

Our findings allow for a number of conclusions to be drawn. For instance, we discovered that *m. frenicus* and *m. plantaris* responded differently to sensitization. The share of fast MF increased in the former, and the relative content of slow MF rose in the latter, this again attesting to diverse plasticity of the fast and slow muscles.

The detected sensitization-induced changes of the contractile characteristics of the fast and slow muscles suggest that slow muscles are more sensitive to allergic processes in the organism than are fast ones.

As was mentioned above, we previously showed [3] that sensitization does not alter the initial histochemical profile of the slow *m. soleus*, whereas in the slow *m. frenicus* we observed changes in the relative content of fast and slow MF. This fact can be explained by the initial absence of MF containing fast myosin in the slow *m. soleus* of guinea pig, i.e., synthesis of this protein in the muscle is prevented at the transcription level. In such a homogeneous muscle sensitization was unable to trigger the synthesis of fast myosin, and the induction of synthesis of this protein in sensitized animals was observed by us only after preliminary denervation of the soleus [3]. It should be mentioned that such alterations of the immunohistochemical profile of the denervated soleus after injection of antigen are qualitative. As for *m. frenicus*, some MF in it already contain fast myosin (Table 1), and sensitization evidently just increases synthesis of this protein, i.e., these changes are quantitative.

An increased relative content of fast MF in the *m. frenicus* of sensitized animals may affect its resistance to fatigue, and this in turn may lead to disturbances of the external respiration. Similar changes of the external respiration, with regard to pathophysiological mechanism, are observed in bronchial asthma [5].

Thus, sensitization acts upon fast and slow muscles differently, changing their immunohistochemical and contractile characteristics accordingly.

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