Immunohistochemical Study of Rat Soleus Muscle in Various Modes of Denervation

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Immunohistochemical study with monoclonal antibodies against fast myosin heavy chains showed that excision of a fragment of sciatic nerve does not change, while compression of the nerve decreased the relative content of fast muscle fibers in rat soleus muscle. The content of fast fibers in the muscles of contralateral limbs decreased in both models of denervation. Hence, contralateral limbs cannot be used as the control in experiments with disturbed neurotrophic function. Possible mechanisms underlying changes in the immunohistochemical profiles of experimental and contralateral limbs are discussed.

Key Words: *denervation; slow muscle; myosins; immunohistochemistry*

Neurotrophic regulation of structural and functional features of skeletal muscles is performed via motoneuron impulse activity and specific trophic substances, neuregulins [6,8]. These substances are transported from motoneuron perikaryon along the axon and secreted into synaptic cleft, where they activate receptor tyrosine kinases and regulate expression of muscle fiber (MF)-specific genes. Disturbances in neurotrophic control leads to various structural and functional changes in skeletal muscles [1,2,5]. There are several modes of denervation differing by the degree of nerve alteration and hence, the denervation-induced changes in the muscle. Excision of a nerve fragment completely prevent natural reinnervation of the muscle, but it can occur after nerve compression [5]. Regulation of expression of various contractile proteins in denervated muscle is poorly studied. We previously showed that disturbances in neurotrophic control produced different alterations in fast and slow skeletal muscles [1,2]. However, changes in myosin composition in denervated and contralateral limbs in various modes of denervation are little studied. Our aim was to study the state of the soleus muscle in denervated and intact contralateral muscles in rat limbs.

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MATERIALS AND METHODS

Experiments were carried out on random-bred albino male rats. In group 1 rats (n=5) the soleus muscle was denervated by excision of a 5-mm fragment of the sciatic nerve, while in group 2 rats (n=5) denervation was

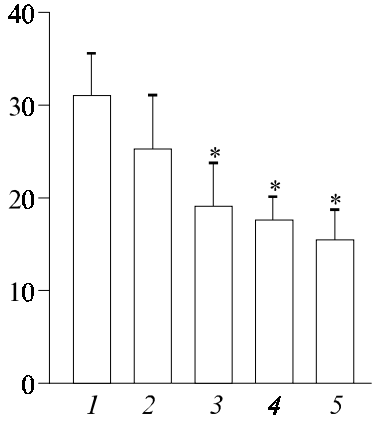

Fig. 1. Effect of various modes of denervation on relative content (%) of fast muscle fibers in rat soleus muscle in denervated and contralateral limb. *1*) intact rats; *2*) denervated limb after excision of nerve fragment; *3*) contralateral limb after excision of nerve fragment; *4*) denervated limb after nerve compression; *5*) contralateral limb after nerve compression. *p<0.05 compared to intact rats.

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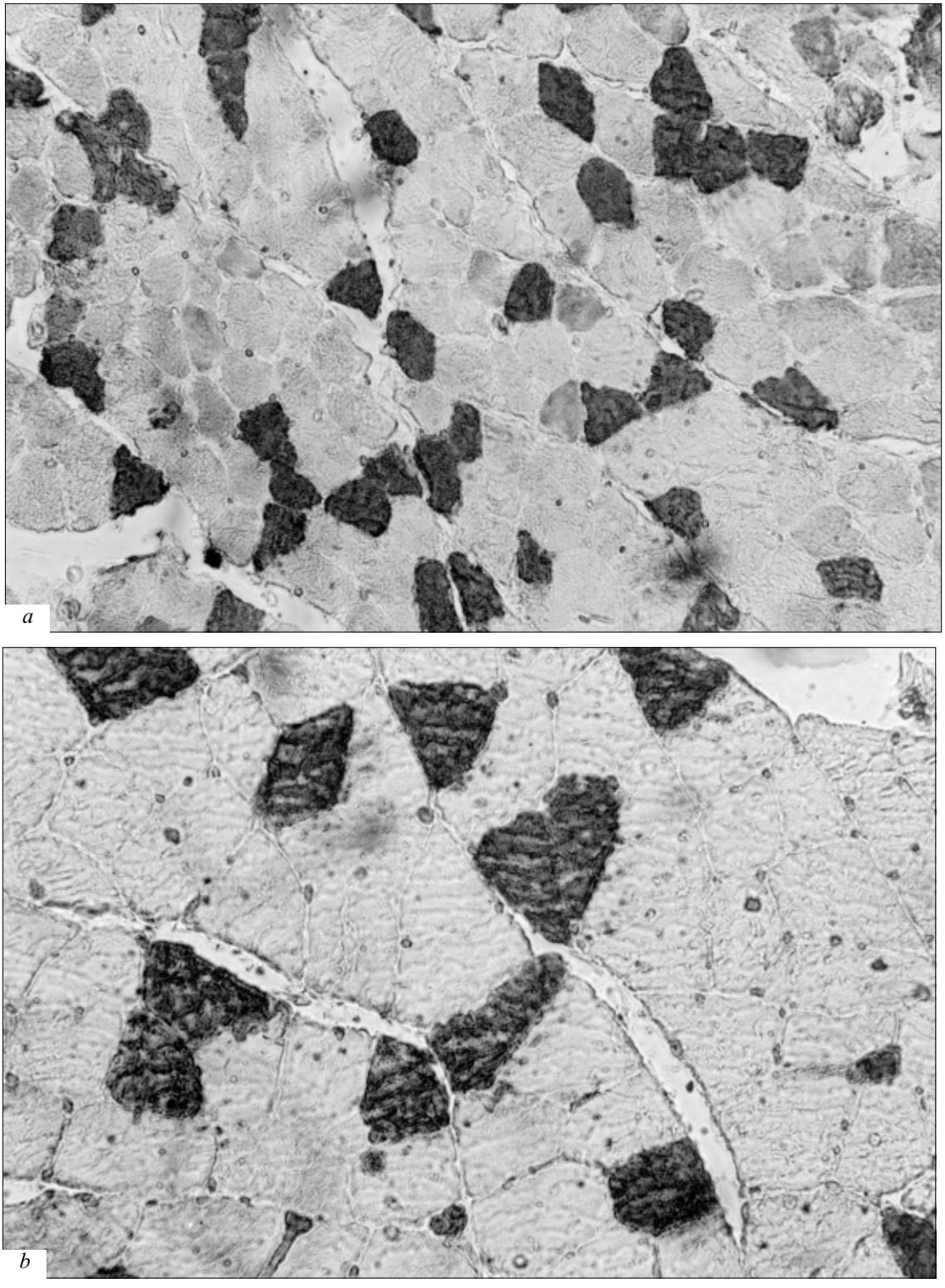

Fig. 2. Rat soleus muscle in denervated (*a*) and contralateral (*b*) limbs after excision of sciatic nerve fragment. Here and in Fig. 3: dark muscle fibers are fast, and light fibers are slow. Immunohistochemical staining with monoclonal antibodies against fast myosin heavy chains, ×400.

made by 30-sec compression the sciatic nerve with hemostatic mosquito forceps [4,7]. On denervation day 30, the rats were decapitated under deep ether narcosis, and the soleus muscle was isolated. In intact rats (n=3, control group) both soleus muscles were examined. Immunochemical staining of paraffin sections with monoclonal antibodies (Sigma) against fast myosin heavy chains was performed routinely [3]. No

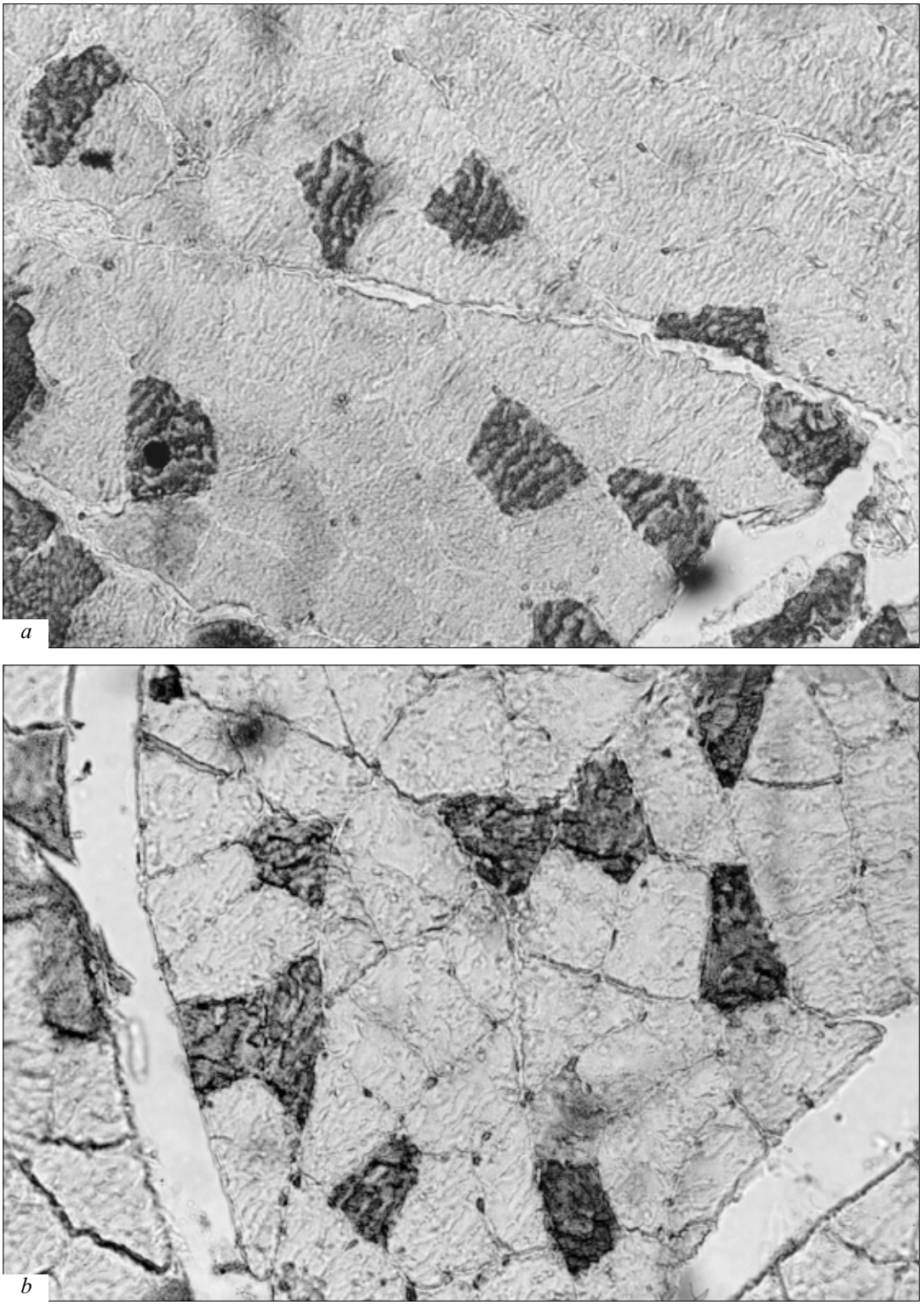

Fig. 3. Rat soleus muscle in denervated (*a*) and contralateral (*b*) limb after compression of the sciatic nerve.

less than 200 MF were counted in each preparations, and the relative content of fast MF were calculated. The results were analyzed by Student's *t* test using Statgraphics software.

RESULTS

In intact rats the numbers of fast MF in both limbs was the same. Excision of a nerve segment did not

change the content of fast MF in denervated muscle, while in the contralateral limb this parameter decreased (Figs. 1 and 2).

Maximum decrease in the content of fast MF was observed after compression denervation, which probably indicates the beginning of reinnervation [5,7]. Similar decrease in the content of fast MF was revealed in the contralateral muscle (Figs. 1 and 3).

Our findings suggest that denervation by excision of a nerve fragment produced no changes in the relative content of fast MF in denervated muscle, but reduced this parameter in the contralateral muscle. Similar decrease in the content of fast MF was observed in both muscles after compression denervation. It should be stressed that the relative content of fast MF in the contralateral limb similar by decrease in both denervation models. Hence, muscles of the contralateral limbs cannot be used as the control in studies of neurotrophic disturbances. The observed changes in the contralateral muscles are probably associated with their enhanced functional activity. Another explanation of this phenomenon can be participation of the extrapyramidal system in the regulation of skeletal muscle phenotype under given conditions. It can be hypothesized that neurons of the red nucleus on the denervated side activate contralateral neurons via the reticular formation, thus changing immunohistochemical profile of the muscle.

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