individual parameters during exposure to SFF to be determined. For example, high values of contributions of parameters describing orientation of dendrites of the apical system, obtained for FN1 cells are evidence of more significant changes in this dendritic system compared with changes in the basal system (small values of contributions of parameters describing orientation of dendrites of the basal system).

By analyzing the character of changes in the whole set of parameters, we could determine the functional importance of structural reorganization of the dendritic system of neurons during exposure to SFF, but this task lies outside the scope of the present publication.

The study of the response of visual cortical neurons to SFF can be regarded as a special case of the frequently arising task of studying the reaction of biological objects to exposure to environmental-factors of complex nature. Our suggested approach, using methods of multivariate data analysis, may prove to be of substantial help when problems of this kind are being solved, especially if variation of the conditions of exposure is difficult or, as is often the case when studying human biology, impossible.

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# IS TRANSFERRIN THE NEUROTROPHIC FACTOR CONTROLLING THE COMPOSITION OF SKELETAL MUSCLE MYOSINS?


**V. V. Valiullin, R. A. Dzamukov, R. R. Islamov, É. L. Prokhorenko, and G. I. Poletaev**



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It is generally accepted that skeletal muscle phenotypes are under neurotrophic control [7]. It is suggested that this control is realized by motoneurons by two mechanisms: through hypothetical trophic factors of peptide nature synthesized in the perikarya of nerve cells and transported to muscle by axonal transport systems, and it may also be determined by the character of spike activity [6, 13]. What are these trophic factors that are transported intraaxonally to muscle? We have as yet no unambiguous answer to this question. It has been shown that certain organoids, enzymes, and high-molecular-weight compounds can be transported by the axonal current to target cells [11]. In 1976 [15] a protein, which was called sciatin, was isolated from nerve tissue, and according to some of its features, it could possibly claim the role of one such neurotrophic factor. It was soon discovered that this protein is none other than transferrin, bound with Fe [14]. Exogenous administration of transferrin and even of trivalent ferric ions could prevent the development of certain denervation disturbances, such as: a decrease in the area of cross section of the muscle fibers (MF), an increase in expression of proteins of acetylcholine receptors, a change in the


Department of Histology and Embryology, and Department of Biology and Biophysics, S. V. Kurashov Kazan' Medical Institute.


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isozyme spectrum of lactate dehydrogenase [5, 7]. This led to the suggestion of a possible neurotrophic role of iron-containing compounds for skeletal muscle [5, 14]. It was not clear, however, whether these substances have any relation to neurotrophic control of the qualitative composition of contractile proteins of MF. To determine the contribution of neurotrophic control to maintenance of the differentiated state of skeletal muscles, several experimental approaches are available. For instance, division of the motor nerve (denervation) deprives MF of both components of neurotrophic control. In experiments with artificial stimulation of MF the role of spike activity of the motoneuron in the maintenance of muscle phenotypes has been studied [6]. The contribution of axonal transport to the regulation of muscle function has been studied in experiments in which it was blocked by application of stathmokinetic agents, such as colchicine, to the motor nerve [4]. Colchicine is known to interact irreversibly with the tubulin of the microtubules, and it has been ascribed a role in the mechanism of axonal transport [8]. Incidentally, in response to application of colchicine to a nerve, conduction of impulses along its axon is not impaired. Numerous experiments have shown that disturbance of neurotrophic control affects different characteristics of the muscle: the functional state of the membrane of MF, morphological properties, velocity parameters and, finally, the qualitative composition of the contractile proteins [3, 12]. Virtually all studies of skeletal-muscle myosins in neurotrophic disturbances have been undertaken by electrophoretic methods [12], but such an approach is not always justified, for certain isoforms of myosins have closely similar electrophoretic parameters [10], and this may lead to an incorrect interpretation of the results. In this connection, the most appropriate methods for detecting different myosins, it must be accepted, are immunologic methods using antibodies (AB) to concrete proteins or to their fragments [9]. The writers showed previously [2] that the guinea pig soleus muscle consists entirely of slow type I MF, with low ATPase activity, and not reacting to AB to fast myosin. Denervation does not change the histochemical profile of the muscle, either according to the level of ATPase activity or during immunohistochemical demonstration of fast myosin. When axonal transport is blocked by application of colchicine to the sciatic nerve, MF reacting with monoclonal AB to fast myosin, but possessing low ATPase activity, appear in this slow muscle [2]. Thus disturbance of neurotrophic control, in the form of blockade of axonal transport, leads to induction of fast myosin synthesis in the guinea pig soleus muscle.

Assuming that iron-bound transferrin in fact possesses a certain neurotrophic function, it can be expected that introduction of its exogenous form into a muscle after termination of axonal transport can prevent expression of fast myosin in it. The aim of this investigation was to test this hypothesis, by undertaking an immunohistochemical study of muscle after blockade of axonal transport and injection of transferrin.

## EXPERIMENTAL METHOD

The investigation was conducted on the slow soleus muscle of male guinea pigs weighing 350-400 g. Indirect immunohistochemical staining (by the PAP method) with monoclonal AB to fast myosin heavy chains (HC; from "Sigma") [16] was carried out on frozen sections 8 $\mu$ thick. Colchicine was applied to the sciatic nerve of the animals of one group (n = 6). A second group consisted of animals into which a solution of transferrin was injected intramuscularly in a dose of 0.5 mg/kg body weight daily for 3 weeks. Finally, in the third group colchicine was applied to the animals and the transferrin preparation was injected into the lower limb undergoing operation, on a similar schedule. The animals' muscles were studied 3 weeks after the experiment began. The operative techniques and design of the controls were described by the writers previously [1].

## EXPERIMENTAL RESULTS

The soleus muscle of the intact guinea pig is formed entirely of slow MF which do not react with monoclonal AB to fast myosin HC (Fig. 1). The initial histochemical profile of the muscle likewise was unchanged after injection of transferrin, for all MF contained slow myosin and did not stain with AB. Blockade of axonal transport, as already stated above, led to the appearance of fast, darkly stained MF, interacting with AB, in the muscle (Fig. 2). When transferrin was injected after application of colchicine to the nerve, we also found MF containing fast myosin (Fig. 3), and they were actually more numerous than in the muscle of the animals after blockade of axonal transport (Table 1).

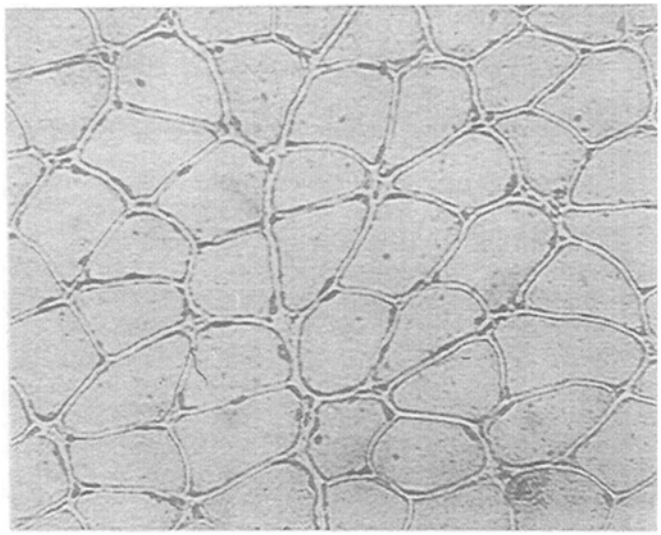

Fig. 1

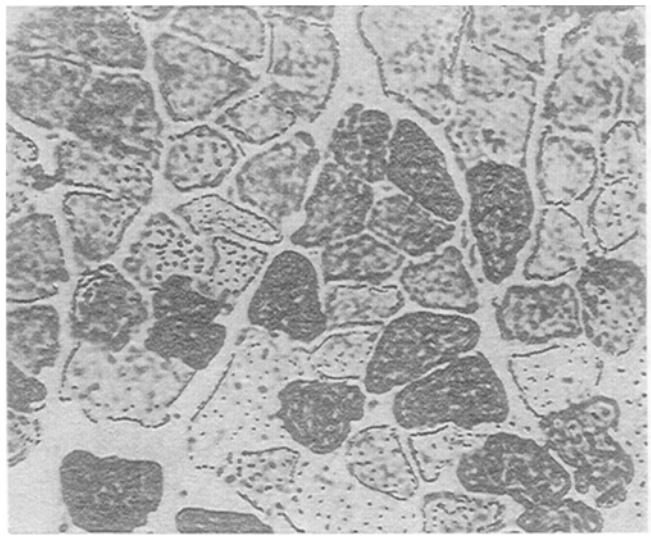

Fig. 2

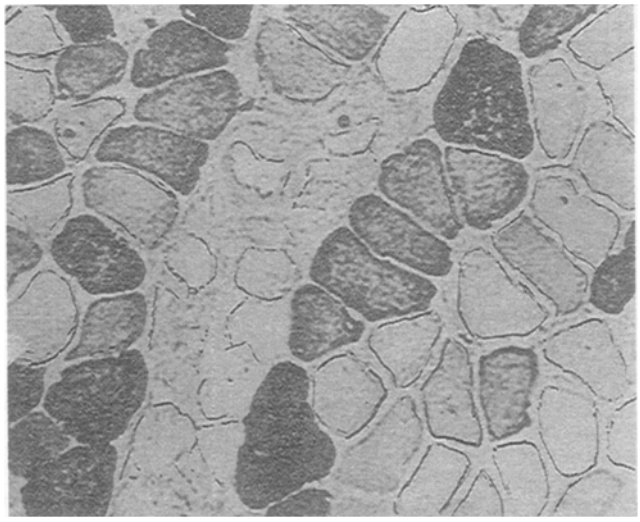

Fig. 3

Fig. 1. Soleus muscle of intact guinea pig. Here and in Figs. 2 and 3 – immunohistochemical staining (PAP method) with monoclonal AB to fast myosin heavy chains. Pale muscle fibers are slow, dark fibers fast.

Fig. 2. Soleus muscle of guinea pig after application of colchicine to sciatic nerve.

Fig. 3. Soleus muscle of guinea pig after application of colchicine to sciatic nerve and injection of transferrin.

TABLE 1. Relative Content (in %) of Muscle Fibers of Different Types from Guinea Pig Soleus Muscle under Different Experimental Conditions ($\bar{X} \pm S_{\bar{x}}$)

| Experimental conditions | Muscle fiber of soleus muscle | |
|---|---|---|
| | fast | slow |
| Control | — | 100 |
| Injection of transferrin | — | 100 |
| Application of colchicine | 13,83±2,33 | 86,17±2,33 |
| Application of colchicine + injection of transferrin | 20,98±1,13* | 79,02±1,13* |

**Legend.** Asterisk indicates significant differences compared with muscle after application of colchicine.

The results indicate that injection of transferrin into a muscle after application of colchicine to the motor nerve did not prevent synthesis of fast myosin in it. It can accordingly be concluded that transferrin is evidently not a neurotrophic factor controlling the qualitative composition of skeletal muscle myosins. Moreover, quantitative analysis of the relative content of MF of different types showed (Table 1) that when axonal transport is blocked transferrin induces fast myosin synthesis in the guinea pig slow muscle.

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