white matter, are axons. Spines are clearly visible on the base of the lower dendrite of the motoneuron on the left.

These results, which in our opinion are very important for physiologists, help to explain some functional features distinguishing the phrenic nucleus and, in particular, the mechanism of synchronous activation of phrenic motoneurons. The arrangement of packing of the dendrites in bands itself suggests the existence of connections between them. These connections should be most probably be electrical. However, Lipski [5] failed to find them, although he does not rule out the possibility that his technique was not completely adequate. In the present investigation morphological structures which can form synapses between dendrites are described. Unfortunately, the final conclusion regarding the existence of synapses can be drawn only on the basis of electron microscopy. It is difficult to evaluate the functional significance of the *en passant* synapses and the spinous formations in the phrenic nucleus [3]. Nevertheless, if our hypothesis [1] that respiratory fibers from the pacemaker zone of the respiratory center reach dendrites of the phrenic nucleus is correct, the existence of synapses between dendrites, and perhaps of electrical communications between them, provides the structural basis and a mechanism of synchronous activation of the motoneurons of that nucleus. The view expressed in one publication, that bands of dendrites in the spinal cord can perform the role of substrate for central programs of stereotyped activity [7], must be recalled.

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EFFECT OF HORMONAL AND NEUROTROPHIC FACTORS ON EXPRESSION OF FAST MYOSIN IN SLOW MUSCLE

V. V. Valiullin and N. P. Razvyakov

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It is now generally considered that the phenotypes of skeletal muscle fibers (MF) are controlled by many different factors [2, 3]. The most important of these factors are neurotrophic and hormonal control. Motoneurons, for instance, determine differentiation and maintenance of the differentiated state of different types of MF by means of neurotrophic substances synthesized in the perikaryon and transported intraaxonally to the muscle [3], and also by the character of the spike discharge of motoneurons [10]. It has also been shown that both increased and reduced concentrations of iodine-containing thyroid hormones in the body may lead to changes in muscle phenotype [6, 8, 9]. For instance, after administration of L-thyroxine (T_4) to guinea pigs MF containing fast myosin appear in a slow (the soleus) muscle [1, 8]. Motor denervation of the muscle followed by injection of T_4 caused an increase in the relative number of fast MF in that muscle [1]. Since both axonal transport of substances and nervous impulses cease after division of the motor nerve to a muscle, it was decided to study how T_4 affects the phenotype of a muscle when axonal transport is blocked but when transmission of impulses along axons of motoneurons still continues.

This paper gives the results of a study of the histochemical, morphometric, and immunochemical characteristics of guinea pig slow muscle under the conditions described above.

Chair of Histology and Embryology, S. V. Kurashov Kazan' Medical Institute.

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TABLE 1. Relative Number (in %) of MF in Soleus Muscle under Different Experimental Conditions ($X \pm S_X$)

Experimental conditions	Type of MF	
	I	II
Denervation	100	—
Application of colchicine	100	—
Injection of T_4	91,5±1,3	8,5±1,2
Denervation + T_4	84,9±1,4*	15,1±1,4*
Application of colchicine + T_4	87,4±5,5	12,6±5,5

Note. *) Differences significant compared with injection of T_4.

EXPERIMENTAL METHOD

Guinea pigs weighing 350-400 g were used. Under sterile conditions a 10 mM solution of colchicine (from Ferak, West Germany) was applied for 6 min to the sciatic nerve of the animals of one group (n = 6). Starting from the day after application of colchicine to the nerve, T_4 in a dose of 200 µg/kg body weight was injected subcutaneously on alternate days for 3 weeks into the animals of another group (n = 6). Guinea pigs of the third group (n = 6) received injections of T_4 by the same schedule, but after division of the sciatic nerve. The animals were decapitated under deep ether anesthesia and frozen sections through the muscles were cut to a thickness of 10 µ, for demonstration of adenosine triphosphatase (ATPase) [7] and succinate dehydrogenase (SDH) [4] activity and for counting the relative numbers of MF of different types. In intact animals (n = 3) the sciatic nerve below the site of application of colchicine and a homologous region of the nerve were isolated, fixed in 2.5% glutaraldehyde solution, stained in 2% OsO_4 solution, and embedded in Epon-Araldite. The number of axon profiles in an area of cross-section of the nerve of 10,000 μ^2 was counted in semithin sections stained with methylene blue. Methods of isolation of myosin from the muscles, of obtaining antibodies to fast myosin, and of immunochemical detection of fast subjected to statistical analysis [5], at the 0.05 level of significance.

EXPERIMENTAL RESULTS

The intact soleus muscle of guinea pigs consists of type I MF (slow MF) with low ATPase activity, and after histochemical demonstration of SDH all the MF had the same intensity of staining, characteristic of MF of the B type. The histochemical profile of the muscle after staining for ATPase and SDH activity remained the same 21 days after application of colchicine to the nerve, but the intensity of staining of MF for SDH was reduced. After application of colchicine to the nerve and injection of T_4, MF with high ATPase activity (fast MF) were found in the soleus muscle, just as in animals with denervation of the muscle and receiving T_4; the relative number of these MF did not differ from that in the homonymous muscle of animals undergoing denervation and receiving T_4 (Table 1). All MF under these circumstances has the same SDH level, identical with the control. The double immunodiffusion test showed that myosin extracted from the soleus muscle of animals to whose sciatic nerve colchicine was applied, like myosin from muscles of the control animals, did not precipitate with antibodies to fast myosin. Myosin from the muscles of animals to whose sciatic nerve colchicine was applied, and which received injections of T_4, formed a precipitation band with antibodies to fast myosin, just as in animals with denervation and receiving injections of T_4 (Fig. 1).

Application of colchicine to the nerve and a hyperthyroid status did not cause anatomical denervation of the muscle, for the study of transverse semithin sections through the sciatic nerve showed that the number of axon profiles under experimental (application of colchicine to the nerve + injection of T_4) and control conditions did not differ statistically significantly (73 ± 3 and 70 ± 2, respectively).

Injection of T_4 induced reprogramming of myosin synthesis in some MF [8]. The increase in the relative number of type II MF after administration of T_4 preceded by denervation can be explained on the grounds that thyroxine increases synthesis of fast myosin in the skeletal muscles, whereas removal of neurotrophic control (by denervation) coupled with injection of T_4 caused an increase in the number of MF synthesizing fast myosin, as the writers showed previously [1]. It can be tentatively suggested that neurotrophic control in the slow muscle represses expression of fast myosin. In that case it becomes clear why the hyperthyroid

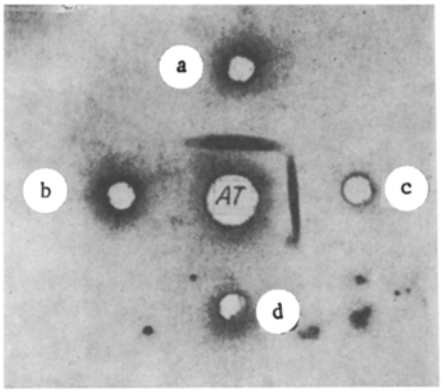

Fig. 1. Immunodiffusion of myosins of soleus muscle against antibodies to guinea pig fast myosin: a) myosins from muscle of control animals; b) after application of colchicine to nerve; c) after application of colchicine and injection of T_4; d) myosins of fast (soleus) muscle of control animals. AB) Antibodies to fast myosin.

status in the denervated slow muscle leads to an increase in the relative number of fast MF compared with that in the innervated muscle of thyrotoxin animals.

Since virtually identical results were obtained in the series of experiments with denervation and injection of T_4 (when both the spike discharge of the motoneurons and axonal transport of materials were abolished) and in experiments with application of colchicine and injection of T_4 (when only transport of materials was blocked), it can be concluded that the neurotrophic control factor, determining expression of fast MF in a slow muscle is not the character of spike activity of the nerve, but neurotrophic substances transported along the axons.

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