EXPERIMENTAL BIOLOGY

Catabolic Effect of Anabolic Steroid in Skeletal Muscle under Conditions of Impaired Neurotrophic Regulation

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Effect of anabolic steroid nandrolone (Phenobolin) on fast (plantar) and slow (soleus) skeletal muscles was studied in guinea pigs with impaired neurotrophic regulation (denervation, axonal transport blockade) or after tenotomy. Immunohistochemical analysis with monoclonal antibodies to fast myosin heavy chains showed that injection of anabolic steroid did not modify the relative content of fast and slow muscle fibers in the studied muscles under all experimental conditions. Injection of anabolic steroid did not modify the weight of the studied muscles and did not prevent its drop after denervation or tenotomy. Axonal transport blockade by colchicine application on the nerve induced the appearance of fast muscle fibers in the slow soleus muscle and an increase in its weight; in the slow muscle, nandrolone did not prevent the induction of fast myosin synthesis. Under conditions of axonal transport blockade, the agent exerted a catabolic effect and considerably reduced the muscle weight.

Key Words: *skeletal muscle; myosin immunohistochemistry; neurotrophic control; tenotomy; anabolic steroid*

The rate of contraction of fast and slow muscle fibers (MF) is determined by the qualitative composition of contractile proteins, primarily skeletal myosins [9]. By the predominance of this or that type of fibers, skeletal muscles (SM) are divided into fast and slow, which exhibit different plasticity under different conditions of functioning [8]. These adaptation processes are regulated by the nervous and humoral systems [3].

Disturbances of the neurotrophic regulation induce a drop in the muscle weight, a decrease in the activities of many enzymes, connective tissue proliferation, *etc.* [4]. These changes, described as the denervation syndrome, involve the composition of contractile proteins, in particular myosins [10]. Blockade of the axonal transport by colchicine application on the nerve [2] leads to the appearance of MF containing fast myosin in homogeneously slow *m. soleus* in guinea pig.

Apart from the nervous regulation, SM are regulated by hormones: excess and deficit of some hormones lead to morphofunctional changes in SM [6]. Sex hormones possess a specific myotrophic effect, and therefore their effects on SM deserve special studies. Androgens and their derivatives anabolic steroids (AS) stimulate protein synthesis in muscles, while estrogens exert a catabolic effect on SM [3]. However, the effects of AS on the qualitative composition of contractile proteins in SM are still little studied.

In addition to nervous and hormonal effects, the SM phenotype depends on functional load [11]. If SM do not function, as is the case when the animal is suspended [14], subjected to tenotomy [12], or under conditions of weightlessness [7], they undergo atrophy and their contractile proteins degrade. In contrast, intense exercise (treadmill running) stimulates the production of contractile and energy proteins [13].

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Disorders in the nervous and humoral regulation of SM function modify their phenotype. The effects of some hormones and of motor neuron on SM have been extensively studied, while the interactions between these two regulating systems received little attention. We do not know how different hormones affect the development of denervation disorders in the muscle, *e. g.*, the qualitative composition of contractile proteins in MF. The considerable myotrophic effect of AS suggests that these hormones will prevent to a certain extent muscle atrophy after disruption of the neurotrophic control or exercise deprivation of the muscle. Therefore, we investigated the effect of AS nandrolone on SM under conditions of impaired neurotrophic regulation and after tenotomy.

MATERIALS AND METHODS

Slow *m. soleus* and fast *m. plantaris* of adult male guinea pigs were analyzed. Oil solution of nandrolone phenylpropionate (1%, Phenobolin, Durabolin,) was intramuscularly injected to animals in a daily dose of 0.9 mg/kg for 3 weeks [15].

Experimental models of impaired neurotrophic regulation were denervation and axonal transport blocking. For muscle denervation, a 3-4-mm fragment of the sciatic nerve was dissected under ether narcosis under aseptic conditions; axonal transport was blocked by 10-min application of 10 mM colchicine (Merck) to the same portion of the sciatic nerve [1]. Exercise deprivation consisted in tenotomy (dissection of the Achilles tendon). Nandrolone injections were started on the next day after surgery and were carried out for 3 weeks.

Six animals were used in each experimental series. Muscles were examined 3 weeks after the beginning of experiments. Before collecting the material, the animals were not fed the evening before and weighed in the morning. Muscles were removed, weighed, and frozen in liquid nitrogen. The weight of examined muscles was expressed in percent of total body weight and of muscle weight in intact animals. Immunohistochemical reaction (PAP-method) was carried out on transverse cryostat sections (8 μ) with monoclonal antibodies to fast myosin heavy chains (Sigma) [5]. The relative content of MF of different types was evaluated by counting no less than 600 fibers per muscle. The results were statistically processed using Student's *t* test.

RESULTS

Soleus muscle from intact guinea pig contains only slow MF which do not react with antibodies to fast myosin (Fig. 1, *a*). *M. plantaris* contains both fast and slow MF, as was shown by immunohistochemical analysis for fast myosin.

Unlike denervation and tenotomy, colchicine application to the sciatic nerve caused the appearance of fast MF reacting with antibodies to fast myosin in *m. soleus* (Fig. 1, *b*). *M. soleus* also contained fast MF after blockade of axonal transport and nandrolone injections (Fig. 1, *c*). Hence, nandrolone did not prevent the production of fast myosin in slow muscle after axonal transport blockade. The relative content of fast and slow MF in *m. plantaris* did not change in all experimental series. Therefore, our results indicate that nandrolone does not modify the qualitative composition of myosins in the studied muscles even under conditions of impaired neurotrophic regulation or tenotomy.

In intact animals, nandrolone did not affect the weight of the examined muscles (Table 1).

Denervation and tenotomy decreased the weight of examined muscles, and AS did not prevent this decrease. Injection of nandrolone after colchicine application to the nerve was associated with rapid weight loss in both fast and slow muscles. Therefore, under conditions of axonal transport blockade, AS produced not anabolic (as expected), but even catabolic effect more pronounced in the slow muscle.

Our results indicate that anabolic effect of androgens can manifest in SM only under conditions of preserved functional activity and innervation.

TABLE 1. Muscle Weight (% of Body Weight $\times 10^3$) under Different Experimental Conditions ($M \pm m$)

Experimental conditions	*M. plantaris*		*M. soleus*	
	no injections	nandrolone	no injections	nandrolone
Control	73.0±2.55 (100.0)	74.7±2.07 (102.3)	32.3±0.99 (100.0)	31.3±1.23 (96.9)
Denervation	38.3±1.74*** (52.5)	38.3±1.79*** (52.5)	23.8±0.76*** (73.7)	21.7±1.31*** (67.2)
Axonal transport blockade	55.6±0.38*** (76.2)	36.3±0.52*** (49.7)	41.2±2.96* (127.6)	19.4±0.99*** (59.8)
Tenotomy	64.5±2.28*** (88.4)	60.5±3.16** (82.9)	22.6±1.16*** (69.9)	21.6±0.77*** (66.9)

Note. Muscle weight in % of muscle weight in intact animals is given in parentheses; *$p<0.05$, **$p<0.01$, ***$p<0.001$ in comparison with the control.

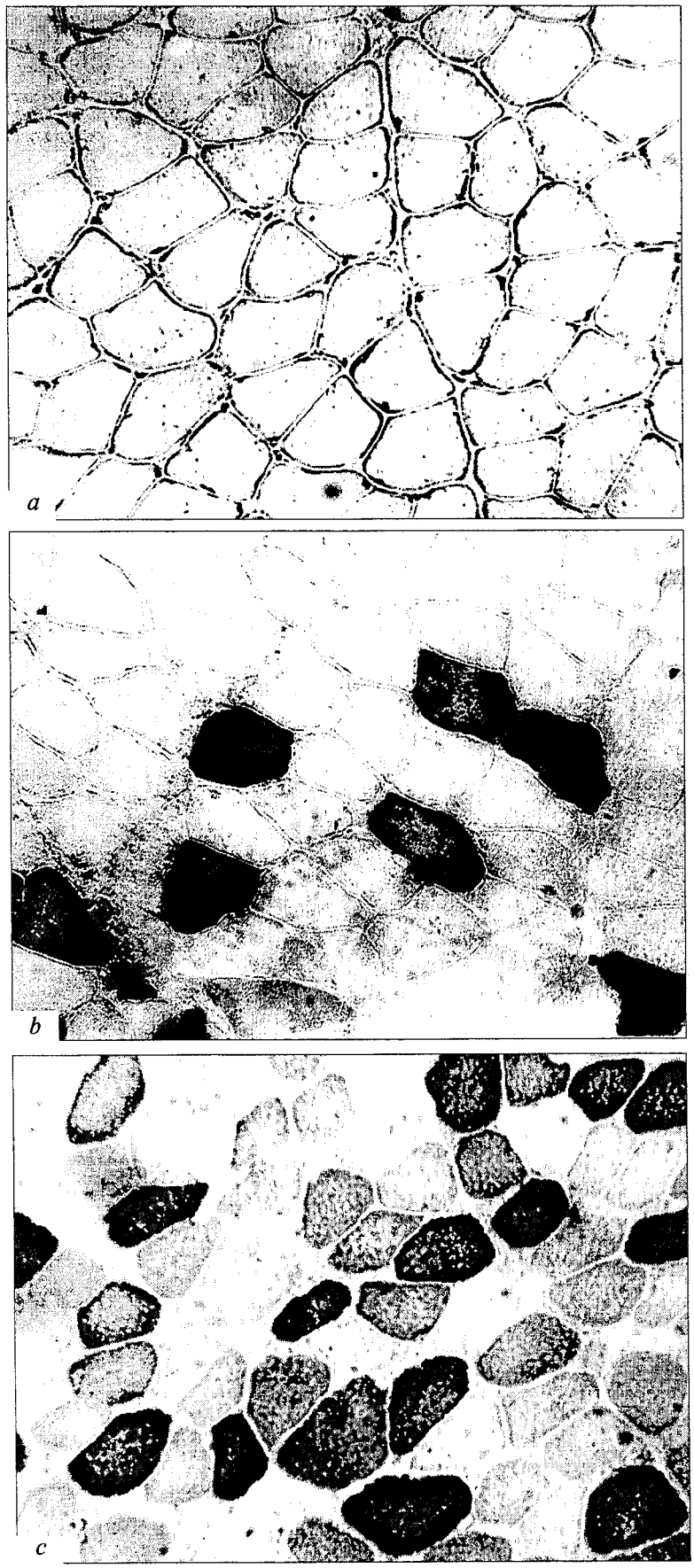

Fig. 1. Soleus muscle from guinea pig, ×140. *a*) control; *b*) after colchicine application to the sciatic nerve; *c*) after colchicine application to the sciatic nerve and nandrolone injections. Immunohistochemical staining with monoclonal antibodies to fast myosin heavy chains. Light (slow) and dark (fast) muscle fibers.

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