The Response of Skeletal Muscles to Anabolic Steroid Is Individual and Does Not Depend on the Motor Activity Mode

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Histochemical methods demonstrated that injection of the anabolic steroid phenobolin and exercise did not modify the content of fast and slow muscle fibers in the slow (*m. soleus*) and fast (*m. plantaris* and *m. semimembranosus*) muscles. Energy metabolism changed only in the fast muscles. After exercise the number of oxidative muscle fibers increased in both fast muscles and after injection of anabolic steroids in *m. semimem-branosus*. Combined exposure produced an additive effect. The content of glycolytic muscle fibers in *m. plantaris* increased under the effect of anabolic steroids.

Key Words: *skeletal muscle; myosins; exercise; anabolic steroid*

The ability of male sex hormones to stimulate protein production in various tissues (anabolic effect) was discovered in 1935 [13]. Under experimental conditions androgens prevent skeletal muscle atrophy after castration (myotrophic effect). Anabolic effect of androgens, *e. g.* testosterone, manifests during diseases associated with high protein degradation: chronic infections, severe injuries, surgical interventions, *etc.* Pronounced masculinizing (androgenic) effect of testosterone prevents its medical application as an anabolic agent. Therefore, anabolic steroids (AS) — drugs with high anabolic and minimal androgenic activity were synthesized on the basis of testosterone.

Study of the effect of AS on skeletal muscles showed that each muscle responded individually to AS treatment [8]. Moreover, responses of the same muscle vary in different animals [9,10].

Classifications of muscle fibers (MF) based on the qualitative composition of myosin or its ATPase activity [6] dividing the MF into fast and slow are the most adequate to the rate of contraction. For identification of MF by the type of energy metabolism, activities of some enzymes, *e. g.* succinate dehydrogenase (SDH) are histochemically detected. This enzyme is found only in the mitochondria and its activity reflects the activity of the Krebs' cycle. Typing of MF by the activity of SDH identifies three types of fibers: with low activity of the enzyme, glycolytic (type A); with intermediate activity, oxidative glycolytic (type B); and with high activity, oxidative (C type). Oxidative and oxidative glycolytic MF are little fatigable, while the glycolytic ones are easily fatigable. Depending on the exercise intensity, this or that type of muscles is mainly working [4]. In movements of low intensity mainly oxidative fibers are working, in moderate exercise oxidative glycolytic ones work, while the glycolytic fibers are involved in potent short-term contractions [1]. As the intensity of aerobic metabolism of MF increases, the muscle is less liable to fatigue and its working capacity is quicker restored.

Anabolic steroids markedly increase the resistance of skeletal muscles to fatigue and to a lesser extent the contraction force [5,7]. The effects of AS on the qualitative myosin composition of the fast and slow skeletal muscles and on energy metabolism, including that during adaptation of the muscle to a new mode of motor activity, are little studied. At present, AS are widely used in medicine for maintaining normal protein metabolism and in athletics during training for purposeful regulation of the strength and contractile characteristics of certain muscles [14].

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We investigated the effects of AS on the myosin composition and energy metabolism of fast and slow muscles of guinea pigs, including those in intense exercise.

MATERIALS AND METHODS

Slow (*m. soleus*) and fast (*m. plantaris* and head of fast *m. semimembranosus*) from adult male guinea pigs were examined. To group 1 animals (n=6), 1% oil solution of AS phenobolin (durabolin, nandrolone-phenylpropionate) was injected daily for 3 weeks in a dose of 0.9 mg/kg. Phenobolin dose was estimated with consideration for AS doses used in athletics [13]. Group 2 animals (n=6) were trained for 3 weeks (treadmill running, 20 m/min, 6 days per week, 45 min a day). For more complicated conditions of running the track was positioned at a 10° angle [12]. In group 3 (n=6) the animals were injected phenobolin in parallel with exercise.

After 3 weeks, the activities of alkali-resistant myosin ATPase [11] and SDH [2] were histochemically evaluated on transverse cryostat muscle sections (10 μ) and activity of fast myosin were evaluated immunohistochemically (PAP method) using monoclonal antibodies to fast myosin heavy chains (Sigma). The relative content of MF of different types was evaluated after counting at least 600 MF per muscle. The results were statistically processed using Student's t test.

RESULTS

ATPase activity and the presence of fast myosin in *m. plantaris* and in the fast part of *m. semimembranosus* of intact animals indicated the presence of fast and slow MF, with the latter predominating. Typing of SDH activity showed three types of MF in this muscle: A, B, and C.

No MF reacting with antibodies to fast myosin with a high ATPase activity were detected in *m. soleus* of intact guinea pigs, *i. e.* all MF were slow. Only type B MF were identified by SDH activity in this muscle.

No changes in the studied parameters were detected in *m. plantaris* in all experimental series.

In fast muscles the relative content of fast and slow MF remained unchanged. This indicates that neither AS, nor strenuous exercise, nor combination thereof modified the qualitative composition of myosins. Changes in the ratio of MF types were detected in fast muscles in all series of experiments. The number of glycolytic MF increased in *m. plantaris* of animals injected with phenobolin, and the number of oxidative muscles increased in *m. semimembranosus* (Table 1). Strenuous exercise increased the proportion of oxidative MF in both fast muscles. The number of oxidative MF in *m. plantaris* of guinea pigs injected with AS during exercise was significantly lower that in animals trained without the hormone, but higher than in intact animals. Hence, phenobolin retained its capacity to shift the energy metabolism towards glycolysis under conditions of intense muscular activity. In *m. semimembranosus*, the effects of exercise and AS were similarly directed, therefore their summary effect on the muscle was higher than individual.

The results indicate that the effect of AS on energy metabolism of studied MF is not lost during muscle adaptation to a new mode of functioning, and the effect of the hormone is summarized with the effect of exercise in each muscle.

Anabolic steroids have been created on the basis of testosterone, and their effects are realized through androgen receptors [13]; our findings suggest that the reaction of each skeletal muscle to the level of sex hormones is genetically determined. This apparently determines the individual response of the muscles to seasonal fluctuations of sex hormone levels in warm-blooded animals.

Extrapolating these results to human muscles, we conclude that use of AS as a dope is not desirable for all athletes. AS can be effective only if the muscles work in the mode maintained by the agent.

TABLE 1. Relative Content (%) of MF in Guinea Pig Fast Muscles ($M \pm m$)

Experimental conditions	MF types					
	m. plantaris			*m. semimembranosus*		
	A	B	C	A	B	C
Control	46.10±0.97	44.21±1.12	9.70±1.46	57.84±2.25	38.00±2.44	4.16±0.48
Phenobolin	58.82±1.31***	32.13±0.88***	9.05±0.88	57.64±1.87	31.51±0.92*	10.85±1.09**
Exercise	45.25±1.65	28.79±1.62***	25.96±1.45***	47.65±4.68	42.84±4.89*	9.51±0.74***
Exercise+ phenobolin	48.48±2.06	31.58±1.90***	19.94±1.29**	55.12±2.74	27.38±3.97	17.50±1.73***

Note. *p<0.05, **p<0.01, ***p<0.001 *vs.* the control.

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