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ANABOLIC STEROID METABOLISM IN SKELETAL MUSCLE

V. A. ROGOZKIN
Research Institute of Physical Culture, Leningrad, U.S.S.R.

SUMMARY

Three hundred and sixty male albino rats weighing 180 to 200 g were used to determine the effect of anabolic steroid hormones on adaptive changes in the synthesis of ribosomal RNA both in sedentary animals and in animals involved in a training programme. One injection of Retabolil (0.1 mg/100 g body weight) increased the α-amanitin insensitive RNA polymerase activity of nuclei from skeletal muscles. Fourteen h after this hormone injection the enzyme activity was 45% higher than in control animals and it remained at this level for 4 days. Under these conditions a selective binding of 19-nortestosterone with cytoplasmic proteins of skeletal muscle was found. Physical training increased the RNA polymerase activity by 50% ($P < 0.05$). It was found that the testosterone binding capacity of a cytoplasmic extract from trained animals was 70% greater than that of the control animals ($P < 0.05$). Four injections of Retabolil during training resulted in an additional increase of RNA polymerase activity of 40% ($P < 0.05$) but reduced the testosterone binding capacity of the cytoplasmic proteins that occurred with training by 21%. These results demonstrate the effect of anabolic hormones in the regulations of RNA synthesis in skeletal muscle nuclei in the process of their adaptation to systematic physical training.

INTRODUCTION

Study of the different stages of protein synthesis in skeletal muscle has established the mechanisms by which modifications of the basic processes are controlled by the genetic information contained within the cell. This process includes the transcription, transport and translation of genetic information to a final expression in protein synthesis. It is these processes that provide for the increase in the functional capacity of the skeletal muscles that occurs with physical training.

Evidence of changes in protein metabolism during systematic physical training [1] gives rise to the question of how the activity of the system for the regulation of protein synthesis in skeletal muscle is influenced by the endocrine system. Some data are available indicating that the androgenic hormones may take part in the adaptive activation of the anabolic phase of protein metabolism [2]. These previous research findings, along with contemporary ideas that the primary effect of steroid hormones on the major target tissues is through a modification of RNA synthesis [3], have made it possible to suggest that the presences of a dynamic mechanism for the control of protein synthesis in skeletal muscle is at the level of transcription of ribosomal RNA (rRNA).

In view of the above considerations the present studies were undertaken to examine the effects of the anabolic steroids Retabolil (17β-hydroxestr-4-en-3-one-17-decanoate) and of the combined effects of physical training and Retabolil on the RNA polymerase activity of skeletal muscle. Testosterone binding in skeletal muscle of sedentary animals and of animals subjected to an experimental exercise programme has also been examined.

MATERIALS AND METHODS

The experiments were performed on male albino rats weighing 180–200 g. The animals in the trained group were exposed to a daily 10-min period of swimming in water at 32°C. A load of 6% of the body weight was attached to each rat. The duration of the daily swim was gradually increased from 10 min at the beginning to 20 min after 10 days of training. A dose of 0.1 mg/100 g body weight of the anabolic hormone Retabolil was injected intramuscularly during training period. Four injections were given. The first injection was given 2 days before beginning training programme and other injections were given every 4 days.

Nuclei were prepared from the thigh muscle of the animals by differential centrifugation in a medium of 2.2 M sucrose [4]. The entire preparative procedure was performed at 4°C. Animals were sacrificed by decapitation. Muscles (30 g) from the hindlimbs of 2 animals were homogenized in 3.5 vol. of 2.47 M sucrose containing 3 mM $MgCl_2$ in a Woring homogenizer for 5 min. The suspension was filtered through nylon filter (150 mesh) and centrifuged for 2 hr at 45,000 *g*. The nuclear pellet was resuspended in 60 mM Tris–HCl buffer, pH 8.0, containing 12.5% glycerol and 6 mM $MgCl_2$.

The hormone binding capacity of the cytoplasmic proteins was determined after separation of the free radioactive hormone and steroid which binding with proteins on columns of Sephadex G-25. Testosterone binding and corticosterone binding capacities in the cytoplasm from the skeletal muscles were analyzed by standard procedures [5]. The cytoplasm (supernatant after centrifugation homogenate muscles in 3 vols. Tris–HCl, pH 7.4, contained 10 mM dithiotreitol,

1.5 mM EDTA at 20,000 *g* for 30 min) was treated with charcoal to remove most of the endogenous hormones before determinating the testosterone binding capacity. The cytoplasmic extract was incubated with [^{3}H]-testosterone (10^6 c.p.m.) or [^{3}H]-corticosterone (10^6 c.p.m.). Tubes containing the cytoplasmic extract along with labelled steroids were incubated in parallel with those containing the unlabelled steroids as well as labelled steroids. Following incubation the extracts (1 ml, 6 mg protein) were transferred to columns 16 cm, 1 cm diameter, containing Sephadex G-25 and the free radioactive hormone and the hormone bound with protein was separated by collecting in fractions (1.0 ml). The levels of radioactivity were determined with Mark III liquid scintillation system.

RNA polymerase activity of the nuclei was determined in a low ionic strength medium as described elsewhere [6]. Reaction mixtures contained the following components: 60 mM Tris–HCl, pH 8.0; 50 mM ammonium sulphate; 6 mM magnesium chloride: α-amanitin, 2 μg/ml; 12.5% glycerol; 3 mM dithiothreitol; ATP, CTP, GTP, 0.4 mM; [^{3}H]-UTP or [^{14}C]-UTP 0.01–0.02 mM; and nuclear suspension. Incubation was performed at 25°C. Aliquots (0.05 ml vol., 20–30 μg DNA) were put on filter (FII-track-390) every 5 min during period of incubation. Filters were washed with 50 ml of cold 0.5 M $HClO_4$ − 0.1 M $Na_4P_2O_7$. The incorporation of radioactivity into RNA was determined by counting dried filters in a liquid scintillation spectrometer. The capacity of nuclei to synthesize RNA under these conditions is determined by the activity of the enzyme DNA-dependent RNA polymerase and results in the synthesis of rRNA.

For the determination of RNA-se activity in the reaction medium after 11 min of incubation unlabelled UTP (final concentration 5 mM) was added. The subsequent procedure was as described above.

RESULTS AND DISCUSSION

In the first experiment the effect of Retabolil on the synthesis of rRNA in skeletal muscle of sedentary rats was studied. It was found that a single hormone injection resulted in a significant ($P < 0.05$) increase in RNA polymerase activity of nuclei prepared from the rat hindlimb muscles (Table 1). Thus, 14 h after the hormone injection the enzyme activity increased by 45% over the control value. It remained elevated for 4 days (by 48%). In a second part of this experiment the rats received 5 hormone injections (0.1 mg/100 g) over a 13-day period every 3 days. Following this treatment RNA polymerase activity was 23% higher than that of the control group. Hence the response was less than that which was observed in the animals after a single injection of Retabolil. These data suggest that with repeated hormone injections the muscles may become refractory to the stimulatory effect of the hormone on protein synthesis.

Following the demonstration that the sensitivity of skeletal muscle to steroid hormones could be changed depending upon the experimental conditions, a study was undertaken to ascertain whether a competition existed between the several biologically activity hormones for the receptor sites in the cytoplasm. In these experiments the competition of testosterone, corticosterone, 19-nortestosterone, methandrostenolone and Retabolil for the receptors for the androgens and glucocorticoids was determined. This was estimated from the change in the percent of either [^{3}H]-testosterone or [^{3}H]-corticosterone when incubated with unlabelled hormones. The data (Table 2) demonstrated that 19-nortestosterone competes more with testosterone than with corticosterone for the hormone binding protein. From this we have concluded that the stimulating effect of Retabolil on the synthesis of rRNA is mediated by a selective receptor for 19-nortestosterone in the cytoplasmic protein of skeletal muscle. This

Table 1. Effect of Retabolil on nuclear RNA-polymerase I activity in rat skeletal muscle (M ± S.E.M.; $n = 4$)

Experimental conditions	pmoles [^{14}C]-UMP (mg DNA)	Change (%)
Without hormone	109 ± 14	—
With Retabolil, once	173 ± 10	58
With Retabolil 5 times	134 ± 3	23

Table 3. Effect of training and Retabolil on nuclear RNA-polymerase activity in rat skeletal muscle (M ± S.E.M.; $n = 4$)

Experimental conditions	pmoles [^{3}H]-UMP (mg DNA)	Change (%)
Control	73 ± 5	—
Training	107 ± 9	47
Training with Retabolil	141 ± 9	93

Table 2. Competition of different steroid for skeletal muscle receptors ($n = 5$)

Hormone	Androgen receptors % binding with [^{3}H]-testosterone	Glucocorticoid receptors, % binding with [^{3}H]-corticosterone
Testosterone	36	97
Corticosterone	119	58
19-Nortestosterone	36	81
Methandrostenolone	69	96
Retabolil	95	—

Table 4. Effect of training and Retabolil on testosterone binding capacity of sarcoplasmic proteins (M ± S.E.M.; $n = 4$)

Experimental conditions	c.p.m. (mg/protein)	Change (%)
Control	108 ± 8	—
Training	185 ± 16	71
Training with Retabolil	146 ± 11	35

observation agrees with the recent report of Dube *et al.*[5].

The presence of receptors in the cytosol that selectively bind anabolic hormones made it possible to study their participation in changes in rRNA induced either by Retabolil administration or by training. These experiments were carried out on three groups of rats. From Table 3 it can be seen that physical training resulted in a 47% increase in RNA polymerase activity of nuclei isolated from skeletal muscle. Four injections of Retabolil during training resulted in an increased RNA polymerase activity that was 93% greater than the control value.

The kinetics of the enzyme reaction of the nuclei from skeletal muscle was the same in all experimental groups with the same time constant for the completion of RNA synthesis [6]. These data demonstrate that transcription of RNA increased due to the increased activity of the RNA polymerase.

The possibility of the involvement of the endocrine system in adaptive changes in rRNA synthesis of nuclei from skeletal muscle during training was studied in another series of experiments. These studies (Table 4) demonstrated that systematic physical training induced increased ($P < 0.05$) testosterone binding capacity of the cytoplasmic proteins. This could lead to the stimulation of RNA synthesis even if the level of androgens in the blood were unchanged as a result of training. It is interesting to note that the Retabolil injections during systematic physical training induced significantly smaller increases in hormone binding capacity of the sarcoplasmic protein compared to the data demonstrating that transcription of RNA increased due to the increased activity of RNA-polymerase.

However, in this case, additional increases of RNA synthesis could be explained by hormone–receptor interaction as the injection of Retabolil was accompanied by the appearance of anabolic steroid 19-nortestosterone in blood.

Finally, our previous experiments have demonstrated that a single injection of methandrostenolone induced increased incorporation [^{14}C]-leucine into myosin, myofibrillar and sarcoplasmic proteins [7].

Thus, it can be concluded by a scheme which although conditional reflects the participation of anabolic steroids in the control of transfer and realization of genetic information in skeletal muscle (Fig. 1). In blood anabolic steroids are transported within a complex with steroid bound proteins, synthesized in liver. By an equilibrium mechanism maintaining the size of steroid charged transport proteins there occurs a permanent release from complex of a definite part of steroid hormones which then enter the muscle cell. Here it associates with cytoplasmic receptors and is transported to nuclei where the steroid–receptor complex interacts with intranuclear structures, namely, chromatin. Steroids exert their effect by activating DNA-dependent RNA polymerase enzymes I and II. As a result the synthesis of ribosomal RNA with ribosomal formation and labile messenger RNA increases with subsequent activation of protein synthesis (sarcoplasmic and myofibrillar proteins)—consequently resulting in expansion of functional poten-

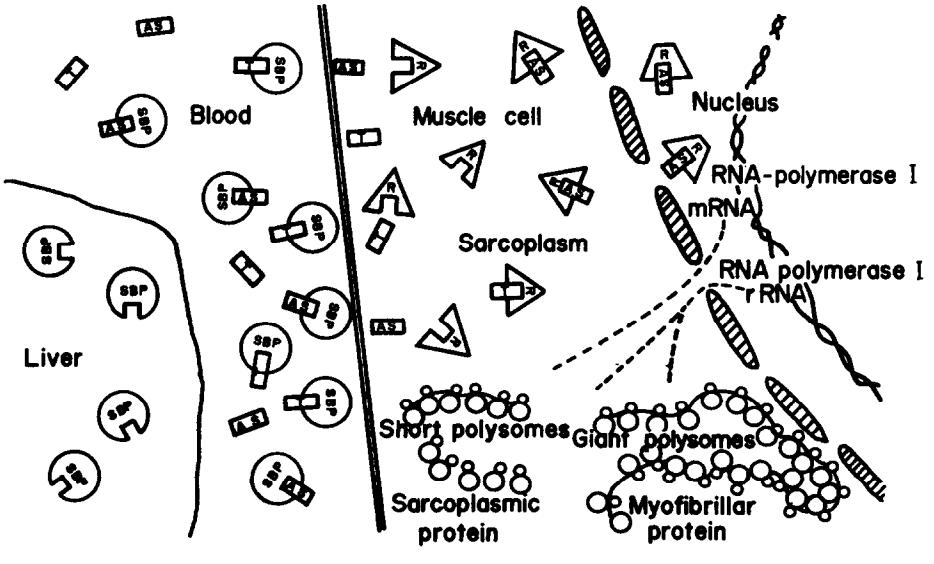

Fig. 1. A possible way in which anabolic steroids could participate in the regulation of skeletal muscle metabolism.

tialities of skeletal muscles. Thus anabolic steroids can be regarded as regulators of the complex process of protein synthesis in skeletal muscle.

Our conclusions are that the anabolic steroids have a stimulating effect on the transcription of ribosomal RNA in skeletal muscle and, they can play an important role in the control of protein metabolism and muscle content during systematic physical training.

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