Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (4)

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Sport und Buch Strauß, Köln, 1997

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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (4). Sport und Buch Strauß, Köln, (1997) 13-19

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Feasible Mechanism Of the Testosterone Anabolic Action

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Abstract

The purpose of this study was to evaluate the role of testosterone in skeletal muscle androgen receptor regulation and to determine the effects of 4 wk of intensive or aerobic training on testosterone content in blood and androgen receptor in skeletal muscles and brain in rats. Adult male rats (weight 180-200 g) received an intraperitoneal injection of either testosterone (0.1 mg/100 g) alone, cycloheximide (3 mg/100 g) alone or their combination. Free and total quantity androgen receptors was measured by radioligand method. After a single injection of testosterone total amount of androgen receptor was decreased from 4.1± 0.3 fmol/mg protein to 1.6± 0.2 fmol/mg already in 15 min after injection. The maximal androgen receptor depletion occurred at 1 hour (1.2± 0.1 fmol/mg) and the replenishment of androgen receptor at 6 hours (4.6±0.5 fmol/mg) was observed. Cycloheximide treatment alone had not effect on receptor levels. The administration of cycloheximide at 1 hour after testosterone significantly suppressed androgen receptor level (3.0±0.2 fmol/mg) at 6 hour. Full replenishment of androgen receptor in skeletal muscle cytosol is depended on testosterone induced androgen receptor synthesis and the course of events observed in this study is possible to occur during the recovery after physical exercise. Forty animals were divided into four groups and trained during a month with different physical activity. The systematic intensive exercise consisted in animals swimming during 15 min. With gradual increase of the load from 3 to 12% of body mass. The systematic aerobic exercise consisted in swimming of animals with load 4% of body mass with a gradual increase of the duration of swimming from 3 till 40 min. The results indicated that 4 wk of intensive or aerobic training to the level of androgens in blood, their reception in the organ target depends upon the nature of physical exercises.

Introduction

The mechanism of testosterone action in an organism included a successive interaction with transport protein, receptors and enzyme binding proteins (1). Testosterone act through intracellular receptors that are not associated with the cell membrane. It is believed that the testosterone pass through the cell membrane without the help of any membrane-associated protein, bind the androgen receptors in the cell and transform them into active transcription factors(2). The most basic method of identification of androgen receptors is based on their capability to selectivity and their relative strength bind to androgens and their metabolites. There is little information about the effects of exercise on androgen receptor in skeletal muscles. However strenuous acute exercise may increased blood testosterone concentration in athletes. This may be due to increased testosterone secretion during exercise (3,4). Since regular exercise is used for athletes as a means to achieve several performance results, it is very important to determine the impact of aerobic training on testosterone content in blood and androgen receptor in skeletal muscles. The aim of this study was to evaluate the role of testosterone in skeletal muscle androgen receptor regulation and to determine the effects of 4 wk of intensive or aerobic training on testosterone content in blood and androgen receptor in skeletal muscles and brain in rats. The results indicated that 4 wk of intensive or aerobic training to the level of androgens in blood, their reception in the target organ depends upon the nature of physical exercises. The change of these indices reflects the trend of the hormone regulation of skeletal muscles metabolism under the influence of training. The finding of this study suggest that the full replenishment of androgen receptors in skeletal muscles cytosol depends on testosterone induced androgen receptors synthesis. The course of events observed is similar to the recovery after physical exercise.

Material and Methods

The investigation were carried out on 210 white male rats with body mass 180-200 g. In order to investigate the influence of physical exercises on the level of blood testosterone and androgen receptor in skeletal muscles 80 rats were performed intensive exercise consisted in swimming 15 min with a load 12% of body mass or swimming 40 min with a load 4% of body mass. The animals were investigated after exercise and in 1, 2, 4, 6, 8, 16, 24 hours of rest after them. For study the intensity of protein synthesis in skeletal muscles 90 rats were administrated inside the peritoneum testosterone (0.1 mg/100 g) alone, cycloheximide (3 mg/100 g) alone or their combination. The animals were investigated in 0, 0.5, 1, 2, 4, 6, 8 hours after injection of testosterone or cycloheximide. By studying the influence of systematic physical exercise of various metabolic trend on the level of rats blood testosterone and androgen receptor in skeletal muscles and brain their investigated 40 animals that during a month received a standard ration. All the animals were divided into four groups with different physical activity. The systematic intensive exercise consisted in animals swimming during 15 min with gradual increase of the load from 3 to 12% of body mass. The systematic aerobic exercise consisted in uninterrupted swimming of animals with load 4% of body mass with a gradual increase of the duration of swimming from 3 to 40 min. The systematic aerobic exercise with speed up consisted in swimming of animals with a load 4% of body mass with a gradual increase of duration of swimming from 3 till 35 min. After that the animals swimming during 30 sec with a load 8% of body mass and with 30 sec rest for 5 times. The testosterone concentration in rat blood was measured by RIA. Determination of androgen receptor in skeletal muscle cytosol by radioligand with testosterone. Selected muscles of rat hindlimbs vigorously mince with scissors and homogenise in ratio 1:2 (w/v) in ice-cold buffer solution which is consisted of 20 mM TRIS-HCl (pH 7.4) 25 mM KCl, 1.5 mM EDTA, 2 mM DTT, 10 mM Na_2MoO_4 10% glycerol, 1% charcoal (Norit A) 0.1% Dextran T-70. It was shown early that Na_2MoO_4 and 10% glycerol effective stabilise steroid receptors and dextran coated charcoal (DCC) adsorbs free endogenous steroids. Homogenates are centrifuged at 100.000 g for 1 hr and the resulting clear supernatants are refers to as cytosols. Total quantity AR (free and occupied with endogenous hormone) were analysed by two-step incubation for 6 hr at 15 degrees C followed by an overnight incubation at 0 degrees C. The last procedure is sufficient for AR elaboration and binding with ^3H-testosterone without AR degradation. After the end of the incubation period unbound steroid will be removed with 0.15 ml of DCC, left for 30 min at 0 degrees C, centrifuged and the radioactivity in aliquot of the supernatant will be measured. The radioactivity in the protein-bound fraction which will no be displaced by excess of unlabelled testosterone was calculated in all instances. Dissociation constant and maximum binding was calculated by the Scatchard analysis. Brain androgen receptors were measured using the same procedure. Protein was analysed by the Bradford method (5).

Results and Discussion

The results of the ^{3}H-testosterone binding in cytoplasm skeletal muscle as well as its content in rats blood, are given in Figure 1.
After intensive exercise the number of free cytoplasmic receptors rapidly reduced on 15 to 20% of the initial level. In 2 hours an increase in receptor binding is observed and in 4 hours it twice exceeds the initial level. 4 hours later the testosterone binding in cytoplasm is practically reduced to initial level. The change in testosterone concentration in the blood has an impulsive character with first increase of in 1 hour after exercise, the maximum value of testosterone was observed 8 hours after exercise. The findings show that after exercise in skeletal muscle, there occurs a testosterone binding to an androgen receptor, translocation of hormone-receptor complex to the nuclei and subsequent return to cytoplasm. After aerobic exercise are shown in Figure 2 there is similar picture, but without sharp changes of the testosterone level in the blood and binding of testosterone in cytoplasm skeletal muscle during the recovery period. The effects of exercise to the level of testosterone in blood, their reception in the skeletal muscles depends upon the nature physical exercises, its metabolism.
To elucidate the role of protein synthesis on AR we investigated the influence of testosterone and cycloheximide on the level of androgen receptor in rat skeletal muscle cytosol. After a single injection of testosterone an appreciable alteration in the content of androgen receptor was observed in skeletal muscle cytosol. Total amount of androgen receptor was decreased from 4.1±0.3 fmol/mg cytosol protein to 1.6±0.2 fmol/mg already in 15 min after injection (Figure 3). The maximal androgen receptor depletion occurred at 1 hour (1.2 fmol/mg) and the replenishment of androgen receptor at 6 hours (4.6 fmol/mg) was observed. The quantity of occupied AR did not significantly change within this period. These results demonstrated that testosterone administration is followed by the rapid translocation of androgen receptor into nucleus rather than occupancies.
Table 1 gives the data on the level of androgen receptor in skeletal muscle cytosol of the rats after injection of testosterone and cycloheximide. As shown in Table 1 the administration of cycloheximide alone had no effects on androgen receptor levels. The administration of cycloheximide at 1 hour after testosterone significantly suppressed androgen receptor level at 6 hours, it was shown that androgen receptor reappeared in cytosol due to the replenishment after testosterone or testosterone and cycloheximide injection were functionally active in the ability to depletion stimulating by second testosterone injection in the same extent as after the first one.
In order to determine the effect of various training programs on the level of testosterone in rat blood and on androgen receptor in skeletal muscle and brain we compered the amount of testosterone and androgen receptors from animals after training with intensive exercise, aerobic exercise and aerobic exercise with speed up. As shown in table 2, after training of intensive exercise the amount of androgen receptor in skeletal muscle increased on 25% to compare the training of aerobic exercise and on 85% to compare the control animals. The aerobic exercise led to increase of androgen receptor in skeletal muscle by 47% to compare the control animals. As shown in table 2 any differences between the level of testosterone in the groups of rats with different physical activity were not revealed. The four-week training

Table 1
Effect of cycloxemidine on angrogen reception in rat skeletal muscle cytosol (M ± m, n = 5)

Experimental conditions	Androgen Receptor		
	Free	Total	
	fmol/mg	fmol/mg	%
Control	1.05 ± 0.17	1.39 ± 0.11	100
Control + cycloheximide (3 mg/100 g, 5 hours)	0.95 ± 0.16	1.36 ± 0.12	98
Testosterone (0.1 mg/100 g, 6 hours)	1.36 ± 0.38	1.82 ± 0.22	131
Testosterone + cycloheximide	0.65 ± 0.09	0.90 ± 0.17	65

by a systematic aerobic exercise did not influence on the level rats blood testosterone. By

Table 2
Effect of various training programs on testosterone content in rat blood and on androgen receptor in skeletal muscle and brain (M ± m, n = 5)

Experimental conditions	Testosterone (mg/ml)	Androgen receptor (fmol/mg)	
		skeletal muscle	brain
Control	0.87 ± 0.12	2.1 ± 0.1	58 ± 6
Intensive exercise	1.20 ± 0.13	3.9 ± 0.5	89 ± 7
Aerobic exercise	0.89 ± 0.15	3.1 ± 0.1	37 ± 4
Aerobic exercise with speed up	0.71 ± 0.08	3.1 ± 0.3	38 ± 4

examining the data stated above two questions arose. First is which molecular mechanisms ensure the rise testosterone concentration during the performance of intensive physical exercise ? Secondly and in our opinion more essential, is what functional significance may the increased hormone level have ? It should be admitted that at present it is not possible to answer these questions in a reasonable way. We could only notice that the rise of testosterone concentration is apparently not due to an increase of its synthesis under the influence of luteinizing hormone (3). Many questions remain unanswered concerning the mechanism of testosterone action. The ability of androgen receptors to rapidly locate their target sites with the genome following their import into nuclei has been realised for some times, bur has yet to be explained at the molecular level. We can look forward in future years to more in-depth molecular and biochemical investigation, the goals of which will be to provide a detailed molecular description of the mechanism of testosterone action.

Conclusion

The findings show that the full replenishment of androgen receptors in skeletal muscles cytosol depends on testosterone induced androgen receptors synthesis. The course of events observed in this study is similar to the recovery after physical exercise.

The effect of training on the level of androgens in blood, their reception in the target organ depends upon the nature of systematic physical exercises. The change of these indices reflects the trend of the hormone regulation of skeletal muscles metabolism under the influence of training.

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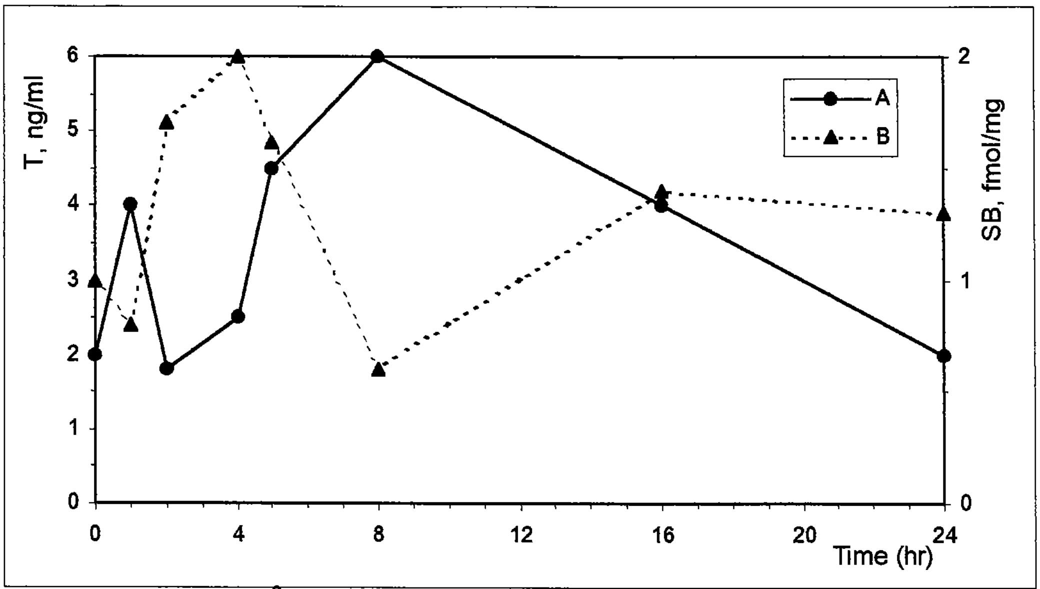

Figure 1. Binding of ^{3}H-testosterone in rat skeletal muscle cytosol (B) and its concentration in blood (A) after intensive exercise (swimming 15 min, load 12%)

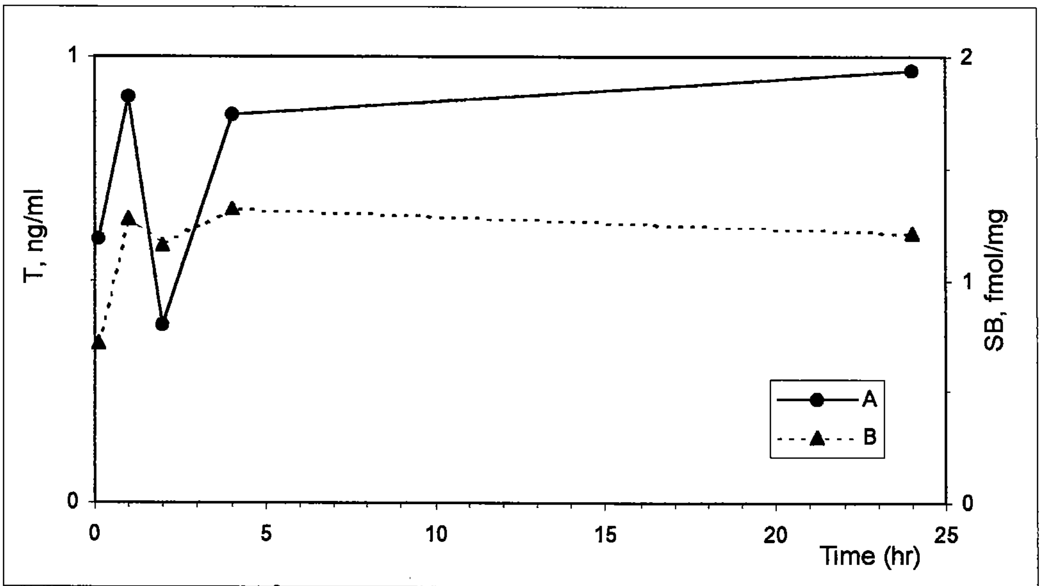

Figure 2. Binding of ^{3}H-testosterone in rat skeletal muscle cytosol (B) and its concentration in blood (A) after aerobic exercise (swimming 40 min, load 4%)

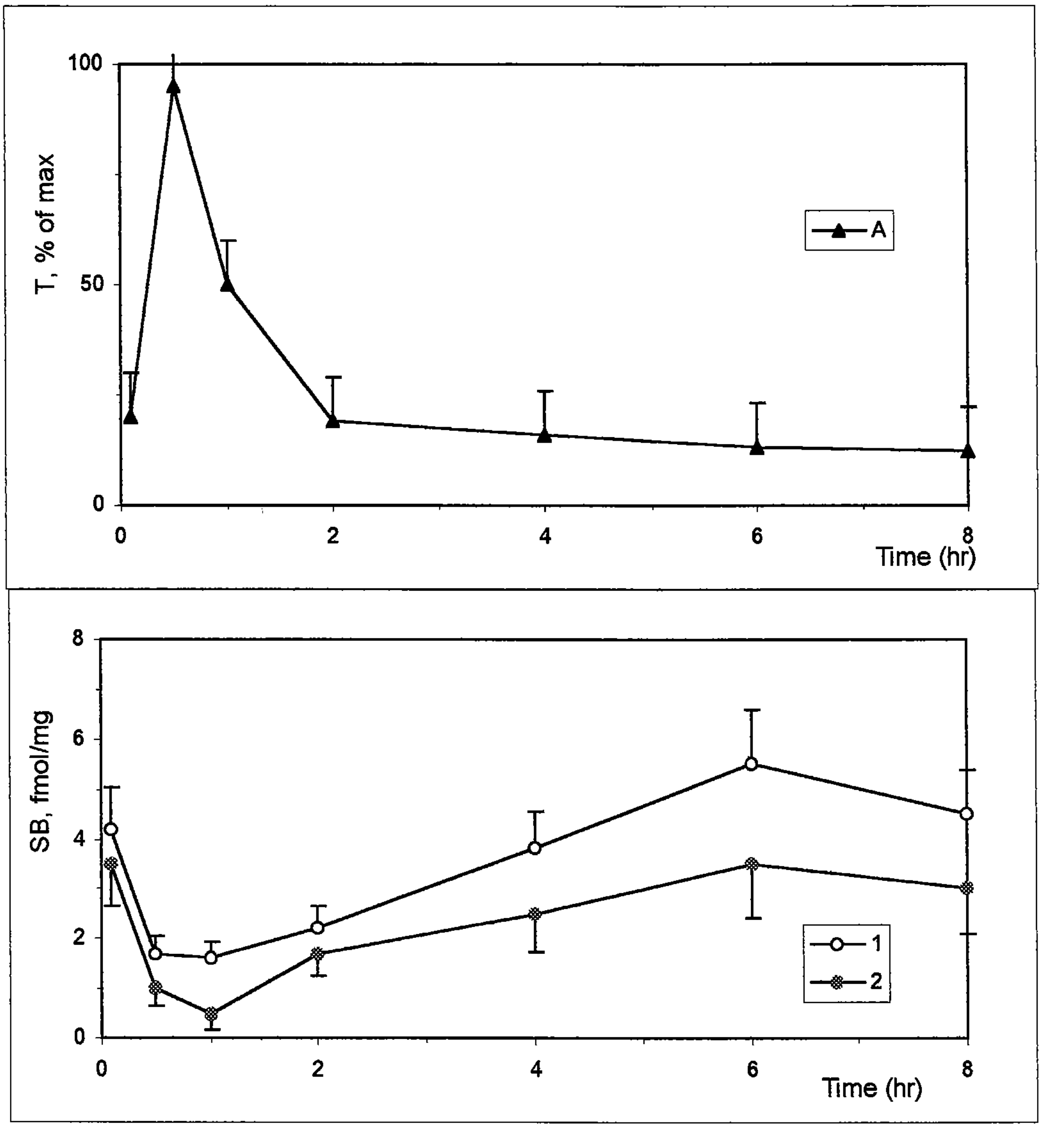

Figure 3.Testosterone concentration in blood (A) and total amount (1) and free (2) of androgen receptor in rat skeletal muscle cytosol (B) after injection testosterone (0,1 mg/100 g)