Effect of work-load exercise on the compensatory muscle hypertrophy in the castrated rat

Shigeru Yamada

Department of Sports Sciences, College of Arts and Sciences, the University of Tokyo,
3-8-1 Komaba, Meguro-ku, Tokyo 153

Summary

Effects of work-load exercise on skeletal muscles of the castrated rat were compared with those of normal rats. Castration has little effect on the development of compensatory muscle hypertrophy and the accumulation of albumin in the hypertrophied muscle.

Key Words: Albumin, Skeletal muscle, Compensatory hypertrophy, Testosterone

Introduction

We reported that the tenotomy-induced hypertrophy in the rat skeletal muscle accompanies a significant increase in the level of albumin in muscle (1). Study employing the albumin-deficient rat (NAR) subsequently demonstrated that the work-induced muscle hypertrophy, which is constantly observed with normal rat, fails to take place in the work-loaded skeletal muscle of this mutant rat (2). However, since NAR is known to exhibit lowered inborn levels of testosterone and growth hormone (3), it has been argued that the inability of NAR to develop the work-induced muscle hypertrophy might be accounted for by the lowered hormone levels intrinsic to NAR.

Present study has been undertaken to asertain whether the serum level of testosterone affects the compensatory of muscle hypertrophy, and changes in the weight and protein composition of the work-loaded skeletal muscles were compared between castrated and normal rats.

Materials and Methods

Animals

Sprague-Dawley rats were obtained from the stock of CLEA Japan, Kanagawa.

Tenotomy

Rats of the same developmental stage were divided into control and experimental groups by weight-matching. Tenotomy and sham-operation of the hind legs were performed in the same manner as described previously (1). The plantaris and the soleus muscles in both operated and contralateral limbs were removed for examination 7 days after operation.

Castration

Rats of approximately 250 g body weight were castrated after anesthesia with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). Sham-operated control received the same anesthesia, but only scrotal incision.

Assay of testosterone

Blood samples were collected from rats 15–20 min after anesthesia and were centrifuged to separate plasma. Testosterone in the plasma was determined by use of a double-antibody radioimmunoassay kit supplied by EIKEN, Tokyo (2).

Preparation of antibody

64KDa protein (rat albumin) was purified from whole skeletal muscle and anti-64KDa protein antibody was prepared in rabbit as described previously (1).

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was performed as described by Laemmli (5) on 10% acrylamide slab gels. Total muscle proteins were dissolved in a sample buffer, containing 25 mM Tris-HC1, 2% SDS, pH 6.5 and heated in boiling water for 2 min. Electrophoresis was performed at 25 mA constant current until the bromophenol blue tracking dye moved to the end of the gel. After electrophoresis, proteins were stained with Coomassie blue (1).

Transfer of proteins onto nitrocellulose and immunoblotting

The methods of Towbin et al. (6) and Legocki and Verma (7) were adopted with some modifications. After electrophoresis, gels were equilibrated with 40 mM glycine containing 5 mM Tris-base (blotting buffer) for 30 min. The protein in the gel were then electrophoretically transfered to nitrocellulose membranes by transverse application of 125 V for 1 hr. The nitrocellulose membrane was successively treated with 0.03 M borate buffer containing 0.15 M NaCl, 10% casein, and 0.1% sodium azide, pH 8.0 (masking solution) for 30 min, then masking solution containing 0.5% specific antibody and 0.02% SDS for 90 min. After washing the membrane ten times with 0.03 M borate buffer containg 0.15 M NaCl pH8.0 (washing solution), the membrane was treated with masking solution containing 0.02% SDS and 0.5% goat anti-rabbit IgG conjugated with peroxidase for 90 min and washed with ten changes of washing solution. The immunocomplex was located by staining for peroxidase activity with 4-chloro-1-naphthol and hydrogen peroxide as described by Hawke et al. (8).

Results

Fig. 1. shows the serum testosterone concentrations of castrated and control rats 7 days after surgery. It is evident that testosterone concentrations in the serum of castrated rats declines to much lower levels than that in the control animals.

To study the effect of work-load exercise on the muscle hypertrophy in castrated rat, the soleus and plantaris muscles of one limb were work-loaded by tenotomy of the synergistic gastrocnemius muscle. The contralateral limb which received only sham-operation served as control. During the week following tenotomy, the work-loaded muscles of the tenotomized limb of castrated rat exhibited statistically significant changes in wet weight as compared to those of control muscles. By contrast, we routinely observed 30–50% weight gain in the work-loaded muscles of normal rats when the synergistic muscle was tenotomized (1).

To verify whether the work-load exercise brings about qualitative or quantitative change in the protein components of the skeletal muscles of castrated rat, total protein from the work-loaded and the control muscle were analyzed by means of SDS polyacrylamide gel electrophoresis and the muscle albumin levels were compared between the hypertrophied and control muscles by means of Western-blotting. As shown in Fig. 1, the amount of albumin significantly increased in the work-loaded muscle of the testosterone-depleted animal (1). Although weights of the work-loaded plantaris muscles are somewhat greater in castrated rats than in control animals, differences between two groups are statistically insignificant (Fig. 3). Therefore, it appears that the serum level of testosterone has little effect on the work-induced muscle hypertrophy.

Discussion

As evidenced previously, compensatory hypertrophy in rat skeletal muscle accompanies an increase in muscle concentration of albumin (1). Studies on the physiological role of albumin in compensatory muscle hypertrophy would be greatly facilitated by the availability of a rat strain defective in albumin biosynthesis (9, 10). In the present experiments, tenotomy was performed on the skeletal muscle of castrated rats and changes in weight and protein composition of the work-loaded synergistic muscles were compared with those of normal rats. In contrast to the case with normal rats, we found that castrated rat succeed to develop significant muscle hypertrophy in response to the work-load training. The result strongly suggests positive involvement of albumin in the work-induced enlargement of skeletal muscle, though mechanisms by which albumin participates in the muscle hypertrophy remain to be elucidated.

Among biochemical parameters which may influence the rate of muscle hypertrophy, serum levels of testosterone is known to exert pronounced effects (11, 12, 13, 14, 15, 16, 17, 18, 19). Testosterone primarily regulates the DNA dependent RNA synthesis in target tissues, and facilitates the synthesis of male specific proteins (20, 21, 22). In addition, this steroid is also known to play an important role in muscle hypertrophy. Although the responses of different tissues to treatment with this hormonesis are not uniform and the mechanisms by which these hormones affect muscle mass and muscle protein composition remain only poorly understood, the accumulated facts raise the possibility that the observed inability of the NAR skeletal muscle to respond to work load training might be the consequence of the lowered inborn levels of testosterone and growth hormone in this mutant rat (10).

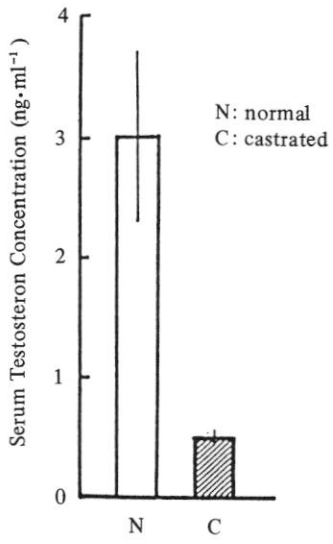

Fig. 1. Effect of castration on the serum testosterone concentration. Blood was withdrawn from the abdominal aorta at 15–20 min after pentobarbital injection and testosterone concentration was determined by the radio-immunoassay as described in "Materials and Methods". N and C indicate the testosterone concentration in the serum of normal and castrated rats, respectively.

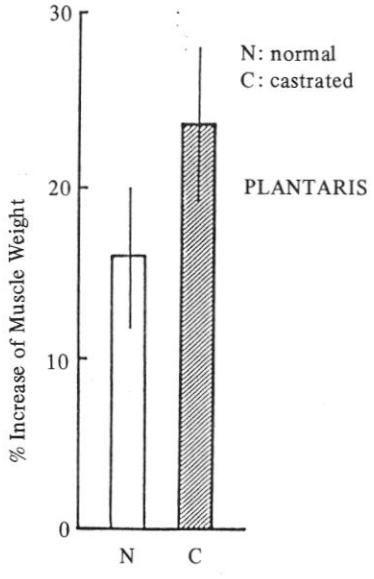

Fig. 3. Effect of tenotomy and training on weight of the plantaris muscle in normal and castrated rats. N and C indicate the muscle weights of the normal rat group and the castrated rat group, respectively. Bars indicate the relative changes in wet weight of the plantaris muscle in normal and castrated rat 7 days after operation. Normal and castrated rats were subjected to training for 7 days after tenotomy. The weights of the plantaris muscles of normal and castrated rats were determined.

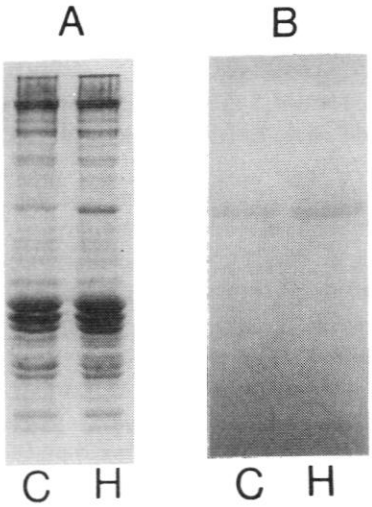

Fig. 2. SDS-polyacrylamide gel electrophoresis and Western blot analysis of the muscle proteins in the muscle proteins in the castrated rat. Normal male rats were castrated and subjected to tenotomy and training as described in "Materials and Methods". The plantaris muscles of the tenotomized (H) and control (C) limbs were solubilized in SDS sample buffer. Each 12 μl solution (20 μg protein) was subjected to 10% SDS-polyacrylamide gel electrophoresis and stained for protein with Coomassie blue (A). Protein in the gel were electrophoretically transfered to nitrocellulose filter. After the nitrocellulose filter was successively treated by anti-rat albumin antibody and goat anti-rabbit IgG conjugated with lactoperoxidase, the rabbit IgG-goat IgG complex was located by staining for peroxidase activity with 4-chloro-1-naphthol and hydrogen peroxide (B).

Therefore, the effect of castration on compensatory muscle hypertrophy was examined in normal rats as respects muscle enlargement and albumin accumulation. The results show that compensatory muscle hypertrophy is inducible in the castrated rats as be shown in the hypophysectomized rats. Moreover, muscle hypertrophy observed with these organ-ectomized rats also accompanied albumin accumulation in the hypertrophied muscle. Successful induction of the compensatory muscle hypertrophy in the castrated rats is well in agreement with the Goldberg's statement that endogenous testicular hormones has no effect on tenotomy induced muscle hypertrophy (23).

Taken together, the present results, suggest that testosterone, in not essential for the induction of muscle hypertrophy and that failure of the NAR muscle to exhibit work-induced hypertrophy is possibly due to albumin deficiency in this mutant rat.

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