Mechanism of Muscle Atrophy (IV)

Changes of Muscle Protein Contents and Components with Muscle Atrophy Induced by the Tail Suspension of Rats

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Abstract

A state of hypokinesia and hypodynamia has been induced in the hindlimb muscles of the rat (200g) using a suspension model. After 4 weeks, the slow-twitch postural soleus muscle underwent a much greater atrophy than the fast-twitch phasic plantaris muscle. The protein content per muscle weight in atrophied soleus was less 28% compared with that of the control muscle, while that in atrophied plantaris muscle was not significantly different with control muscle (6.5%). Muscle protein components were analyzed by SDS-PAGE. A specific and significant reductions were seen in bands of muscle proteins of molecular weights of 20K-Da and 30K-Da, both of which are included more in soleus than in plantaris. Further physiological and biochemical analysis are now under study.

Key words: muscle protein, atrophy, rat, soleus

Introduction

Recent years, disuse atrophy of muscle induced by tail suspension model of rats is extensively being investigated [5,11,15,16,20,21], because the method is simple and non-invasive for animals and rapid effects can be obtained. In previous reports, we examined the tail suspension model developed by Morey et al.[15] and reported a marked reduction of soleus muscle weight and especially myofibrillar protein in soleus muscle using paired rats[2].

The loss of muscle mass in disuse is due at least in part to the loss of muscle protein[6,10]. Slower protein synthesis, faster protein degradation, or concurrent changes of both processes can decrease the deposition of muscle protein[9]. It is known that atrophy of muscle in immobilized rat hindlimbs showed the changes of both processes to be responsible[10], however, the mechanism to induce muscle atrophy can not be elucidated by the analysis of overall turnover studies.

A primary purpose of this study was to identify the protein content and component of soleus muscle that changes specifically in association with muscle atrophy and growth failure.

Methods

Animal Fourteen male Wister rats (7~8 week, 200g) were divided into 2 groups of tail suspension group (SG) and control group (CG). The suspension was induced by a model of Morey et al.[15] The method in detail was described in the previous literature[2]. After 4 weeks of tail suspension

muscles of hindlimb were excized and analyzed.

Analysis of muscle protein. Non-collagen protein content of muscles was measured by dye-binding assay (Bio-Rad Chemical Co., Ltd. USA) after the extraction of muscle protein with 0.2N NaOH solution[13].

The measurement of the protein composition in muscle was carried out by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) as described by Laemmli[12] using 10% and 15% (includes 2 M Urea) acrylamide gel prepared in a slab gel electrophoresis apparatus with 1.0 mm gel thickness. The muscle of rats was homogenized with a glass homogenizer with 10 vol. of 0.03 M Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose (buffered sucrose solution). Total muscle proteins were solubilized in the SDS sample buffer[12] by heating in boiling water for 2 min. Electrophoresis was performed at 25mA constant until the tracking dye had reached the end of gel. Proteins in gels were stained by Coomassie blue[3]. After electrophoresis and staining for protein were completed, polyacrylamide gel was placed on glass plate and scanned for densitometry at 640 nm using a Helena scanner.

Table 1 The body weights of experimental rats.

Group (n)	Body weight (g)	
	Initial	4 wk
Suspended (7)	190 ± 5	253 ± 38**
Control (7)	189 ± 9	363 ± 27

**p<0.01

Table 2 The muscle weights of the experimental rats.

	Soleus (mg)	Plantaris	Soleus (mg/100g BW)	Plantaris
Suspended	44 ± 3***	180 ± 14***	18 ± 1***	71 ± 3
Control	163 ± 7	308 ± 8	45 ± 2	85 ± 3

Mean ± SE, ***p<0.001

Results

The mean body weight of SG was significantly lower than that of CG after 4-week suspension (Table 1). The muscle weights of soleus and plantaris of SG were significantly lower than that of CG (Table 2). No significant difference was found in the muscle weight per body weight in plantaris muscle between SG and CG, while in the soleus muscle of SG was significantly lower, compared with that of CG. The protein content of total soleus in SG was significantly lower both in absolute and relative (per body weight) values (Table 3). But that of plantaris muscle in SG was lower only in absolute value compared with that of CG.

The quantitative or qualitative changes in the protein components of the muscle were analyzed by means of SDS-PAGE (10%). The total and soluble proteins of the soleus muscle in suspended rat were compared with those in control rat (Fig. 1). The ratios (%) of 30k-Da and 20k-Da protein in soluble protein in suspended soleus muscle were significantly lower than that of control soleus (Fig. 2).

Table 3 The non-collagen protein content of total muscle of the experimental muscles.

	Soleus	Plantaris	Soleus	Plantaris
	(mg/muscle)		(mg/g muscle)	
Suspended	5.0 ± 0.3**	37.2 ± 2.5**	114 ± 7**	201 ± 4
Control	25.8 ± 1.4	67.0 ± 2.0	158 ± 4	215 ± 9

Mean ± SE, ** $p<0.01$.

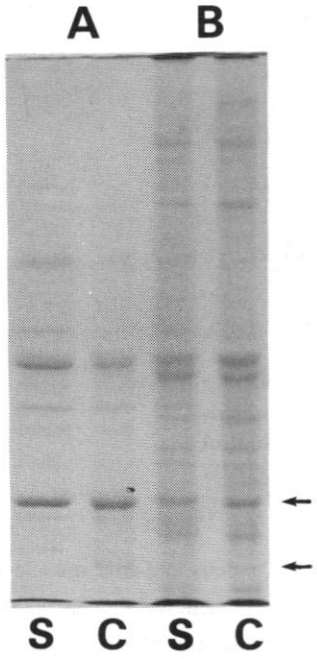

Fig. 1 SAS-polyacrylamide gel (10%) electrophoresis of total muscle proteins (B) and soluble muscle protein (A) in soleus muscle from the suspended (S) and the control (C) rats. Arrows show 30k-Da and 20k-Da proteins.

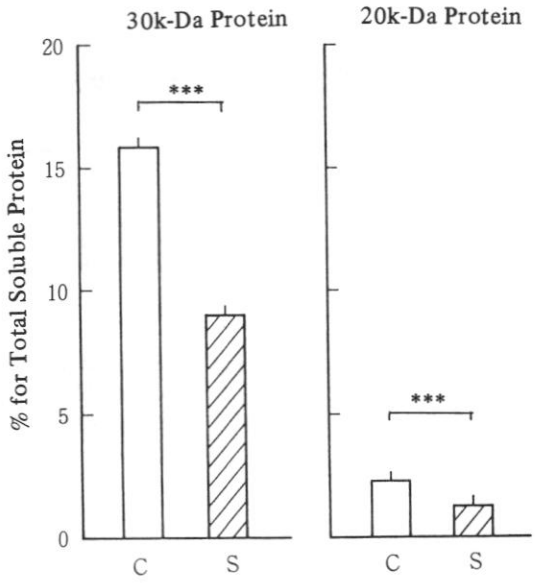

Fig. 2 The relative decrease of 30k-Da and 20k-Da proteins in soluble fraction. *** $p<0.001$

In order to examine the relationship between these two proteins and muscle atrophy, the changes of these two proteins in plantaris muscle were determined (Fig. 3). Both proteins are specifically much in soleus. No marked change was seen in the components of plantaris muscle.

Discussion

In the present study suspended soleus muscle showed marked reduction (70%) in muscle protein content as well as muscle weight both in absolute and relative (per body weight) values, while suspended plantaris muscle showed only one-third of soleus atrophy, despite that both muscles were maintained in shortened position. Passive stretch, that is, the length of muscle maintained in the immobolization or inactivity is a very important factor influencing muscle atrophy[10,14,19]. Spector et al.[19] examined the architecaural alterations of soleus, medial gastrocnemius (MG) and tibialis

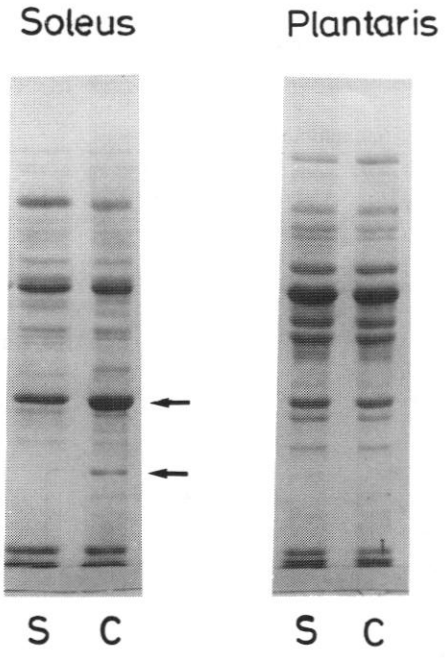

Fig. 3 SDS-polyacrylamide gel (15%) electrophoresis of soluble proteins in soleus and plantaris muscles from the suspended (S) and control (C) rats. Arrows show 30k-Da and 20k-Da proteins.

anterior (TA) muscle immobilized at different lengths. As a result, soleus and MG muscle showed a marked atrophy in the shortened position, and were able to maintain muscle weight, better than when immobilized neutrally, in the lengthened position. The level of the changes of both muscle atrophy were almost equal. A same change was not found for TA muscle. They speculated that the difference was due to that in the nature of the anatomic characteristics of the muscle-skeletal units crossing the ankle joint. The histochemical analysis did not show a consistent change due to fiber types.

According to Fell et al.[7] and Musacchia et al.[17], atrophy is more extensive and rapid in a slow-twitch postural muscles than in fast-twitch phasic muscles[6,26]. In the suspended model, Templton et al.[21] showed a specific and significant decline in Type I myosin isozyme content occurred without a change in that of Type II. These data shows a selective damage in slow twitch fiber by suspension model. Recently Loughha et al.[14] examined the effect of passive stretch on the synthesis and degradation rate of soleus and EDL (extensor digitorum longus) muscle and basically agreed with previous studies[11,17] describing a greater atrophy of slow-twitch postual muscle compared with fast-twitch phasic muscles in response to suspension, although the responses of the different muscle types have previously been exaggerated by planterflexed position and different lengths at which muscles were held. They also showed that the extent of the atrophy in stretched muscle in response to hypokinesia and hypodynamia was greatly reduced, although protein breakdown remained elevated in that muscle.

In the present study, specific reductions of 30k-Da and 20k-Da soluble proteins were observed in the suspended atrophied muscle. Since these proteins contained more in postural tonic soleus muscle

than in fast phasic plantaris muscle, there might be possible to have cause-result relationship with specific soleus atrophy. The function of these proteins and the mechanism of the reduction of these proteins in unstretched hypokinesia and hypodynamia have not been elucidated. It seems that many factors induce or modify the atrophy of muscle in the tail suspension model excluding stretching (the length of muscle) and fiber type, such as the decrease of nervous stimulus[1,14] and the increase of stress on the whole body (the increase of glucocorticoid,[1,9]). It is necessary to examine the relationship between the 20k-Da or 30k-Da protein and these factors, mentioned above, in order to determine the function of these specific proteins. Further analysis is now under investigation.

In conclusion, the marked atrophy was associated with the decrease of muscle protein in soleus muscle after 4-week suspension. Specific reductions of 30k-Da and 20k-Da protein were observed simultaneously.

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