Mechanism of Muscle Protein Degradation

3. A Model of Muscle Atrophy with the Tail Suspension of Rat.

by

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Abstract

A modification of the Morey tail suspension model was examined to determine atrophic responses of rat muscle unloading of hindlimbs for 60 days. The conspicuous decrease in the wet weight of soleus muscle was observed. Ratios of decrease in muscle weight to that of the control rat after 60 days-suspension of hindlimbs were 70% for soleus muscle and 17-28% for the other muscle groups. Muscle protein components were analyzed by SDS-PAGE. The conspicuous difference was seen in bands of muscle proteins of molecular weights of 43 k-Da and 200 k-Da, which respectively correspond to actin and myosin heavy-chain in the soleus muscle of the suspended hypokinesia rat. In conclusion, the Morey method of suspension can be used as a suitable model for the evaluation of the influence of hypokinesia on contractile proteins of skeletal muscles.

Key Words: Muscle atrophy, Protein degradadation, Rat

Introduction

Disuse atrophy of muscle is currently being investigated with an extensive variety of techniques including cage restraine[21], cast fixation[2], denervation[6], space flight[11,20] and tenotomy[17]. In addition to these more established techniques, another technique, which was recently developed by Morey[18] for the purpose of simulating zero gravity, has also been found to induce muscle atrophy[18,19]. The present investigation was undertaken to determine the possibility of the Morey model in order to elucidate the molecular mechanism of disuse muscle atrophy.

The loss of muscle mass in disuse is due at least in part to the loss of muscle protein[7,10]. Slower protein syntheses, faster protein degradation, or concurrent changes of both processes can decrease the deposition of muscle protein[9]. Although it is known that atrophy of muscle in immobilized rat hindlimbs showed the changes of both processes to be responsible[10], the mechanism to induce muscle atrophy can not be elucidated by the method of overall turnover studies.

A primary purpose of this study was to evaluate this Morey model of tail suspension as a means of identifying the specific muscle protein that changes specifically in association with muscle atrophy and growth failure.

Materials and Methods

Animal model

A Rat was subjected to suspension hypokinesia using an apparatus modified from that originally described by Morey[18]. After the rat was lightly anesthetized with ethylether, a 1-mm stainless steel ring inserted through the second caudal vertebra. The stainless steel ring causes little or no tissue reaction when it is inserted cleanly through the vertebral body. A stainless suspending wire was attached to the ring by a swivel that allows the rat 360° mobility. The length of the suspending wire was adjusted so that the hindlimbs did not touch the cage bottom. This form of suspension permitted the rats to walk on their forelimbs and gain access to food and water (Fig. 1).

Two female Wistar strain rats were individually housed in stainless steel cage, of which the floor and the front panel were made of wire mesh. Water and food were set on the front panel.

Measurement of muscle protein composition

Measurement of the protein composition in muscle was carried out by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophyoresis (PAGE) as described by Laemmli[14] using 10% 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, containning 0.25 M sucrose (buffered sucrose solution). Total muscle proteins were solubilized in the SDS sample buffer[14] by heating in boiling water for 2 min. Electrophoresis was performed at 25 mA constant until the tracking dye had reached the end of the gel. Proteins in the gels were stained Coomassie blue[5]. After electrophoresis and stainning for protein were completed, poryacrylamide gel was placed on a glass plate and scanned for densitometry at 640 nm using a Helena scanner.

Results

Body weights of the control and the suspended rat before and after 60 days of experiment were

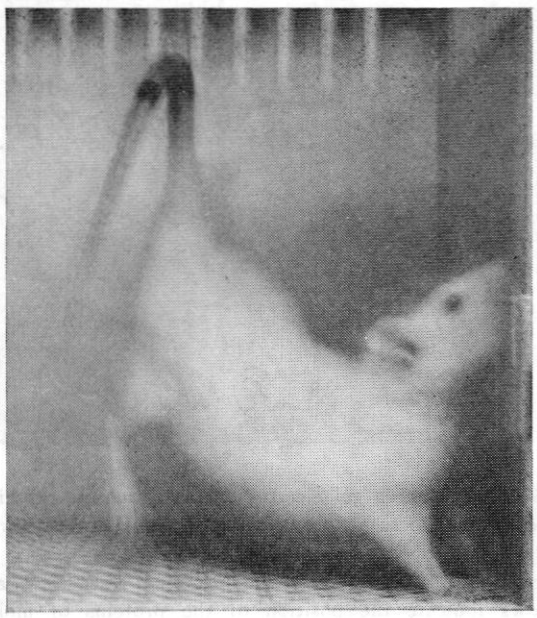

Fig. 1 The tail suspension of the rat.

Table 1 Muscle weights of experimental rats (gram).

Muscles		Control	Suspended	Difference	% Difference
Plantaris	r	0.311	0.222		
	l	0.312	0.229		
	mean	0.311	0.226	0.086	27.5
Soleus	r	0.157	0.046		
	l	0.148	0.044		
	mean	0.153	0.045	0.108	70.6
EDL	r	0.138	0.116		
	l	0.142	0.117		
	mean	0.140	0.116	0.024	16.9
Gastrocnemius	r	1.140	1.142		
	l	1.456	1.076		
	mean	1.448	1.109	0.339	23.4
TA	r	0.575	0.471		
	l	0.550	0.471		
	mean	0.562	0.471	0.092	16.3

r: right leg, l: left leg, difference: difference between the control and the suspended leg.

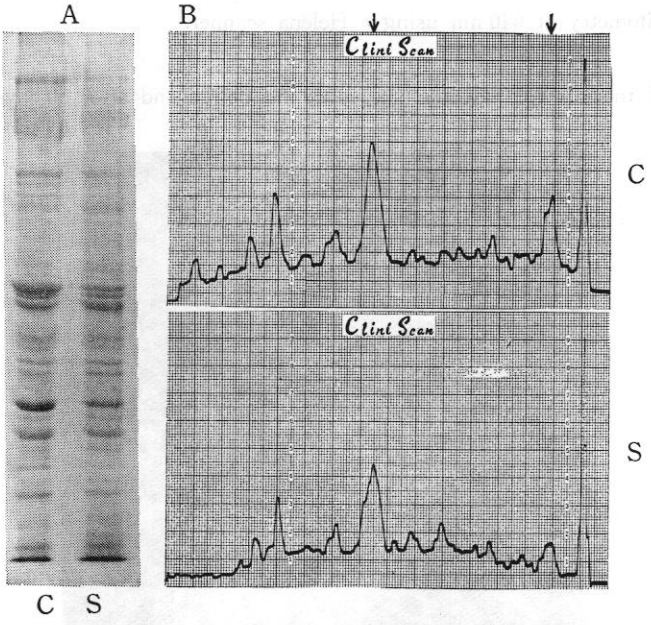

Fig. 2 A SDS-polyacrylamide gel electrophoresis of muscle proteins. The soleus muscle from the suspendered (S) and control (C) rats were removed at 60 days after operation.

B Electrophoretograms of muscle proteins traced by a densitometer. Arrows indicate the position of the proteins of molecular weights corresponding myosin heavy-chain and actin.

188 and 289 g, and 197 and 260 g, respectively.

Muscle weights of hindlimbs of rats were shown in Table 1. The wet weight of the soleus muscle of the suspended rat was 70% smaller than that of the control rat. The other musclular weights of the suspended rat were 17 to 28% smaller than those of the control.

The quantitative or qualitative changes in the protein component of the muscle were analyzed by means of SDS-polyacrylamide gel electrophoresis. Total protein of the soleus muscle in suspended rat were compared with those of the control rat (Fig. 2). Marked differences in the staining intensity were seen in the protein bands of molecular weights which correspond to actin and myosin heavy chain. Differences in the amount of these proteins in the muscle preparation weres quantitated by the densitometric tracing of the stained gel (Fig. 1-b). Evidently, peak area of tracing of the these proteins was markedly smaller in the preparation from the suspended muscle than that from the control.

Discussion

The rat adjusted quickly to the suspended position, as indicated by their eating, drinking and grooming activities, while a little difference in the growth of the body weight was seen in the suspended rat of the Morey model.

The suspension for 60 days with the Morey method in the present study induced the conspicuous atrophy of the rat soleus muscle. The duration of 14 to 30 days might be enough for the examination of the atrophy of the skeletal muscle compared with other data[12,15,22] obtained by the same Morey method[18]. This marked decrease observed in tne soleus muscle weight agreed with the soleus atrophy consistently found in other disuse models of the rat muscle[8,17,20] and with the observation of protein degradation elicited by the Morey model[18].

The mechanism of the disuse muscle atrophy has not yet been elucidated. The analysis of muscle protein components may give the solution of the mechanism of the disuse muscle atrophy. Our investigation found that the Morey model for hypokinesia might evoke a decrease in the soleus contractile protein. Templeton et al.[22] observed that the Morey model for hypokinesia evoked a decrease in soleus contractile function and the decrease in type I myosin content. A reduction of slow-twich fibers in the soleus has been reported from rats[3] and guinea pigs[6]. Small-cage confinement of rats also evoked a decline in slow-twich fibers[21]. Similarly, the vastus intermediate muscles, the slow muscle in the thigh analogous to the soleus showed a decline in the percentage of slow-twich fibers with immobilization without a change in fiber numbers[4]. According to the analysis of myosin isozyme data by Templeton et al.[22], which show no significant change in the fast-twich component after either 2 or 4 week of suspension while, showing a contineous and significant decline in the slow-twich component, the preferential slow-twich fiber atrophy was evident.

The marked atrophy of the rat soleus was evident in our previous study[1], in which determined the effect of denervation, compared with other hindlimb muscles. Jakubiec-Puka et al.[13] have reported evidence that the denervation atrophy of the rat soleus produces a lower propotion of myosin heavy-chain to actin. Whether a change in a neural activity is related to soleus atrophy with the Morey model will require further investigation.

In conclusion, the Morey method of suspension can be used as a suitable model for a simulation of the influence of hypokinesia on characteristics of the contractile muscle protein in the rat.

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