Neuromuscular Adaptation to Gravitational Unloading or Decreased Contractile Activity

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Introduction

There is a close association in the physiological, biochemical, and morphological properties between a motoneuron and the muscle fibers it innervates. This association has been hypothesized to be so because of the activity imposed by the motoneuron on the muscle fibers. Among those studies which support this view are those in which chronic electrical stimulation at low frequency (1-10Hz) changes the properties of fast-twitch muscles toward those of slow-twitch muscles (24,25,59,77,86,95). Some of the characteristics of muscle fibers are also altered following cross-innervation (9-12). Buller *et al.* (10) suggested that neural influence on muscle could be due to neurotrophic effect as well as via the nerve impulses. These experiments demonstrate that there is a significant level of neural and muscular interdependence.

The pattern of muscle activity is also reported to influence the morphological, metabolic, and contractile properties of skeletal muscles. For example, the metabolic capacity of muscles is affected specifically by the types of exercise training (45, 46). Increased activity or over-loading by removing the synergists causes a compensatory hypertrophy (19, 31, 32, 37, 50, 65, 71, 96). Hypertrophy is also induced by stretching of matured muscles *in vivo* (3,29) and cultured myotubes and fibroblasts (38,113-115). On the contrary, the exercise-induced metabolic adaptation of muscles are lost when exercise training is stopped (18). Further, muscle atrophy is induced by some models of reduced neuromuscular activity. But it is apparent that the level of use is not the only factor involved in the atrophic process. In the current study, the responses of skeletal muscles to gravitational unloading or reduction of contractile activity and mechanism responsible for the changes are briefly reviewed.

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I. Effects of Gravitational Unloading

A. Morphological properties

Gravitational unloading by exposure to weightlessness causes an atrophy mainly in anti-gravity muscles (4-6,16,22,23,36,48,51,57,61,67, 72,89,91). These studies show a greater atrophy of a slow extensor, modest atrophy of a fast extensor, and lesser or no atrophy in an ankle dorsi-flexor following spaceflight. Muscle atrophy is also induced by simulation models such as hindlimb suspension (20,34,35,39,40,51,56,68,72,78, 80, 82, 84, 94, 108, 117, 118), denervation (71), deafferentation (71, 73), spinal cord transection (93), tenotomy (33, 73, 75), or joint immobilization (7,8,42,83,84).

The differences in the muscle weight between the suspended rats and the age-matched cage controls are greater in the ankle extensors than the flexors (49, 78). The weights of ankle extensors in the hindlimb-suspended rats are significantly less than those of pre-suspension levels, suggesting that the muscles atrophied (83). The weights of suspended ankle flexors are also less than those of the age-matched cage controls. However, these weights are not different from the pre-suspension levels. Thus, one could argue that the lower weight of ankle flexors of suspended rats is caused by growth retardation but not due to atrophy.

The cross-sectional area (CSA) of both slow- and fast-twitch fibers of rats after 14-day spaceflight and hindlimb suspension was less than those in the age-matched ground controls

(72). However, the degree of atrophy was greater in slow- than fast-twitch fibers even in the antigravity muscle (61,72). Therefore, soleus composed of approximately 80% of slow-twitch fibers atrophies more than other ankle extensors (48,61,91,97). Although there is a clear selective atrophy of muscle that is related to the predominant fiber type generally, the magnitude of the atrophy can not be attributed primarily to the fiber type.

Riley *et al.* (91) reported that regional interstitial edema was noted in adductor longus (AL) and soleus, but not in plantaris and extensor digitorum longus (EDL), of rats orbited for 12.5 days and returned to Earth 2 days before the sampling. More aberrant fibers, consisting of small angular fibers, were seen in flight AL (approximately 3.6%) and soleus (approximately 6.8%) than in the respective synchronous controls (approximately 0.17 and 0.9%, respectively). These fibers often contained central nuclei and more than 80% of the aberrant fiber population demonstrated some necrotic fibers with invasion by mononucleated cells. Mean Z line length was significantly less in these flight AL than in ground controls. Further, myofibrils often showed longitudinal streaming and loss of sarcomere banding in the midbelly region of the flight AL. Muscle fiber damage was similar to that observed 1-2 days after strenuous eccentric exercise (2,70). Therefore, it is speculated that such muscle damage may be caused by weight-bearing exercise during 2 days after spaceflight (91). Unloading of muscle by spaceflight or hindlimb suspension may not directly cause fiber damage, but may increase the susceptibility to exercise-induced injury.

B. Metabolic properties

Enzyme activity

In young adult rats, the specific activities of mitochondrial enzymes measured in whole homogenates are generally lower in unloaded muscles than normal levels (20, 28, 69, 78, 99). However, succinate dehydrogenase (SDH) activity measured in single muscle fibers is often maintained or even elevated in atrophied muscle (35,39,61,67,72,94). These different observations and phenomena may be caused by the greater decrease in fiber size and relative increase in connective tissues or interstitial volume (28, 41, 60,103).

Some observations on fibers of young adult rats following 14 days of flight suggest that subsarcolemmal mitochondria may be preferentially altered (5, 90, 91). The effect of spaceflight on the distribution of mitochondria in soleus muscle fibers were studied by Bell *et al.* (5). The distribution of SDH activity determined quantitatively was studied throughout the cross section of the fibers. The fibers were also classified as slow-twitch oxidative or fast-twitch oxidative-glycolytic in histochemically prepared tissue sections. In all fibers, the distribution of SDH activity was significantly higher in the subsarcolemmal than intermyofibrillar region. After 12.5 days of spaceflight, the entire regional distribution of SDH activity was significantly altered in the slow-twitch oxidative fibers. The fast-twitch oxidative-glycolytic fibers of the spaceflight muscles exhibited a significantly lower SDH activity only in their subsarcolemmal region. These data suggest that the relative loss of SDH activity in the subsarcolemmal vs. intermyofibril region following spaceflight is fiber type dependent. Riley *et al.* (91) also reported that the distribution of mitochondria in the subsarcolemmal area of flight AL was 31% less than that of synchronous controls. Thus, the activities of SDH and NADH dehydrogenase in the peripheral region were also decreased.

It has become evident that the adaptive response of skeletal muscle to spaceflight is different across muscles, within different fibers in a muscle, and between different proteins in a fiber. These findings also suggest that, when considering the influence of spaceflight on oxidative enzymes, it may be of functional importance to consider how and where those enzymes are distributed within a fiber. The functional effect of a selective loss of mitochondria in the subsarcolemmal vs. the more central intermyofibrillar regions is not clear.

Phosphorus compounds

The high-energy phosphate contents in calf muscles of rats measured by using ^{31}P-nuclear magnetic resonance spectroscopy tended to be elevated by approximately 30 days of suspension (84). The PCr/(PCr + Pi) ratio, which indicates the relative content of PCr, was significantly elevated (where PCr: phosphocreatine and Pi: inorganic phosphate). The ankle dorsi-flexors were not influenced by suspension. The Pi/PCr ratio in the ankle extensors, but not in flexors, was significantly decreased by hindlimb suspension. The rate of adenosine triphosphate synthesis, estimated by using the method reported by

Chance *et al.* (13) as $1/(1+0.6\times PCr/Pi)$, was lowered in unloaded muscles. Such results may suggest that the metabolic rate, as well as the mitochondrial biogenesis indicated by decreased enzyme activities (20,28,69,78,99), in ankle extensors might be lowered by unloading.

β-Adrenoceptors

The function of β-adrenoceptors (βAR) in skeletal muscle is not fully understood. However, its density in muscle is positively correlated with the activities of mitochondrial enzymes (55, 116). The density of βAR is considerably greater in slow-twitch oxidative soleus than in fast-twitch gastrocnemius (116). The βAR density in type I fibers is three-fold greater than in type II fibers in the same muscle (62). It is, in general, increased by exercise training (104,116) and continuous electrical stimulation at 10 Hz (55) which both stimulate the mitochondrial enzyme activities (55, 104, 116), although Martin *et al.* (62) showed that the βAR density in human muscles was unchanged by 12 weeks of exercise training which increased the activity of citrate synthase. Our previous study showed that the maximum binding capacity (Bmax) of βAR in frog and rat hindlimb muscles was decreased by both spaceflight and hindlimb suspension (80). Because the dissociation constant or affinity of βAR was unchanged, the reduction of Bmax appears to have been due to a decrease in the number of receptors. Such decrease in βAR density may be closely associated with the reduction of specific activities of mitochondrial enzymes measured in whole muscle homogenates (20,28,69,78,99).

C. Fiber phenotype

Hindlimb unloading by spaceflight and/or suspension causes a progressive decrease in the % distribution of slow (type I) fibers in soleus (61,72,103), but not in fast muscles such as medial portion of gastrocnemius and tibialis anterior (TA) (34, 51, 94). Similar results have been indicated by both qualitative and quantitative histochemical staining of myosin adenosine triphosphatase (ATPase) as well as immunohistochemical analysis using antibodies specific to myosin heavy chain (MHC) (51,72). The fibers stained intermediately dark by qualitative staining for myosin ATPase with alkaline pre-incubation (pH 8.75) increased by approximately 9-14% in the soleus muscles following 2 weeks of spaceflight and hindlimb suspension. These fibers stained darkly after acid pre-incubation (pH 4.35), and reacted positively with both fast and slow MHC antibodies. The results indicated that the % distribution of fibers which expressed only slow MHC was decreased because some of the "pure" slow fibers began to express fast MHC as well after unloading.

The activity (Mean ± SEM) of myosin ATPase in fast soleus fibers of control rats ($29.8\pm2.9\times10^{-3}$, ΔOD/min) measured quantitatively was significantly greater than in slow fibers ($15.8\pm1.4\times10^{-3}$, $p<0.01$, Table 1). However, that in the intermediate fibers ($16.7\pm1.7\times10^{-3}$) was similar to the level of slow fibers. The activities (ΔOD/min) of SDH and α-glycerophosphate dehydrogenase (GPD) also tended to be greater in fast than slow fibers. The SDH and GPD activities of the fibers that expressed both slow and fast MHC also tended to be intermediate. However, none of the quantitatively measured activities of myosin ATPase, SDH, and GPD in any types of fibers changed significantly following spaceflight or hindlimb suspension (72).

These studies suggest that some fibers were shifted from slow to fast type, although the type was not completely reversed. If the transformation of fibers occurs normally, the myosin ATPase and mitochondrial and glycolytic enzyme activities may change in a predictable manner. For example, if fiber becomes fast, its glycolytic enzyme activities increase. It is also indicated that the shift of MHC expression may be resulted from a relative increase of fast characteristics due to a loss of slow MHC expression, although the absolute level of fast MHC expression may not have been affected dramatically. Although 9-14% of slow fibers expressed both slow and fast MHC after unloading, the remaining slow fibers were unchanged even though they also atrophied.

These changes in muscle fiber type seems to be due to a transformation of some fibers from a pure slow to a hybrid (expresses slow and fast MHC), but not due to a *de novo* synthesis of new fast fibers. It is not clear why some slow fibers respond differently. Some possibilities are that the slow fibers that remained unchanged did not possess the fast MHC expression genetically, or that slow MHC expression in these fibers did not respond to unloading.

It is found that 10 days of hindlimb suspension resulted in an increased expression of type IIa and IIx MHC in the soleus of hypophysectomized rats (Talmadge, Roy, Grindeland, and

Table 1 Fiber-type composition and enzyme activities of soleus in cage control and hindlimb-suspended rats with or without ankle-joint immobilization

	Control	Susp-Free	Susp-DF	Susp-PF
Slow-twitch fibers				
n	287	234	186	212
CSA	2,269 ±42	1,563 ±47***	2,307 ±61†††	1,768 ±55***, ††, §§§
% fiber	88.3±5.0	71.7±5.0*	76.9±7.2	86.5±8.0
SDH	33.8±4.8	34.0±3.8	49.1±1.4*, †††	45.3±0.6*, ††, §
ISDH	76.7±2.0	53.1±1.8***	106.8±3.1***, †††	75.3±1.7†††, §§§
GPD	0.4±0.2	0.9±0.3	1.0±0.1*	1.0±0.1*
IGPD	0.9±0.1	1.4±0.1**	2.5±0.2***, †††	1.7±0.1***, †, §§§
ATPase	15.8±1.4	15.1±1.8	19.5±0.2*, †	15.5 ±0.1§§§
IATPase	35.9±0.6	23.6±0.8***	46.2±1.2***, †††	27.2±0.8***, ††, §§§
Fast-twitch fibers				
n	33	66	35	18
CSA	1,373 ±46	994 ±39***	1,835 ±163**, †††	1,044 ±71***, §§
% fiber	10.2±4.5	20.3±2.1	15.0±5.0†	7.4±4.2
SDH	57.0±6.9	45.3±4.6	57.1±5.1	61.4±4.1
ISDH	78.3±3.2	45.0±1.8***	93.3±8.8†††	60.6±2.7***, †††, §
GPD	1.4±0.5	3.1±0.3	1.4±0.2	1.7±0.7
IGPD	1.9±0.2	3.1±0.3**	2.6±0.3	1.7±0.5†
ATPase	29.8±2.9	31.9±3.6	23.1±1.2*	22.7±1.6
IATPase	40.9±1.3	31.7±1.4***	44.9±3.5†††	24.7±3.5***, †, §§§
Slow & fast-twitch fibers				
n	5	26	19	14
CSA	1,078 ±164	906 ±76	1,657 ±213†††	1,031 ±115§
% fiber	1.5±0.7	8.0±3.6	6.1±3.9	8.1±2.4*
SDH	54.0±12.6	38.8±4.3	54.2±6.3†	63.7±4.7†††
ISDH	58.2±20.7	35.2±3.3	78.4±9.3†††	62.1±7.1†††
GPD	0.9±0.3	1.8±0.4	1.1±0.4	0.7±0.3
IGPD	1.0±0.5	1.6±0.3	1.7±0.5	1.1±0.5
ATPase	16.7±1.7	17.6±1.4	20.2±1.3	17.1±0.6§
IATPase	18.0±2.8	15.9±1.1	34.2±3.6*, †††	17.0±1.5§§§

Mean ± SEM. Control: cage control, Susp-Free: hindlimb-suspended without ankle joint immobilization, Susp-DF: hindlimb-suspended with ankle joint immobilization at a dorsi-flexed position, Susp-PF: hindlimb-suspended with ankle joint immobilization at a plantar-flexed position. n: number of analyzed fibers, CSA: cross-sectional area (μm^2), SDH: succinate dehydrogenase; △ optical density (OD)/min $\times 10^{-3}$, ISDH: integrated SDH (△OD/min × μm^2), GPD: α-glycerophosphate dehydrogenase (△OD/min $\times 10^{-3}$), IGPD: integrated GPD (△OD/min × μm^2), ATPase: myosin adenosine triphosphatase (△OD/min $\times 10^{-3}$), IATPase: integrated myosin ATPase (△OD/min × μm^2). *: $p<0.05$, **: $p<0.01$, and ***: $p<0.001$ vs. Control, †: $p<0.05$, ††: $p<0.01$, and †††: $p<0.001$ vs. Susp-Free, and §: $p<0.05$, §§: $p<0.01$, and §§§: $p<0.001$ vs. Susp-DF (Yasui, W., Y. Ohira, R.R. Roy and V.R. Edgerton. In preparation for publication).

Edgerton, Unpublished observations). The expression of MHC IId (most likely analogous to IIx) has also been observed in the soleus of rats suspended for 21 and/or 28 days (101). The fiber phenotype of the human vastus lateralis muscle also tended to be shifted toward fast-type similarly after 11 days of spaceflight (23). However, the exposure of carp fishes to microgravity for 8 days did not affect the fiber types of various muscles (Ohira *et al.*, Unpublished observations). Hindlimb unloading is accompanied by a progressive decrease in the concentration of myofibrillar and myosin protein soleus (106-108, 110, 111). The activity of myofibrillar ATPase

(72,105,107,108) and the composition of myosin light chain isoform (87,107-109) in soleus muscle homogenates and single fibers appear to be unchanged.

D. Contractile properties

Following the induction of atrophy, the magnitude of decrease in the maximum tetanic tension is greater than that in muscle mass (41, 85, 117). Thus, the specific tension per unit weight or CSA is lowered. Such phenomena may be due to the greater decrease in the concentration of myofibrillar protein (108,112) and/or the relative increase in the non-contractile tissue (28, 41,60,103) and interstitial volume (53). The results reported by Stevens *et al.* (98) showed that the specific tension of skinned soleus fibers in rats suspended for 15 days was similar to the cage controls. The specific tension in predominantly fast muscles such as medial gastrocnemius (41,85,117), TA (117), and EDL (27) are not influenced by suspension and may even be increased (66).

The speed-related properties in slow soleus are shifted toward fast-type, although fast muscles, both ankle extensors and flexors, are not markedly affected by unloading (21, 27, 41, 85, 103, 117). The time-to-peak tension is reduced. The maximum shortening velocity of whole muscle (27,41,85,117) and single fibers (30,64,87) is increased following unloading. Interestingly, the change in myosin ATPase activity has not always been observed to be proportional to an increase in shortening velocity after unloading (21,72,107, 108). Further, one-half relaxation time is decreased may be due to changes in sarcoplasmic reticulum kinetics.

The fatigue resistance remains remarkably high after a chronic unloading even in soleus muscle (26,41,85,117), although it seems to be affected more after a prolonged fatigue test (63). The maintenance of fatigue resistance in atrophied muscles may be attributable to, in part, 1) lowered absolute tension production, 2) relatively stable oxidative enzyme levels in fibers, and/or 3) shorter diffusion distance to the center of fibers due to decreased CSA.

E. Locomotor capability

Postural stability of Skylab crew members was found to be particularly compromised after spaceflight when the eyes are closed (47). Similar phenomena were seen after 18-day Soyuz-9 mission (14,15). Such effects were marked immediately after flight but were normalized after approximately 10 days. After 140 and 185 days of spaceflight, Kozlovskaya *et al.* (54) found that the ratio of electromyogram (EMG) amplitude to the perturbation force during standing posture was more than double compared to the pre-flight level. They also reported that the time taken for balance recovery after external disturbances increased and that the thresholds of corrective EMG responses decreased and the EMG amplitudes and durations were longer than in pre-flight.

Sensory informations from the otolith organs and other sensory receptors that respond to gravitational loading and vectors under normal gravitational conditions are altered dramatically in a microgravity environment. Such changes can be expected to contribute to modifications of motor behavior during weightlessness which result in altered patterns of muscle activity and morphological and metabolic properties of muscles. For example, there are lowered levels of soleus activity and elevated levels of TA activity (58) and a diminished H-reflex excitability in the medial gastrocnemius muscle after vestibular stimulation during spaceflight (88). Furthermore, there appears to be an adaptation of the H-reflex response to vestibular stimulation throughout the duration of spaceflight resulting in major increases in the response after the return to normal gravity which takes many days to return to pre-flight levels (88).

The results obtained in one flight rhesus monkey from the COSMOS 2044 flight indicated a significant modulation in the recruitment strategy used activating a slow and a fast ankle extensor muscles following 14 days of spaceflight (44). Upon return to 1-G environment, there appears to be an increased activation of the medial gastrocnemius muscle relative to the soleus muscle. This adaptation of the motor system persisted up to 5 days after the return to normal gravity and returned to a normal pattern within 2 weeks in 1-G environment.

II. Why Does Muscle Atrophy?

A. Electromyogram activity

The EMG of soleus and medial gastrocnemius disappeared immediately in response to hindlimb suspension of rats (1). And the total amount of daily EMG activity in these muscles remained significantly reduced on the day of suspension. The activity remained lower than normal for up to 2 weeks. However, the EMG

activity was gradually increased and maintained near-normal thereafter. In contrast, daily EMG activity of ankle flexor, TA, was above the normal during suspension. From these results it remains unclear as to whether the direct cause of the atrophy is related to disuse of the muscle. The soleus muscle atropied following spaceflight within 4 days (52) during the period of a marked reduction in the EMG activity. However, a greater atrophy was still seen after 28 days of hindlimb suspension (117). It is clear that the recovery of EMG toward normal is not associated with a recovery of the muscle mass lost.

The tonic EMG activity in human soleus, which account for about 80% of plantar-flexor torque, was reduced during spaceflight, whereas that in TA, dorsi-flexor, was higher than in preflight trials during a standardized postural test such as standing erect (17,58). Such reversal in the roles of ankle extensors and flexors has also been seen in parabolic flight in human (17) and monkeys after 14 days of spaceflight (44). Riley *et al.* (92) reported that the EMG activity in rat soleus was shifted from "tonic to phasic" following hindlimb suspension. Generally, these studies suggest that chronic changes in gravitational loading in the adult has a significant effect on the way the nervous system recruit units in one motor pool compared to another. This raises an obvious question of the role of 1-G environment in guiding the development of the motor system.

B. Electrical stimulation

Effects of electrical stimulation through the sciatic nerve at the gluteal region during suspension on rat hindlimb muscles were studied (74, 77,79,102). In one group of rats, twitch contraction was induced at 1 Hz for 4 hours continuously. In the other groups, train stimulation was performed at either 50 Hz (2-sec stimulation and 3-sec rest) or 100 Hz (1-sec stimulation and 4-sec rest) for 4 hours. The same patterns of stimulation were repeated again after 6 hours of recovery in the same day. Such electrical stimulation for 8 hours per day was performed for 10 consecutive days.

The weights and fiber CSA of plantaris, gastrocnemius, TA, and EDL in limb stimulated at 1 Hz were significantly less than in the contralateral muscles. The 50-Hz stimulation prevented the suspension-related decrease, relative to the age-matched cage controls, in the weight of TA and EDL, but not of the soleus, plantaris, and gastrocnemius. No beneficial effect was obtained in any muscles by 100-Hz stimulation.

Stimulation at 1 Hz caused an increase in citrate synthase activity in tissue homogenates of the TA but not the plantaris, while lactate dehydrogenase was unaffected. However, the suspension-induced effects on enzyme activities and mitochondrial volume in whole muscle or single fibers were not prevented by any types of electrical stimulations generally (102). These experiments suggest that electrical stimulation through the sciatic nerve is not an effective countermeasure for the muscle deadaptation that occurs during hindlimb suspension. The patterns or magnitude of tension production of suspended muscles in response to electrical stimulation are likely to be different from those in the cage controls.

C. Force development due to stretching

It is well-known that *in vivo* stretching (3,29) or increased load by elimination of synergistic muscle (19,31,32,37,50,71,96) causes muscle hypertrophy. Stretching of cultured myotubes and fibroblasts without nerve innervation also induces hypertrophy (114,115). However, atrophy was seen in our study when denervated sartorius muscles of adult frogs were stretched (approximately 110%) in an organ culture system (76). These results indicate that not only tension production or loading, but an intact nerve supply is requested for the normal regulation of muscle mass.

As mentioned before, the EMG in ankle extensors is reduced immediately by hindlimb suspension (1, 81). This may be due, in part, to shortening-relared unloading (1,81,92). Immobilization of the ankle joint in a plantar-flexed position also reduced the EMG activity in the soleus (43,81). On the other hand, stretching of muscle by dorsi-flexion helped to maintain EMG activity (43,81) during hindlimb suspension (81). In both cases, the muscles were active electrically. However, plantar flexion causes a passive shortening of ankle extensors and tension production of these muscles are inhibited as is shown below.

The chronic tension that muscle produces or is imposed on it seems to regulate the muscle mass. During hindlimb suspension, the ankle joints of rats are extended (81,92), so that the length of ankle extensors are passively shortened. For example, the length of soleus muscle, excluding the tendons, of rat with body weight of 318g was approximately 28 and 20mm when

the anterior angle of ankle joint was fixed at 50° and 160° during suspension, respectively (81).

Tension was produced by the plantaris muscle with the ankle joint fixed at 90°, when the EMG was present (81) (Fig. 1). A greater tension was developed when the angle of joint was changed to 30°. However, no tension was detected when the joint angle was 160°, which is approximately equivalent to the angle of freely suspended ankle. It is suggested that such a reduction of tension production, even with active EMG, may have a close association with atrophy in ankle extensors.

The wet weight of ankle extensor, soleus, was significantly decreased from the pre-suspension level following hindlimb suspension at a shortened muscle length (Fig. 2). Although the number of sarcomeres or optimum length of muscle was not measured, the reduced muscle weight was closely associated with fiber atrophy or decreased fiber CSA. This atrophy was prevented, if the muscles were stretched by dorsi-flexion of the ankle joint. Although the weight of soleus was significantly lighter than the post-suspension cage control, the suspension-induced atrophy of soleus, compared with the pre-suspension level, was prevented by stretching. But the atrophy of plantaris and gastrocnemius was not fully prevented by dorsi-flexion, although the weight of stretched muscles were significantly greater than that of shortened muscles. Similar results were also reported by Stump*et al.* (100). These results indicate an important role of tension development for maintenance of muscle mass, regardless of the type of muscles.

The weights of ankle flexors, TA and EDL, suspended for 10 days were identical to the pre-suspension controls, but were less than the age-matched controls as mentioned before (83). It is suggested that these muscles did not atrophy but the growth rate was inhibited by hindlimb suspension. However, atrophy was induced if the ankle joint was maintained in a dorsi-flexed position (43, 83). Thus, it is indicated that the

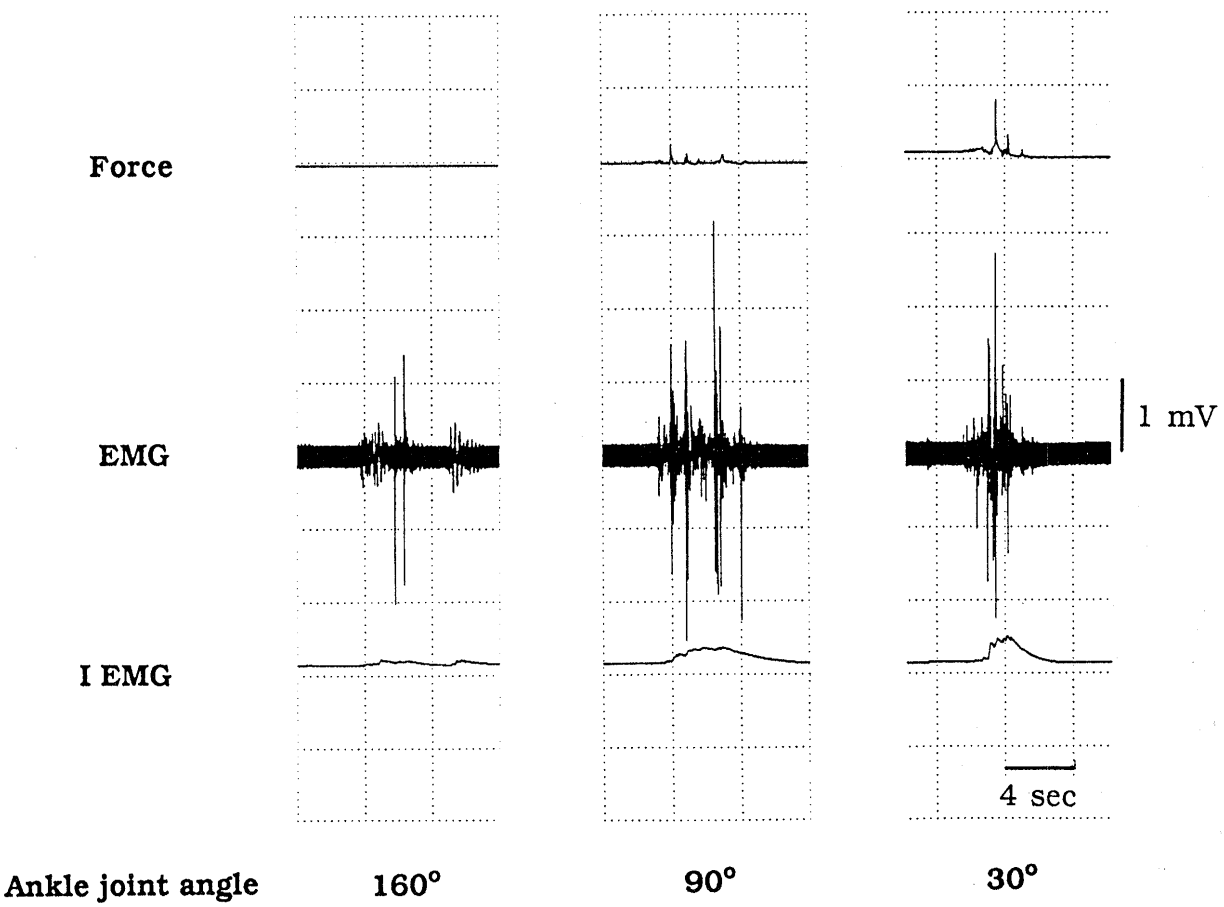

Fig. 1 Isometric force production and electromyogram (EMG) in rat plantaris muscle with various ankle joint angles. I EMG: integrated EMG. Cited from Ref. 81.

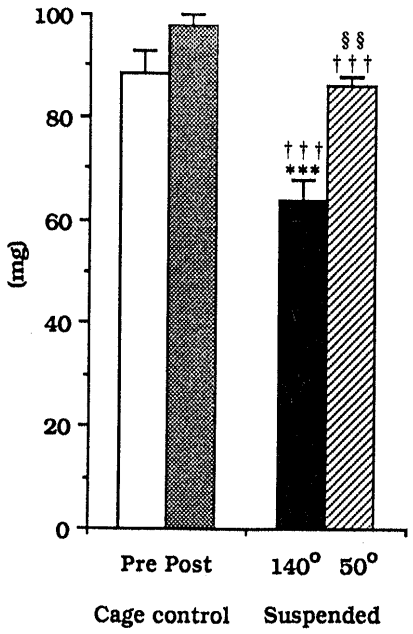

Fig. 2 Wet weigth of rat soleus muscle. Mean ± SEM. ***: $p < 0.001$ vs. pre-suspension cage control, †††: $p < 0.001$ vs. post-suspension cage control, and, §§: $p<0.01$ vs. suspended at 140° ankle joint.

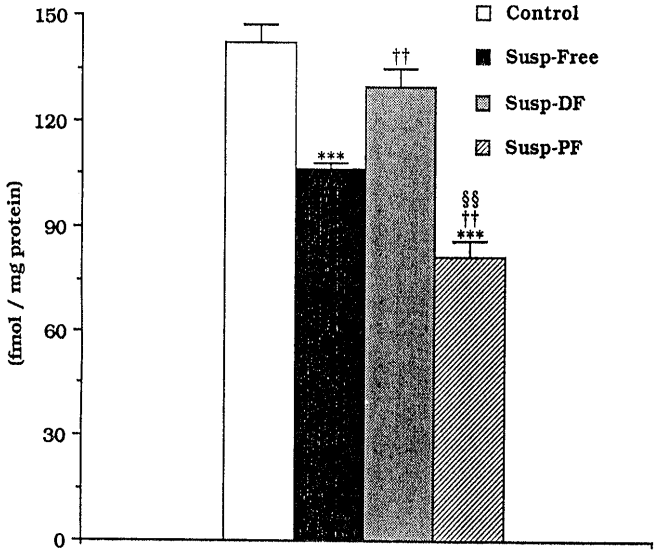

Fig. 3 The maximum binding capacity of β-adrenoceptors in soleus muscle. Mean ± SEM. ***: $p<0.001$ vs. Control, ††: $p<0.01$ vs. Susp-Free, and §§: $p<0.01$ vs. Susp-DF. Susp-Free, Susp-DF, and Susp-PF: hindlimb-suspended with free or ankle joint immobilization at either dorsi- or plantar-flexed position, respectively.

length or degree of loading of ankle flexors was also reduced by dorsi-flexion.

Atrophy of soleus single fibers, as well as whole muscle (Fig. 2), was prevented by stretching (Table 1). The CSA of fast fibers was even increased by stretching. Although the specific activities of SDH, GPD, and myosin ATPase did not change following free suspension, those activities, as well as the total levels in whole CSA, were even enhanced in response to stretching. Fiber phenotype determined histochemically and contractile properties of the stretched muscle were not different from those of the cage controls. Further, stretching of muscle prevented the suspension-induced changes in the levels of phosphorus compounds and/or the Bmax of βAR in the soleus muscle (Fig. 3)

These data indicated that stretching of muscles was useful to prevent the atrophy of whole muscle and single fibers and changes in the metabolic properties induced by suspension. It is further suggested that the unloading of muscle also caused a lowered turnover rate of high-energy phosphates, even though the muscle was active electrically. But the turnover rate and/or content of high-energy phosphates remained normal when the muscle was stretched.

D. Afferent input

Compensatory hypertrophy in soleus and/or plantaris following the tenotomy of gastrocnemius did not occur if deafferentation was performed (73), suggesting an important role of afferent input for the induction of hypertrophy. Further, a similar degree of atrophy was induced in the gastrocnemius by tenotomy and deafferentation (73). Since our results suggest that afferent input may be reduced if the muscle is shortened during hindlimb suspension in some respect (82), tenotomy at the early stage could be similar, functionally, to deafferentation by dorsal root transection. Within a few days, however, the muscle begins to regrow connective tissue reforming connection with other tissues which recovers the ability to produce forces. Although the plantaris EMG and efferent neurogram measured at L_5 were maintained during 3 days of hindlimb suspension, the magnitude of the afferent neurogram tended to be reduced. These results may also indicate an involvement of afferent input in the regulation of muscle mass.

Conclusion

The responses of skeletal muscle to gravita-

tional unloading and the possible mechanism responsible for the neuromuscular adaptation were discussed. Skeletal muscles atrophy rapidly in response to gravitational unloading. Ankle extensors are more susceptible to unloading than flexors. The magnitude of the decrease in CSA is greater in slow- than fast-twitch fibers. Therefore, a prominent atrophy is induced in soleus muscle which is composed of approximately 80% of slow-twitch oxidative fibers. Shifts of contractile and metabolic properties toward fast type are associated with the atrophy. Although the activities of mitochondrial enzymes measured in single fibers do not change generally, these levels analyzed in whole muscle homogenates are lowered by unloading. Such disagreement may be caused by a greater atrophy of fibers, not the connective tissues, which results in a relative increase in the non-fiber volume. Although the EMG activity in rat ankle extensors disappears in response to unloading, it is recovered gradually during suspension. Ankle joints are extended during suspension. Thus, the ankle extensors, especially soleus, are passively shortened and tension development is inhibited even when the EMG is present. A reduction of afferent input was also seen following the passive shortening of muscle or unloading. These results suggest that the adaptations of morphological, metabolic, and contractile properties of skeletal muscles to unloading may be closely related to the decreased levels of tension production and/or afferent input.

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