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Effects of Intermittent Sciatic Nerve Stimulation on the Soleus and Medial Gastrocnemius Muscle Atrophy in Hindlimb Suspended Rats

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=ABSTRACT=

The present study was designed to evaluate effects of intermittent electrical stimulation of the sciatic nerve on the atrophic response of antigravity muscles, such as the soleus (slow m.) and medial gastrocnemius (fast m.) muscles. Rats (Sprague-Dawley, 245-255g) were subjected to a hindlimb suspension and divided into three groups : one was with hindlimb suspension (HS) and another with hindlimb suspension plus intermittent electrical stimulation of the sciatic nerve (HS-ES). Control group (CONT) was kept free without strain of the hindlimb. After 7 days of hindlimb suspension, the soleus and medial gastrocnemius muscles were cut at their insertion sites, and were then connected to the force transducer to observe their mechanical properties.

Optimal pulse width and frequency of electrical stimulation were 0.2ms, 20Hz for the soleus muscle and 0.3ms, 40Hz for the medial gastrocnemius muscle under supramaximal stimulation. Body weight and circumference of the hindlimb were significantly decreased in HS and HS-ES groups compared with the control group. In HS-ES group, however, the weight of the soleus muscle was not different from that in the control group while the weight of the medial gastrocnemius muscle was lower than that in the control group. In HS group, mechanical properties of muscle contraction including contraction time, half relaxation time, twitch tension, tetanic tension, and fatigue index of both muscles were significantly decreased compared with the control group except for twitch tension and tetanic tension of medial gastrocnemius muscle. The degree of atrophy of the soleus muscle in HS group was more prominent than that of the medial gastrocnemius muscle. Twitch tension and fatigue index of the soleus muscle and fatigue index of the medial gastrocnemius muscle in HS-ES group were not different from those of the control group. While mechanical properties of the soleus muscle examined were all significantly increased in HS-ES group compared with HS group, only contraction time and fatigue index of the medial gastrocnemius muscle were significantly increased in HS-ES group. These data indicate that intermittent electrical stimulation may be useful in prevention of muscle atrophy.

Key Words: Muscle atrophy, Intermittent electrical stimulation, Hindlimb suspension

INTRODUCTION

Atrophy of muscle is caused by chronic bed rest, cast immobilization, nerve injury, and weightlessness associated with space flight. Currently, rodent hindlimb suspension is a popular earth - based model to investigate microgravity induced atrophy of antigravity muscles. In the hindlimb suspension model, the hindlimbs are prevented from bearing weight by tail, however, the forelimbs are in contact with the cage floor to allow locomotion to food and water (Morey, 1979; Musacchia et al, 1980; Jaspers & Tischler, 1984; Templeton et al, 1984).

Characteristic changes of muscle atrophy are marked within 2-3 days of suspension, but the changes do not progress after 7 days (Alford et al, 1987; Thomason et al, 1987). These acute changes are more prominent in antigravity muscles, such as the soleus and the medial gastrocnemius than in flexor muscles (Hauschka et al, 1987; Graham et al, 1989a). Extensor muscles of the limb bear weight, especially the soleus muscle composed of slow muscle fibers acts as a strong antigravity muscle and the medial gastrocnemius muscle composed of slow and fast muscle fibers acts as a relatively strong antigravity muscle (Graham et al, 1989a). In hindlimb suspension model, weight of antigravity muscles are decreased and mechanical properties of muscle contraction, for example, contraction time, maximum twitch tension, half relaxation time, maximum tetanic tension, and fatigue index, are changed (Templeton et al, 1984; Pierotti et al, 1990). In order to recover from muscle atrophy, exercise has been used in hindlimb suspension (Templeton et al, 1984; Pierotti et al, 1990). Exercise on a treadmill for 10 min every 6 hours during 7 days of hindlimb suspension improved histological and mechanical properties in the medial gastrocnemius muscle but did not in the soleus muscle (Graham et al, 1989b; Pierotti et al, 1990). Also, on the course of spontaneous recovery from atrophy induced by weightlessness in the soleus muscle maximum tension and contraction time were remained without any improvements (Templeton et al, 1984). Therefore, intermittent exercise in weightlessness was not enough to recover the normal mechanical properties in the soleus muscle.

The purpose of this experiment is to determine the optimal parameters of electrical stimulation for different types of muscle and to evaluate the effect of intermittent electrical stimulation on prevention of muscle atrophy in hindlimb suspended rats. Intermittent electrical stimulation was applied to the sciatic nerve of hindlimb suspended rat by means of therapeutic electrical stimulator (Handa et al, 1989 ; Park et al, 1989) for 7 days, and mechanical properties of muscle contraction and physical characteristics in the soleus and medial gastrocnemius muscles were measured.

METHODS

Sprague-Dawley rats weighing 245-255gm were used for our investigation. The rats were subjected to hindlimb suspension using tail cast method (Morey, 1979). In order to suspend hindlimbs, the tails were cleaned with rubbing alcohol, and self-adhesive plaster looped over a proximal portion of the tail. Dental cement secured fish-swivel was mounted over the plaster and fish-swivel was connected to the ceiling of the cage. In hindlimb suspension model, the rats were suspended at a height preventing their hindlimbs from contacting any floor or wall surface, but allowing the forelimbs complete mobility, weight support, and free 360° rotation. Throughout the suspension period, the hindlimbs had no weight bearing such as weightlessness.

The rats were divided into three groups: control group (CONT, n = 10) was kept free without strain of the hindlimb, hindlimb suspension group (HS, n = 10) was with hindlimb suspension for 7 days, and hindlimb suspension plus electrical stimulation group (HS-ES, n = 10). In HS-ES rats, electrical stimulation was applied to the sciatic nerve through teflon coated stainless steel wire (0.45mm in diameter) for 10 min every 6 hours during 7 days of suspension. In order to decrease a muscle fatigue, intensity, frequency, and pulse width of electrical stimulation were considered and therapeutic electrical stimulation with 5 sec ON-OFF type was used (Handa et al, 1989; Park et al, 1989).

The mechanical properties of muscle contraction were measured by using isometric force transducer (Grass, FT03). The isolated sciatic nerve was stimulated supramaximally (threshold = 0.4V) with bipolar silver electrodes using square wave pulse with 0.2ms, 20Hz for the soleus muscle and 0.3ms, 40Hz for the medial gastrocnemius muscle. Maximum twitch

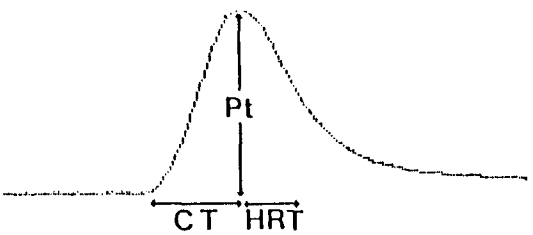

Fig. 1. Measurement of contraction time (CT), half relaxation time (HRT), and twitch tension (Pt). Recordings obtained from average of 5 sweeps.

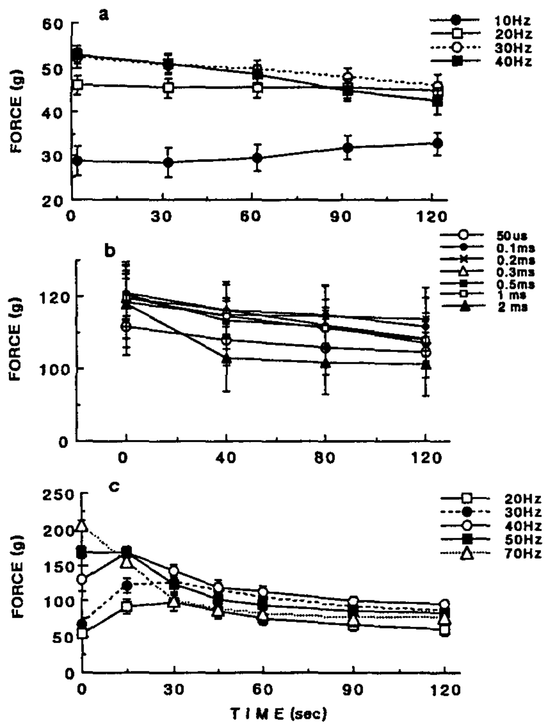

Fig. 2. Time dependent changes of muscle force at various stimulation frequencies and pulse widths. In soleus muscle, pulse width and amplitude were 0.2ms, 1.0 Volt (a), and frequency and amplitude were 20Hz, 1.0 Volt, respectively (b). In medial gastrocnemius muscle (c), pulse width and amplitude were 0.3 ms, 1.0 Volt, respectively. The number of rats in each group is 7. Values are means ± SE.

tension, contraction time, and half relaxation time were determined from a series of twitch responses (Fig.1). Maximum tetanic tension was measured the highest tension achieved during tetanic stimulation. Fatigue properties were determined via tetanic stimulation for 2 min. The tension production at 2 min relative to the maximum tension was used as a fatigue index of the muscle. Fatigue index was calculated by following formula: (maximum initial tension - tension at 2 min)/maximum initial tension.

Contractile events were analyzed by computer (CED 1401) and statistical analyses were used as a student t-test. The 0.05 level of probability was established for statistical significance. Reported values are means ± SEM.

RESULTS

Optimal parameters of electrical stimulation

Time dependent changes of muscle force were measured at various stimulation parameters for 2 min. By supramaximal intensity, pulse width and frequency were applied at the range of 0.1 - 0.3ms, 10 - 40Hz, respectively, for the soleus muscle, and at the range of 0.2 - 0.4ms, 20 - 70Hz for the medial gastrocnemius muscle. In the soleus muscle, 10Hz produced the lowest tension among 4 groups and 30Hz, 40Hz showed fatigue tendency easily. Also, pulse width of 0.2ms produced reasonable tetanic tension. In the medial gastrocnemius muscle, 20Hz produced very low tension. The data for pulse width of the medial gastrocnemius muscle did not show in Fig. 2. Therefore, optimal pulse width and frequency were 0.2ms, 20Hz for the soleus muscle and 0.3ms, 40Hz for the medial gastrocnemius muscle. These parameters produced relatively high tension and the lowest fatigue index in the above experimental ranges (Fig. 2).

Table 1. Physical characteristics of control, hindlimb suspended, and hindlimb suspended plus electrical stimulated rats

	CONT	HS	HS-ES
BW (g)	247 ± 2		
PRE		251 ± 1	247 ± 1
POST		237 ± 3**	238 ± 1**
SOL (% BW)	0.053 ± 0.001	0.040 ± 0.001**	0.052 ± 0.002
MGC (% BW)	0.246 ± 0.005	0.191 ± 0.005**	0.219 ± 0.003**
CIR (mm)	54.2 ± 0.2		
PRE		54.4 ± 0.3	53.7 ± 0.4
POST		48.3 ± 0.5**	50.0 ± 0.6**

Values are means ± SE. The number of rats in each group is 10. BW, body weight; SOL (% BW), relative weight of soleus muscle to body weight; MGC (% BW), relative weight of medial gastrocnemius muscle to body weight; CIR, circumference of hindlimb. * Denotes a significant difference between CONT and HS or HS-ES (** $p<0.01$).

Body and muscle weights

Following 7 days of hindlimb suspension, the HS and HS-ES rats weighed significantly less than the control rats ($p<0.01$). The mean relative weight of soleus muscle to body weight was 25% ($p<0.01$) and 4% smaller than that of control group in the HS and HS-ES groups, respectively. The mean relative weight of medial gastrocnemius muscle to body weight was 22% ($p<0.01$) and 11% ($p<0.01$) less in the HS and HS-ES groups, respectively, in comparison to CONT group (Table 1).

Mechanical properties of muscle contraction

In the control rats, contraction time and half relaxation time of soleus muscle were 2 - 3 times as long as those of medial gastrocnemius muscle. Maximum twitch tension and tetanic tension of the whole muscle were larger in medial gastrocnemius muscle, but relative those tensions to muscle weight were larger in soleus muscle. Fatigue index of soleus muscle was 0.074 and medial gastrocnemius muscle was 0.659, which means that the soleus muscle is more resistant to fatigue. Therefore, the mechanical properties of muscle contraction were quite different between the soleus and medial gastrocnemius muscles (Fig. 3).

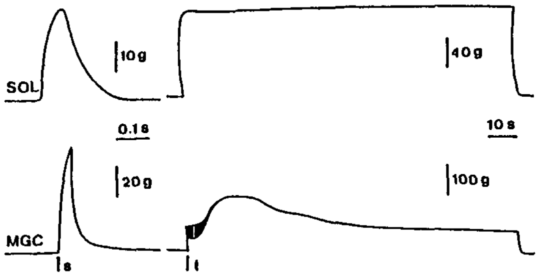

Fig. 3. Records of isometric twitch and tetanic contractions from soleus (SOL) and medial gastrocnemius (MGC) muscles of control rats. s, single shock: t, tetanic stimulation for 2 minutes. Contractions were elicited by 1 V, 0.2ms, 20Hz in soleus muslce and 1 V, 0.3ms, 40Hz in medial gastrocnemius muscle.

In HS rats, contraction time and half relaxation time of the soleus and medial gastrocnemius muscles were significantly decreased compared with the control rats ($p<0.01$). Maximum twitch tension, maximum tetanic tension, and fatigue index of the soleus muscle were decreased in comparison to the control rats ($p<0.01$). Also medial gastro-

Table 2. Mechanical properties of the soleus muscle of control (CONT), 7 days hindlimb suspended (HS), and 7 days hindlimb suspended plus electrically stimulated (HS-ES) rats

	CONT	HS	%Δ	HS-ES	%Δ
CT (ms)	66±1	47±1**	−29	58±1**‡	−13
HRT (ms)	59±1	47±1**	−20	53±1**‡	−9
Pt (g)	28±1	12±1**	−56	27±1	−5
(g/g, MW)	216±6	122±5**	−44	230±16‡	+7
Po (g)	115±3	38±2**	−67	89±5**‡	−23
(g/g, MW)	885±16	397±22**	−55	772±32**‡	−13
FI	0.07±0.01	0.17±0.01**	−128	0.10±0.01‡	−39

%Δ, percent change from control; CT, contraction time; HRT, half relaxation time; Pt(g), twitch tension; Pt(g/g, MW), relative twitch tension to muscle weight; Po(g), tetanic tension; Po(g/g, MW), relative tetanic tension; FI, fatigue index. Values are means±SE. The number of rats in each group is 10. *Denotes a significant difference between CONT and HS or HS-ES (*p<0.05, **p<0.01). † Denotes a significant difference between HS and HS-ES (†p<0.05, ‡<0.01).

Table 3. Mechanical properties of the medial gastrocnemius muscle of control (CONT), 7 days hindlimb suspended (HS), and hindlimb suspended plus electrically stimulated (HS-ES) rats

	CONT	HS	%Δ	HS-ES	%Δ
CT (ms)	29±0	26±0**	−11	27±0**‡	−6
HRT (ms)	20±0	18±0**	−10	19±0*	−6
Pt (g)	65±2	59±2	−9	66±3†	−2
(g/g, MW)	105±4	124±3**	+18	134±5**	+28
Po (g)	168±5	142±12	−16	160±8	−5
(g/g, MW)	277±7	306±18	+11	325±11**	+17
FI	0.66±0.02	0.77±0.01**	−17	0.71±0.01‡	−5

Values are means±SE. The number of rats in each group is 10. Notations are as in Table 1.

cnemius muscle showed significantly increased fatigue index and relative twitch tension to muscle weight (p<0.01), but twitch tension and tetanic tension of whole muscle were not significantly changed.

In HS-ES rats, contraction time of the soleus muscle was decreased compared with the control rats, but increased compared with HS rats (p<0.01). Twitch tension of whole muscle and relative twitch tension to muscle weight of the soleus muscle were increased as twice as those of HS rats. Tetanic tension of whole muscle and relative tetanic tension to muscle weight of the soleus muscle were significantly decreased compared with the control rats (p<0.01), but increased as three to four times as those of HS rats. Also, muscle fatigue of the soleus muscle was similiar to that of the control rats and significantly increased compared with HS rats (p<0.01). However, in the medial gastrocnemius muscle, only contraction time (p<0.01), twitch tension of whole muscle (p<0.05), and fatigue index (p<0.01) were significantly increased compared with HS rats (Table 2, 3 and Fig. 4, 5).

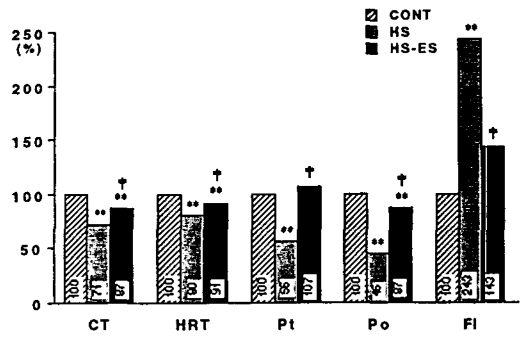

*Fig. 4. Percent ratio of mechanical properties of the soleus muscle in control (CONT = 100%), 7 days hindlimb suspension (HS), and 7 days hindlimb suspension plus electrical stimulation (HS-ES) rats. CT, contraction time; HRT, half relaxation time; Pt, relative twitch tension; Po, relative tetanic tension; FI, fatigue index. * Denotes a significant difference between CONT and HS or HS-ES (*<0.05, **p<0.01) † Denotes a significant difference between HS and HS-ES (‡p<0.01)*

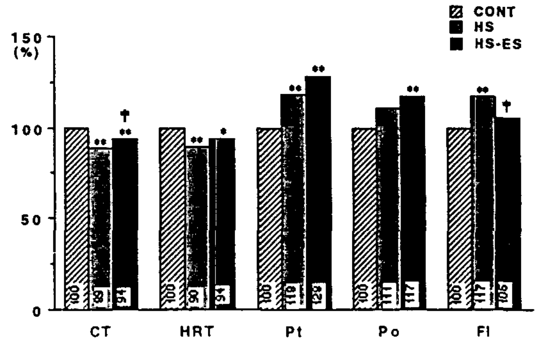

Fig. 5. Percent ratio of mechanical properties of the medial gastrocnemius muslce in control (CONT = 100%), 7 days hindlimb suspension (HS), and 7 days hindlimb suspension plus electrical stimulation (HS-ES) rats. Notations are as in Fig. 4.

DISCUSSION

Decreased body weight by the hindlimb suspension is caused by reduced food intake, stress, or changed basal metabolism (Fitts et al, 1986 ; Herbert et al, 1988). Change of muscle weight was more prominent in the extensors than in the flexors, and circumference of the hindlimb decreased markedly in 7 days of the hindlimb suspension. The atrophy by the hindlimb suspension is known to result from decreased protein content in muscles (Loughna et al, 1986) and decreased size of muscle fiber rather than decreased number of muscle fiber (Graham et al, 1989a ; Thomason & Booth, 1990). In muscle atrophy, the soleus muscle is more prominent than the medial gastrocnemius muscle. The soleus and medial gastrocnemius muscles are synergists as an ankle extensors, but they have very different fiber type compositions and recruitment patterns. The soleus muscle is composed primarily of slow muscle fibers, which showed long contraction time and long half relaxation time, whereas the medial gastrocnemius muscle is comprised of a mixture of fiber types and has predominantly fast muscle fibers, which showed short contraction time and short half relaxation time. Functionally, the soleus muscle is near maximally activated even during simple weight support, and it is called static antigravity muscle, whereas the medial gastrocnemius muscle becomes highly active only when high activity demands are required, and is called dynamic antigravity muscle. Therefore, there are many difference in mechanical properties of muscle contraction between the soleus and medial gastrocnemius muscles (Hutchison et al, 1989). Since the mechanical properties are different between the soleus and medial gastrocnemius muscles, optimal parameters of electrical stimulation in both muscles should be different in order to get a good contractile force without muscle fatigue (Park et al, 1989). Therefore, 20Hz, 0.2ms in the soleus muscle and 40Hz, 0.3ms in the medial gastrocnemius muscle will be very useful to prevent from muscle atrophy without any change of mechanical properties of muscles.

Following hindlimb suspension, contraction time and half relaxation time of both muscles were faster than the control rats. These findings are consistent with other experiments (Graham et al, 1989a ; Pierotti et al, 1990), and are supported by that slow myosins in muscle fiber change into fast myosins (Gardetto et al, 1989). However, contractile responses of the soleus muscle in HS rats were slower than those of the medial gastrocnemius muscle in control rats. This suggests that the soleus muscle contains a lot of slow myosin continuously even though in hindlimb suspension. Also, the suspended soleus muscle contains the characteristics of fast muscle which Ca^{++} activation occurs easily at muscle contraction. Tetanic tension of muscle contraction reflects a number of active cross bridge (Fitts & Holloszy, 1978). Decreased tetanic tension in both muscles during hindlimb suspension resulted from decreased number of active cross bridge and this effect was more prominent in the soleus muscle. However, it was difficult to explain theoretically that relative twitch and tetanic tensions to muscle weight were increased in the suspended medial gastrocnemius muscle. This relative tension suggests that the medial gastrocnemius muscle is less prominent antigravity muscle than the soleus muscle, and the medial gastrocnemius muscle has less effect in muscle atrophy by hindlimb suspension. In muscle fatigue properties, fatigue index of both muscles increased in hindlimb suspension, especially prominent in the soleus muscle. These findings were different from those of other researchers, who reported that fatigue properties were not changed in the soleus muscle and not changed or increased in the medial gastrocnemius muscle by hindlimb suspension (Fell et al, 1985 ; Winiarski et al, 1987 ; Herbert et al, 1988). Potential reasons for this discrepancy between studies may be related to different laboratory techniques.

In HS-ES rats, intermittent electrical stimulation of the sciatic nerve for 7 days produced more improved mechanical properties of contraction than in HS rats. Contraction time and half relaxation time of both muscles were slower in comparison to HS rats. Also, maximum twitch tension and maximum tetanic tension were increased, especially relative tensions to muscle weight were increased. These improvements by intermittent electrical stimulation may result from electrical stimulation, which increases metabolism by changing from anaerobic into aerobic metabolism, and increasing blood flow, oxygen consumption, and glucose consumption (Altman et al, 1979). Also, chronic intermittent electrical stimulation can alter the characteristics of muscle fiber by stimulus frequency which acting on myosin (Salmons & Vrbova, 1969 ; Cotter & Phillips, 1986). Especially, decrease of muscle fatigue by electrical stimulation results from decreased production of lactic acid in addition to the previous explanations (Altman et al, 1979). Our results also show that the intermittent electrical stimulation is more effective on the prevention of muscle atrophy in comparison to the effect of exercise in hindlimb suspended rats (Pierotti et al, 1990).

Consequently, intermittent electrical stimulation used in the present study resulted in an amelioration in the physiological adaptations to hindlimb suspension in the soleus and medial gastrocnemius muscles. Also, an intermittent electrical stimulation may be useful for prevention of muscle atrophy.

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