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Effectiveness of daily eccentric contractions induced via kilohertz frequency transcutaneous electrical stimulation on muscle atrophy

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ABSTRACT

The effects of daily repeated bouts of concentric, isometric, or eccentric contractions induced by high frequency (kilohertz) transcutaneous electrical stimulation in ameliorating atrophy of the soleus muscle in hindlimb unloaded rats were determined. Five groups of male rats were studied: control, hindlimb unloaded for 2 weeks (HU), or HU plus two daily bouts of concentric, isometric, or eccentric high-frequency electrical stimulation-induced contractions of the calf musculature. Soleus mass and fiber size were smaller, the levels of phosphorylated Akt1 and FoxO3a lower, and atrogin-1 and ubiquitinated proteins higher in the HU, and the HU plus concentric or isometric contraction groups than in the control group. In contrast, daily bouts of eccentric contractions maintained these values at near control levels and all measures were significantly different from all other HU groups. These results indicate that daily bouts of eccentric contractions induced by high-frequency stimulation inhibited the ubiquitin–proteasome catabolic pathway and enhanced the Akt1/FoxO3a anabolic pathway that resulted in a prevention of the atrophic response of the soleus muscle to chronic unloading.

Introduction

Skeletal muscle atrophy results from a variety of conditions that involve a decrease in the levels of neuromuscular activity, i.e., muscle activation and/or loading, such as hindlimb unloading (Morey et al., 1979), joint immobilization (Booth, 1982; Pachter and Eberstein, 1984), denervation (Dow et al., 2004; Russo et al., 2007) and spaceflight (Sandonà et al., 2012). A variety of negative effects occur in the skeletal muscles under these conditions, e.g., reduction of muscle mass, decrease in fiber cross-sectional area, and increase in the protein degradation (Edgerton and Roy, 1996; Sandonà et al., 2012; Tsika et al., 1987). In addition, these effects are more prominent in primary extensor than flexor muscles and in muscles that are comprised predominantly of slow fibers, e.g., the soleus muscle (Roy et al., 1996). These slow extensor muscles function as anti-gravity muscles, i.e., they are heavily recruited to maintain posture and during activities that require low loads such as walking (Nagatomo et al., 2009; Richardson et al., 1999; Roy et al., 1991; Sandonà et al., 2012). In effect, the activity patterns of these muscles are the most affected under conditions of decreased loading and activation. Therefore, it is important to develop and identify countermeasures for the detrimental effects of chronic decreases in their activation and loading.

Muscle atrophy induced by hindlimb unloading is associated with a decrease in protein synthesis and an increase in protein degradation (Jackman and Kandarian, 2004). Deactivation of the Akt pathway has been shown to be involved in the regulation of muscle atrophy (Jackman and Kandarian, 2004) and forkhead box O (FoxO) class of transcription factors has been identified as a key regulator of this process downstream of the Akt signaling pathway (Sandri et al., 2004; Stitt et al., 2004). In addition, the Akt signaling pathway can suppress the activation of the ubiquitin–proteasome

Abbreviations: ATPase, adenosine triphosphatase; BSA, bovine serum albumin; CSA, cross-sectional area; HU, hindlimb unloading; ES, electrical stimulation; FoxO, forkhead transcription factor of the O; SDS-PAGE, SDS polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; PBST, phosphate-buffered saline with Tween 20; TBST, Tris-buffered saline with Tween 20.

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pathway, a regulator of protein degradation, via phosphorylation of FoxO (Sandri et al., 2004). In the muscles of hindlimb-unloaded rats, the rates of protein degradation increase through activation of the ubiquitin proteasome pathway (Jackman and Kandarian, 2004). It also has been suggested that ubiquitinated protein and ubiquitin ligases such as atrogin-1 are indicators for the activation of the ubiquitin proteasome pathway (Fujita et al., 2011; Judge et al., 2007). In addition, the phosphorylation of FoxO3a can suppress increased ubiquitin ligases (Sandri et al., 2004). Consequently, activation of the Akt/FoxO signaling pathway might inhibit hindlimb unloading-induced activation of ubiquitin proteasome pathway and prevent the atrophy process.

Generally, resistance exercise training is an effective therapeutic intervention for preventing muscle atrophy induced by a variety of conditions, such as hindlimb unloading, joint immobilization, and spaceflight (Booth, 1982; Morey et al., 1979; Sandonà et al., 2012). The effect of resistance exercise is known to be dependent on the intensity of muscle loading (Milne and Noble, 2002). Exercise involving eccentric contractions has a greater protecting effect against muscle atrophy because the level of loading is greater during eccentric compared to concentric or isometric contractions (Kirby et al., 1992; Fitts, 2003). Additionally, muscle contractions induced by electrical stimulation have been used as a countermeasure to prevent muscle atrophy (Fujita et al., 2011; Kim et al., 2007, 2008; Petrofsky and Laymon, 2002). Similar to resistance exercise, the effectiveness of muscle electrostimulation is determined by the intensity and magnitude of the induced contractions. To elicit high intensity contractions in muscles that are located in the deep region (close to the bone) in a limb segment using transcutaneous stimulation, it is important to set an appropriate current frequency and waveform (Petrofsky, 2008; Petrofsky et al., 2009; Tanaka et al., 2013, 2014). In a previous study, we showed that electrical stimulation using a kilohertz frequency was more effective in preventing hindlimb unloading induced atrophy of muscles located in the deep region of the rat calf than low frequency stimulation producing isometric contractions (Tanaka et al., 2013). This high-frequency stimulation protocol, however, did not maintain the muscle masses at control levels (Tanaka et al., 2013, 2014).

Based on the above results, we hypothesized that a daily high frequency stimulation protocol producing eccentric contractions would be more effective than isometric or concentric contractions in preventing the atrophy of the soleus muscle normally associated with chronic hindlimb unloading. Thus we compared the effects of daily bouts of each type of contraction on soleus mass and fiber size during two weeks of hindlimb unloading. To begin to understand the role of signaling pathways in producing these effects, we also determined the levels of Akt, FoxO3a, rpS6, atrogin-1, and ubiquinated proteins.

Material and methods

Experimental groups

Twenty-four adult male Sprague-Dawley rats (mean body mass, 521 ± 3 g; 20 weeks old; Japan SLC, Hamamatsu, Japan) were used in the present study. The rats were divided randomly (n = 6/group) into control (Cont), hindlimb unloaded (HU), hindlimb unloaded plus electrical stimulation (HU + ES), and hindlimb unloaded plus the combination of electrical stimulation with eccentric contractions (HU + eES) groups. The rats receiving electrical stimulation were subjected to therapeutic electrical stimulation, twice/day during unloading. Rats in the HU + ES group received concentric contractions in the left calf (HU + cES) and isometric contractions in the right calf (HU + iES) simultaneously. Rats in the HU + eES group received eccentric contractions in the right calf only. This study was approved by the Institutional Animal Care and Use Committee and was performed according to the Kobe University Animal Experimentation Regulations. All experiments were conducted in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (National Research Council, 1996).

Hindlimb unloading

Hindlimb unloading was induced by suspending the rats by their tails for two weeks according to the method described by Morey et al. (1979). Briefly, the rats in the HU groups were fitted with a tail harness and suspended by a string just high enough to prevent the hindlimbs from bearing weight on the floor or sides of the cage. The forelimbs were allowed to maintain contact with the floor of the cage, and the rats were fed food and water ad libitum. The animals were housed in an isolated and environmental controlled room, at 22 ± 2 °C in a 12 h:12 h light–dark cycle.

Electrical stimulation protocol

Electrical stimulation was initiated on the second day of hindlimb suspension and continued for 2 weeks. The rats in the HU groups were anesthetized during the electrical stimulation sessions with sodium pentobarbital (50 mg/kg body weight, i.p.). To exclude the effects of the repeated periods of anesthesia, the rats in the HU group were subjected to an anesthetic protocol identical to that experienced by the rats in the stimulated groups. Electrical stimulators that permitted changes in the electrical parameters were used to stimulate the calf muscles transcutaneously (ES-360, Ito, Tokyo, Japan). Two surface electrodes (1 cm in diameter) were adhered to the medial and lateral calf muscles. The stimulation was a sinusoidally modulated waveform composed of 2500 Hz alternating current delivered at a frequency of 100 Hz. This stimulation paradigm induced strong plantarflexor contractions and the current intensity was adjusted daily to produce supra-maximal contractions. Each day prior to initiating the bouts of contractions, a movable transducer was attached at the sole of the foot to measure plantarflexor force. The intensity of the electrical current was increased until a plateau in the force was reached. Then the current intensity required to reach a plateau in force plus 10 mA was used to elicit the contractions during the stimulation bouts. Each session consisted of one burst of electrical stimulation delivered every 3 s (time on: 1 s and time off: 2 s) for 1 min, followed by 5 min of rest. Six consecutive stimulation sessions were performed twice per day, separated by a 9-h interval. Collectively, this resulted in a stimulation duration of 240 s/day. During stimulation, the rats were supported in a prone position on a platform and the knee joint was secured in a fully extended position. The lower thigh was fixed to a stainless steel plate with athletic tape and the foot was attached to a movable footplate with athletic tape. For the isometric contractions the footplate was locked with the left ankle at 0° of dorsiflexion (90° ankle joint angle). For the concentric contractions, the footplate was moved without resistance and the ankle was plantarflexed while being stimulated. The range of the concentric contractions was from 0° to 90° of plantarflexion in the ankle. For the eccentric contractions, a pulley-bar apparatus was attached to the footplate and an actuator (Oriental Motor, Tokyo, Japan) was used to move the footplate such that the ankle was dorsiflexed while being stimulated. Eccentric contractions were induced at an angular velocity of 30°/s as described previously (Ochi et al., 2010). The range of the forced lengthening contractions was from at 0° to 30° of dorsiflexion in the ankle.