Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental Neurology 200 (2006) 191–199
Experimental Neurology
www.elsevier.com/locate/yexnr

Adaptation of rat soleus muscle spindles after 21 days of hindlimb unloading

C. Rosant, M.D. Nagel, C. Pérot *

UMR-CNRS 6600 Biomécanique et Génie Biomédical, Université de Technologie de Compiègne, BP 20529, F-60205 Compiègne, France

Received 18 November 2005; revised 16 January 2006; accepted 6 February 2006
Available online 19 April 2006

Abstract

Spindle discharges are affected by muscle unloading, and changes in passive stiffness of the muscle–tendon unit may contribute to the changes in spindle solicitation. To test this hypothesis, we determined the spindle sensitivity from electroneurograms of the soleus nerve, and, concomitantly, we measured the incremental passive muscle tension. Both measurements were done from ramp and hold stretches imposed to the soleus muscle after the Achilles tendon was severed. The ratio between the spindle sensitivity and the passive stiffness gave a "spindle efficacy index" (SEI). The experiments were conducted on control rats (C, $n = 12$) and on rats that had undergone hindlimb unloading (HU, $n = 12$) for 21 days. The muscle threshold lengths for electroneurogram to discharge (neurogram length, L_n) and for detecting passive tension (slack length, L_s) were determined, and, when these lengths differed, the stretches were imposed at these two initial lengths. The contralateral muscles were used to count muscle spindles and spindle fibers (ATPase staining) and to identify MyHC isoforms by immunostaining.

L_n and L_s values were identical for the C muscles, while after HU, L_n was significantly shorter than L_s, which indicated that spindle afferents were more sensitive since they discharged before any passive tension was developed by the soleus muscle.

At L_n, spindle sensitivity and passive stiffness did not differ for C and HU muscles. Consequently, when calculated at this relatively short initial muscle length, the SEI was maintained (or even slightly increased) after HU. This held under dynamic conditions (ramp phase of the stretch) and under static conditions (hold phase of the stretch).

At L_s, the dynamic and static incremental stiffness values increased significantly after HU. Under dynamic conditions, the spindle sensitivity also increased after HU but to a less degree than incremental stiffness, which led to a significant decrease in SEI. Under static conditions, the spindle sensitivity presented a high increase, and, consequently, SEI was not modified. These functional changes were associated with structural adaptations: HU did not alter the total number of muscle spindles, but the number of spindles containing three nuclear chain fibers increased significantly. The main change in intrafusal MyHC content concerned the slow type I MyHC isoform.

In conclusion, after a period of muscle unloading, the spindle discharges were maintained or even enhanced in several experimental conditions. This may be due to a better transmission of the external stretch to muscle spindles through stiffer elastic structures but also to own muscle spindle adaptations which reinforce the spindle sensitivity, notably under static conditions.

Keywords: Electroneurography; Passive incremental stiffness; Soleus muscle; Muscle spindle; Plasticity; Immunohistochemistry

Introduction

The neuromechanical adaptations of muscles in response to microgravity (real or simulated) are well documented (Edgerton and Roy, 2000; Fitts et al., 2000, 2001; Ohira, 2000). Some of the muscle–tendon changes may be due to fewer proprioceptive messages being transmitted by postural muscles placed in microgravity (Kawano et al., 2002). During hindlimb unloading (HU), the rat soleus muscle is kept shortened and the consequent reduction in spindle discharges may contribute to muscle atrophy (Riley et al., 1990). Recently, Picquet and Falempin (2003) have demonstrated that deafferentation and HU have similar effects on muscle, including significant atrophy, decreased fiber cross-section area with a loss of muscle force.

The T-reflex, the muscle response to a tendon tap, is altered in rats (Anderson et al., 1999) and humans (Lambertz et al., 2003) subjected to microgravity, and, in these two studies, the reflex changes were related to changes in the elastic properties

* Corresponding author. Fax: +33 3 44 20 48 13.
E-mail address: chantal.perot@utc.fr (C. Pérot).

0014-4886/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2006.02.003

of the muscle–tendon unit. These results confirm the earlier proposal of Rack et al. (1983) that muscle spindle solicitation depends on the compliance of the elastic structures linked to them. However, several studies have now demonstrated that muscle spindles can adapt to different situations. Changes in intrafusal structure can be caused by denervation (Zelena, 1957), tenotomy or immobilization (Jozsa et al., 1988), swimming training (Yoshimura et al., 1992) and hindlimb unloading (De-Doncker et al., 2002). A few studies have also shown significant increases in the discharges of isolated spindle afferents in response to muscle stretch after immobilization (Gioux and Petit, 1993) or simulated microgravity (De-Doncker et al., 2003). These studies suggest that changes in muscle passive stiffness contribute to changes in spindle discharges. To verify this hypothesis, we have analyzed the contribution of passive stiffness to changes in muscle spindle responses to stretch after muscle disuse. Discharges of the soleus muscle spindles were characterized from electroneurograms (ENGs) of the soleus nerve. The ENGs represented the cumulated discharges of all muscle spindle afferents activated by the muscle stretches. They were used to characterize the spindle sensitivity under dynamic and static phases of ramp and hold muscle stretches. The passive incremental stiffness was calculated at the same stretching conditions. By relating spindle sensitivity to passive stiffness, we propose to determine a spindle efficacy index (Rosant and Pérot, 2006). After muscle unloading by hindlimb suspension, this calculation of the SEI allows us to estimate the contribution of changes in elastic properties of the muscle–tendon unit to the changes in spindle sensitivity. Both spindle sensitivity and passive tension were measured at two initial lengths: the length threshold for spindle discharges (neurogram length, L_n) and the length threshold for the development of passive tension (slack length, L_s). These two series of measurements were carried out in order to appreciate the eventual differences in spindle sensitivity due to the prestretching of the muscle. The entire protocol was applied to control (C) and hindlimb unloading (HU) groups of rats. Furthermore, histochemical and immunohistochemical analyses were carried out to compare, in C and HU soleus muscles, the number of spindles per muscle, the number of fibers per spindle and the amounts of the various myosin heavy chain (MyHC) isoforms along the intrafusal fibers.

Materials and methods

Rats

A total of 24 male Wistar rats (body weight: 280–300 g) were randomly assigned to control (C) or hindlimb unloaded (HU) groups (12 rats per group). The rats were housed individually in conventional plastic cages and had free access to food and water. The room temperature was 25°C, and a 12:12 h light:dark cycle was used. All the experiments and the housing conditions were approved by the Agricultural and Forestry Ministry (authorization 04910). HU was imposed for 21 days using the tail-suspension model of Morey et al. (1979). Briefly, an orthopedic tape-adhesive plaster, covering less than half of the cleaned and dried tail, was connected to the top of the cage where a swivel allowed 360° rotation. At the end of the hindlimb suspension period, the rat was anesthetized with sodium pentobarbital (30 mg kg^{-1}). Supplementary injections (15 mg kg^{-1}) were used as necessary. Right or left soleus muscles were randomly selected for the neuromechanical study or for immunohistochemistry.

Neuromechanical study

The protocol used was that of Rosant and Pérot (2006). Briefly, the knee and ankle joints were flexed to 90° in order to measure soleus muscle length in situ (L_i). A 12–15 mm length of the soleus nerve was then dissected out under a binocular lens over its insertion into the muscle. The nerve was severed proximally to remove motor control of the muscle spindles, and the Achilles tendon was severed at its insertion into the calcaneum. The rat was placed in a tank, and the Achilles tendon was fixed to a mechanical device that included a servo system to impose ramp and hold stretches and to measure the resultant passive force (force transducer Entran type, ELF-TC13-5) and the muscle length (Schaevitz transducer, LVDT M12F005). The muscle was immersed in Ringer's solution, and its length was adjusted to L_i using a 3D micrometric bench. Petroleum jelly was added to allow the ENG reception in an isolated environment. The nerve was placed on two silver electrodes, 1.5 mm apart. The ground silver electrode was placed at the level of the knee and helped anchor this joint. The electrodes were connected to a GRASS amplifier (gain: 80 dB, bandwidth: 10 Hz to 10 kHz). The raw filtered and amplified ENG signals were sent to a electronic device that calculated their Root Mean Square values (RMS-ENG, time constant: 25 ms) on-line. Force, displacement and RMS-ENG values were sent to an A/D converter, force and displacement were digitized at 1 kHz and RMS values at 5 kHz and stored on a PC.

The experiment began with slow muscle stretches imposed manually to determine two reference lengths: (i) the slack length (L_s) from which a resting tension was detected and (ii) the length corresponding to the acoustic threshold of the ENG (L_n). The muscle was placed at L_s (or L_n), and ramp and hold stretches were randomly imposed of 0.5, 1 and 2 mm and at velocities of 1, 2, 5, 10, 30, 50 and 80 mm/s. The stretch was maintained for 1500 ms. Three trials were carried out at each condition. A second series was then done at L_n (or L_s) if L_s and L_n differed by more than 0.05 mm.

The dynamic index (DI) and static index (SI) were measured on RMS-ENG. DI is defined as the difference between the peak of RMS-ENG at the end of the ramp and the mean RMS-ENG value during the plateau. SI is the difference between the mean RMS-ENG value during the plateau and the mean RMS-ENG value at the reference length. DI and SI are expressed as a percentage of the maximal RMS-ENG value obtained during the series of stretches. Passive tensions were measured during the dynamic (peak tension, F_p) and static phases (steady tension, F_s) of the ramp and hold stretch and later correlated with the muscle cross-sectional area to give peak stress (σ_p) and steady stress (σ_s) values. The strain (ε) value is given by $\Delta L / L_s$