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An index of spindle efficacy obtained by measuring electroneurographic activity and passive tension in the rat soleus muscle

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Abstract

While muscle spindle afferent discharges are known to change with altered muscle use, the way in which the changes in spindle discharge are affected by modifications to the elastic properties of the muscle–tendon unit remains to analyze. This paper describes a methodology to define, in the rat, a spindle efficacy index. This index relates the spindle afferent discharges recorded from electroneurograms (ENG) due to muscle stretch to the passive elastic properties of the muscle–tendon unit quantified during the stretch imposed for the ENGs recordings. The stretches were applied to the rat soleus muscle after the Achilles tendon was severed. The spindle afferent discharges were characterized from the root mean square (RMS) values of electroneurograms (ENGs) recorded from the soleus nerve. The first step of the study was to validate the definition of dynamic and static indices (DI and SI) of spindle discharges from RMS-ENG as classically done when isolated afferents are studied. The slopes of the DI-stretch velocity or SI-stretch amplitude relationships gave the indices of spindle sensitivity under dynamic and static conditions, respectively. Incremental stiffness was calculated to describe the passive elastic properties during the dynamic and static phases of ramp and hold stretches applied at different amplitudes and velocities. The spindle efficacy index (SEI) is the ratio between the indices of spindle sensitivity and incremental stiffness values. Both spindle discharges and incremental stiffness increased with stretch amplitude under dynamic and static conditions. The corresponding SEI values were constant whatever the stretch amplitude. This result validates the relationship between spindle discharges and passive incremental stiffness. This method can be proposed to study, in the rat, the spindle function when the muscles are suspected to present changes in their neuromechanical properties.

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

1. Introduction

The capacity of the neuromuscular system to adapt to a period of training or disuse is now well documented. However, few studies have examined the plasticity of muscle spindles. Some changes in the structure of the intrafusal fibers of rat muscles have been found when the animals were trained to swim (Umnova et al., 2000; Yoshimura et al., 1992), after denervation (Kucera et al., 1993; Walro and Kucera, 1999; Zelena, 1957), immobilisation (Jozsa et al., 1988), simulated microgravity (De-Doncker et al., 2002) and hypergravity (Picquet et al., 2003), but there have been fewer studies of functional adaptations. Hnik et al. (1977) reported quantitative changes in electroneurograms (ENGs) of the rat soleus nerve after the muscle had been de-efferented at birth. Changes in the spindle afferent discharges of cat muscles due to immobilization have been reported (Gioux and Petit, 1993; Nordstrom et al., 1995) and the Ia and II fibers of rat soleus muscle spindles were recently found to be more sensitive after a period of hypodynamia–hypokinesia (De-Doncker et al., 2003b). These changes in spindle afferent discharge may be due to changes in the spindle structure and/or to changes in the elastic properties of the muscle–tendon elements linked to the muscle spindles. It has been reported that more compliant muscle–tendon elements ensure that

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the muscle spindles "see" less of the imposed external displacement (De-Doncker et al., 2003b; Rack et al., 1983). All these studies attempted to look at the adaptations of the discharges of the muscle spindles, or the adaptations of the elastic properties of muscle–tendon unit, without measuring the contribution of each one. In the present study, both spindle afferent discharges and muscle elastic properties were quantified during a given muscle stretch to define a spindle efficacy index (SEI). The first step was to validate the definition of dynamic and static indices (DI and SI) of spindle discharges from RMS-ENG as classically done when isolated afferents are studied. The slopes of the DI-stretch velocity or SI-stretch amplitude relationships gave the indices of spindle sensitivity under dynamic and static conditions, respectively. We describe the method proposed to calculate this SEI from RMS-ENG recordings and passive mechanical tension data obtained during ramp and hold stretches imposed to the rat soleus muscles after the Achilles tendon was severed and linked to the servo-controlled apparatus. The SEI may be used to assess changes in spindle discharge due to neuromuscular adaptation and to estimate the relative contributions of spindle plasticity and of changes in the elastic properties of the structures linked to the muscle spindles.

2. Materials and methods

2.1. Animal care and procedures

Experiments were carried out on 12 male Wistar rats (age = 4 months; body weight = 340 ± 31 g). Rats were anaesthetized with an intraperitoneal injection of pentobarbital sodium (3 mg/100 g body mass). Supplementary injections (1.5 mg/100 g body mass) were provided when necessary. The knee and ankle joints were flexed at 90° to measure the length of the soleus muscle in situ (L_i). L_i was measured between the soleus proximal insertion and the insertion of the gastrocnemius muscle on the Achilles tendon. As the range of motion is between 30° and 180°, and the angular excursion is commonly 30°–90° in caged rats (Ohira et al., 1993), the muscle initial length was set within its physiological range. The joint positions were maintained throughout the dissection phase. The soleus muscle and nerve were carefully dissected out under a binocular microscope so as to preserve the vascularization. The nerve was cleared for 12–15 mm above its muscle insertion and then severed at this level to block motor control of the muscle spindles. The Achilles tendon was severed at its insertion into the calcaneum. The rat was then placed in a tank with an inclined plane (so that its head was up) and held in position by a strap around the thorax beneath the upper limbs. The Achilles tendon was fixed to a force transducer (Entran, model ELF-TC13-5) in series with a length transducer (Schaevitz, model LVDT M12F005) and connected to an electromagnetic vibrator (Dynamic Line System, model VP 201). The tank and the mechanical device were fixed on a 3D micrometric bench so that the link between the muscle and the transducers and the muscle initial length could be precisely adjusted. The muscle was set at L_i. The leg was immobilized by inserting needles through the knee and the foot into a polystyrene plate covering the bottom of the tank. The tank was filled with Ringer's solution until the soleus muscle was totally immersed. The rat and solution were kept at 37 °C by a regulated heating platform placed under the tank. 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. At the end of the experiment, the soleus muscle was excised, stretched to its resting length, immediately frozen in isopentane cooled in liquid nitrogen and stored in liquid nitrogen (−180 °C). The soleus muscle was cut into serial frozen transverse sections (10 μm thick) in a cryostat (Leica, Jung Frigocut 2800 E) at −20 °C to determine the anatomical cross sectional area of the whole muscle. The sections were processed for eosin staining. Anatomical cross sectional areas were calculated with Leica-Qwin software. This anatomical cross-sectional area corresponded to the physiological cross sectional area except for a constant which takes into account the pennation angle, since all soleus muscles were believed to have similar pennation angles.

The protocol was approved by the University hygiene, safety and ethics committee.

2.2. Measurements of ENG and passive tension

A PC was used to pilot the length changes and to record the data. Raw ENG data were amplified (gain: 80 dB) and filtered (bandwidth: 10 Hz to 10 kHz) and used to compute, in real time, their root mean square (RMS) values (gain of 15 dB, time constant of 25 ms). Raw ENGs were also sent to a loud-speaker for continuous acoustic monitoring. Force and displacement data were digitized at 1 kHz and RMS values at 5 kHz for storage on the PC. Mechanical and RMS-ENG signals were also monitored on an oscilloscope. The experiment began with slow manual stretching of the muscle to determine two reference lengths, the slack length (L_s, used to deduce the resting tension) and the length corresponding to the acoustic threshold of the ENG (L_n). These reference lengths were considered to be the same if the difference between L_s and L_n was less than 0.05 mm (around 0.15% of total muscle length). The muscle was then placed at L_s and ramp and hold stretches were performed at three amplitudes (0.5, 1 and 2 mm) and seven velocities (1, 2, 5, 10, 30, 50, and 80 mm/s). The stretch plateau was maintained for 1500 ms. Three trials were carried out at each condition for a total of 63 random trials lasting approximately 20 min. The time between two ramp and hold stretches was 15 s so that the passive tension returned to its resting value in all cases. This rather long delay was chosen to avoid history dependence of spindle afferent responses in successive muscle stretch as recently evidenced in the rat (Haftel et al., 2004). The relatively short stretch plateau and the relative low stretch