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Macroscopic–microscopic characterization of the passive mechanical properties in rat soleus muscle

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Abstract

The purpose of the study was to investigate changes in passive mechanical properties of the soleus muscle of the rat during the first year of life. These mechanical changes were quantified at a macroscopic (whole muscle) and a microscopic level (fiber) and were correlated with biochemical and morphological properties.

Three passive mechanical tests (a relaxation test, a ramp stretch test and a stretch release cycle test) with different amplitudes and velocities were performed on isolated soleus muscles and fibers in rats at ages 1 (R1), 4 (R4) and 12 (R12) months. Mechanical parameters (dynamic and static forces, stresses and normalized stiffness) were recorded and measured. The morphological properties (size of fibers and muscles) for the three groups of rats were assessed by light microscopy which allowed us to observe the evolution of the fiber type (I, IIc and IIa) in the belly region and along the longitudinal axis of the muscle. In addition, biochemical analyses were performed at the level of the whole muscle in order to determine the collagen content.

The results of the passive mechanical properties between the macroscopic (muscle) and microscopic (fiber) levels showed a similar evolution. Thus, an increase of the dynamic and static forces appeared between 1 and 4 months while a decrease of the passive tension occurred between 4 and 12 months. These mechanical changes were correlated to the morphological properties. In addition, the size of the three fibers type which grew with age could explain the increase of forces between 1 and 4 months. Furthermore, the biochemical analysis showed an increase of the collagen content during the same period which could also be associated with the increase of the passive forces. After 4 months, the passive tension decreased while the size of the fiber continued to increase. The biochemical analysis showed a decrease of the collagen content after 4 months, which could explain the loss of passive tension in the whole muscle. Concerning the similar loss at the fiber level, other assumptions are required such as a myofibril loss process and an increase of intermyofibrillar spaces. The originality of this present study was to compare the passive mechanical properties between two different levels of anatomical organization within the soleus muscle of the rat and to explain these mechanical changes in terms of biochemical and morphological properties.

Keywords: Passive stiffness; Morphological properties; Soleus muscle; Skinned isolated fibers; Collagen

1. Introduction

The originality of this present work was to assess the passive mechanical properties of the muscular tissue at different scales. According to our knowledge no previous studies have evaluated the relationship between the macroscopic (whole muscle) and the microscopic

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(muscle fibers) passive mechanical behaviour of soleus muscle.

At the whole muscle level, Kovanen et al. (1984a, b) is one of the major references related to the evolution of the passive mechanical properties (stress–strain relationship) as a function of age. This study involved applying passive mechanical tests until the yield point on soleus muscles (rats) at ages 1, 2, 4, 10 and 24 months. Two categories of rats were used: trained and untrained rats. The passive mechanical results showed an increase of the mechanical strength until 10 months followed by a tendency to rise during the remaining period in the trained rats. In the untrained rats, the passive mechanical properties (ultimate tensile strength) showed an increase between 1 and 2 months while a decrease occured after 2 months.

The passive elastic component, which is the source of the stiffness, is mainly contributed by collagen substances located in epimysium, perimysium, endomysium and sarcolemma. In addition, there are different types of collagen (type I, III, IV and V) which are differently distributed within the muscle and which contribute to the passive strength. Many studies (Kovanen et al. 1984a, b; Kovanen and Suominen, 1987, 1989; Alnaqeed et al., 1984; Ducomps et al., 2003) analysed the role of collagen in the mechanical and physiological properties of the muscle. These studies led to the conclusion that the passive mechanical properties of muscle are dependent on the content of collagen.

In addition to the connective tissue, titin filaments are a source of passive tension at the muscle fiber level. The passive mechanical properties of titin were characterised by different passive mechanical tests on isolated fibers (Anderson et al., 2002; Colomo et al., 1997; Joumaa et al., 2002; Mutungi et al., 1996). Moreover, the stiffness of the muscle fibers is due to an interplay between endosarcomeric and exosarcomeric protein networks (Bartoo et al., 1997; Wang et al., 1993). For instance the desmin network adds passive resistance to stretch at high sarcomere lengths (Salviati et al., 1990). A recent study (Toursel et al., 2002) documented changes in passive elastic properties of rat soleus muscle fibers subjected to hindlimb unloading conditions. It was concluded that this experimental condition did not change titin isoform expression in the soleus muscle but rather indicated a loss of titin content which decreased the stiffness of the muscle. Moreover, Horowitz et al. (1986) concluded that the developed passive forces were related proportionally to the content of titin.

The purpose of this study was to measure the passive mechanical properties of single slow fibers and muscles (soleus) in 1-, 4- and 12-month old rats. In addition, biochemical (content of collagen) and morphological (fiber characteristics) analyses were performed in order to correlate the mechanical and morphological properties at each level of anatomical organisation. Furthermore, a comparison between the two scales will be made in order to see how the microscopic level influences the macroscopic one.

2. Materials and methods

2.1. Animals

Male Wistar rats aged 1 month (R1) ($n = 16$, average body weight $= 104 \pm 4$ g), 4 months (R4) ($n = 16$, average weight $= 395 \pm 8$ g) and 12 months (R12) ($n = 10$, average weight $= 631 \pm 46$ g) lived in a controlled environment, a 12 h light and 12 h dark cycle, and at constant temperature (23 °C). They were housed in $42 \times 42 \times 18$ cm cages grouped by age (3–4 rats per cage). Food (Teklab global 18% protein rodent diet, Harlan) and water were provided ad libitum.

2.2. Muscles and fibers preparation

The rats were anaesthetised with an intraperitoneal injection of pentobarbital sodium (30 mg/kg) and the soleus muscles were removed from the right and left hindlimbs, weighted and subjected to the following preparation procedures.

The soleus muscles from the right hindlimb were fastened at the proximal and distal extremities of the muscle with a thread separating the muscle from its tendon. The length of the muscle was measured in situ with a micrometric tools by placing the knee and ankle joints at 90°. The removed muscles were then mechanically tested.

The soleus muscle from the other hindlimb was removed and cut longitudinally into different strips. After this, the rats were killed with a lethal intraperitoneal injection of pentobarbital sodium. The muscle strips were then chemically skinned in an EGTA skinning solution (2.5 mM ATP, 20 mM MOPS, 170 mM potassium propionate, 2.5 mM magnesium acetate, 5 mM K_2EGTA, pH = 7.0) for 28 h at 4 °C and stored at −18 °C in a 50–50% glycerol skinning solution for 1 week.

Under a binocular microscope, strips from the different-age muscles (four muscles per age group) were dissected into isolated fibers (1–3 mm length). Approximately five fibers per muscle were extracted. A total of 19, 18 and 24 fibers were dissected from a group of 1, 4 and 12 months, respectively.

Solutions. All reagents were obtained from Sigma (St Louis, USA). The composition of all solutions was calculated by the Fabiato computer program (10), with a final ionic strength of 200 mM. pH was adjusted to 7.0 and ATP (2.5 mM) was added to each solution. The skinning solution was made up of (mM) MOPS, 10; K Propionate, 170; Mg Acetate, 2.5; and K_2 EGTA, 5. The