

L-type Ca^{2+} channel mechano-sensitivity in long-term denervated Soleus muscle of the rat

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Abstract

Evidences showed that physical forces, as passive stretching or active contraction, may counteract various kinds of skeletal muscle atrophy due, for instance, to muscle immobilization, pathophysiology or denervation. In accord, active muscle contraction induced by functional electrical stimulation resulted helpful to reduce the muscle atrophic state in denervated man. Moreover, there are evidences that also passive mechanical stimulation of the sarcolemmic membrane may reduce the atrophic muscle state. About the mechanisms by which mechanical stimulation modulates muscle physiology and pathophysiology there is a growing list of facts that signalling pathways to the nucleus concern the cytoskeleton. The anomalous cytoskeleton organization determines leaky plasma-membrane and loss of its mechanical properties causing fragility, less stiffness and disassembling the myofibrils. Actin cytoskeleton is activated by Ca^{2+} -dependent and -independent pathways and its contraction or elongation represent not only a mechanical signal to the nucleus but also a stimulus for many molecular signals that improve the muscle performance. Thus, the abnormal cytoskeleton may also affect the mechanical tension of the T-tubule membrane and consequently the functionality of the L-type Ca^{2+} channel. The aim of this work was to evaluate the mechano-sensitivity of L-type Ca^{2+} channels in long-term denervated Soleus muscle of the rat. Electrophysiological experiments were made in long-term (44 weeks) denervated Soleus muscle of Wistar rats. Currents were recorded in voltage-clamp by intracellular microelectrodes inserted in a single fibre. Results showed that L-type Ca^{2+} channel currents were enhanced by muscle stretching.

Key words: Soleus muscle, long-term denervation, L-type Ca^{2+} channel, mechano-sensitivity, electrophysiology.

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Introduction

Progressive muscle atrophy, weakness and wasting represent a major consequence of denervation [18, 21, 22, 23]. Skeletal muscle denervation causes profound alterations not only in the sarcomeric structure and force generation [21] but also on cytoskeletal organization, sarcolemmic stiffness and gene expression [19], Ca^{2+} handling [2, 5, 6] and new fibres generation from satellite cells differentiation [26]. Thus, denervation while affecting adaptation and repair, compromises the physiological state and the survival of the cells.

Many evidences suggest a significant role of physical forces as passive stretching or active contraction in exercise, in the development and maintenance of skeletal muscle mass and in the onset and maintenance of several muscle disorders [1, 8, 16, 25]. About the mechanisms by which mechanical stress modulates muscle physiology and pathophysiology there is a growing list of signaling pathways that are activated in response to mechanical stimulation in skeletal muscle cells. These include the Ca^{2+} -independent and Ca^{2+} -dependent signalling molecules [13, 15] and the

cytoskeleton [19]. Sarcomeres are connected to the sarcolemma by extrasarcomeric cytoskeletal proteins forming a morphological and functional unit [29]. Titin is a well studied intrasarcomeric cytoskeletal protein capable of producing a passive tension during sarcomere contraction [20]. It is, in fact, a large elastic proteins linking the thick filaments to the Z-disc, so that the sliding of myosin filaments induces changes in the elastic domain of the protein [17]. Among the extra-sarcomeric proteins are desmin that links sarcomeres at Z-disk and sarcolemma [30], and the costameric proteins such as vinculin, spectrin, talin, dystrophin [9, 10, 24], which, being localized in rib-like bands beneath the sarcolemma, connect the cytoskeleton to the cell surface. Other signals acting directly on the nucleus involve Ca^{2+} -activated molecules, such as calcineurin and calmodulin [3]. The most important Ca^{2+} signal for sarcomeric contraction came from excitation contraction (EC) coupling. The first step of EC coupling in skeletal muscle is the Ca^{2+} entry through the L-type Ca^{2+} channels that triggers the second step, that is the Ca^{2+} release from RyRs [3, 14, 27, 28]. Consequently, this Ca^{2+} increase through the sarcolemma is also important as a signal to the nucleus about the entity and type of muscle contraction. Disturbed intracellular Ca^{2+} signalling is implicated in muscle atrophy. The altered Ca^{2+} homeostasis is related to a leaky plasma-membrane and loss of its mechanical properties causing fragility and less stiffness, depending on the anomalous cytoskeleton organization [2, 5, 6]. This in turn, affects the properties of plasmamembrane ionic channels activated by the transmembrane voltage as well as those activated by stretch. Finally, in being the denervated muscle unloaded, the EC-coupling alteration has been assumed as a major determinant of weakness and fatigue together with the modification of the skeletal muscle fibre type expressed [5]. The aim of this work was to evaluate in long-term denervated Soleus muscle of the rat, the mechano-sensitivity of L-type Ca^{2+} channels before and after long term denervation.

Methods

Animals

Male Wistar rats aged two months were used. Animals were placed in individual cages and fed standard diet without limitations. The hind limbs were surgically denervated as described in [7]; in brief, the sciatic nerve was tightly ligated with silk in two places and the nerve was cut between the sutures. Both proximal and distal nerve stumps were implanted into muscular tissue as far away from each other as possible. This method has produced permanent denervation of the hind limb muscles for as long as 25 months. The animals rapidly recovered and showed moderate impairment of locomotion. 44 weeks later the rats were sacrificed, soleus muscles were removed and immediately used for electrophysiological analyses *in vitro*.

Electrophysiological recordings

The electrophysiological behaviour of Soleus muscle fibres was analysed in current- and voltage clamp by intracellular microelectrodes inserted in a single fibre of a bundle. During the experiments, the cells were superfused at a rate of 1.8 ml min^{-1} with a bath solution containing 150 mM NaCl, 5 mM KCl, 2.5 mM CaCl_2 , 1 mM MgCl_2 , 10 mM D-glucose and 10 mM HEPES. Microelectrodes were filled with a solution containing 150 mM CsBr, 5 mM MgCl_2 , 10 mM EGTA and 10 mM HEPES. pH was titrated to 7.4 with NaOH and to 7.2 with TEA-OH for bath and pipette solution, respectively. When filled, the resistance of the microelectrodes measured 8–10 MW. The microelectrode was connected to a micromanipulator (Narishige International, USA) and an Axopatch 200B amplifier (Axon Instruments, CA, USA). Voltage-clamp protocol generation and data acquisition were controlled by using an output and an input of the A/D-D/A interfaces (Digidata 1200; Axon Instruments, CA, USA) and Pclamp 9 software (Axon Instruments, CA, USA) [4, 14]. All the passive properties parameters were estimated as in [11,12]. To allow comparison of test current recorded from different cells, membrane current amplitudes was normalized to cell linear capacitance C_m (in $\mu\text{A}/\mu\text{F}$), since C_m is an index of cell-surface area assuming that membrane-specific capacitance is constant at $1 \mu\text{F}/\text{cm}^2$. All experiments were performed at room temperature ($20\text{--}23^\circ\text{C}$). Data are as mean $\pm$ s.e.m.

Results

The mean fibre diameter in 44 weeks denervated muscle was smaller respect to that of the normally innervated controlateral muscle being 5 ± 1.5 and $40 \pm 5 \mu\text{m}$, respectively. To evaluate the sensitivity to stretch of L-type Ca^{2+} current (I_{Ca}), single fibres were held at a holding potential of -90 mV and step pulses, 5 s long, were applied from -80 to 40 mV in 10 mV steps. I_{Ca} was first recorded from fibres of long-term (44 weeks) denervated Soleus muscle at resting length. I_{Ca} could be recorded in only the 8% of the fibres investigated. In fact, most of the fibres showed great leak currents and I_{Ca} could not be recorded. In the excitable fibres (Fig. 1) the voltage threshold for I_{Ca} was at about -40 mV and its maximal size was elicited at a depolarization of about 0 mV . The I_{Ca} size determined at the peak of the currents elicited at 0 mV was about $0.26 \pm 0.05 \mu\text{A}/\mu\text{F}$ that is about the 5% respect to that recorded in innervated Soleus fibres (unpublished observations). Subsequently the fibres were stretched to 120% of their resting length. The same stimulating protocol was applied again. In such a condition the number of fibres that could elicit I_{Ca} increased to the 18% of the investigated fibres. Moreover, I_{Ca} was enhanced to $0.6 \pm 0.1 \mu\text{A}/\mu\text{F}$ (150% respect to resting length). Voltage threshold and depolarizing value eliciting the maximal I_{Ca} size were not changed significantly.

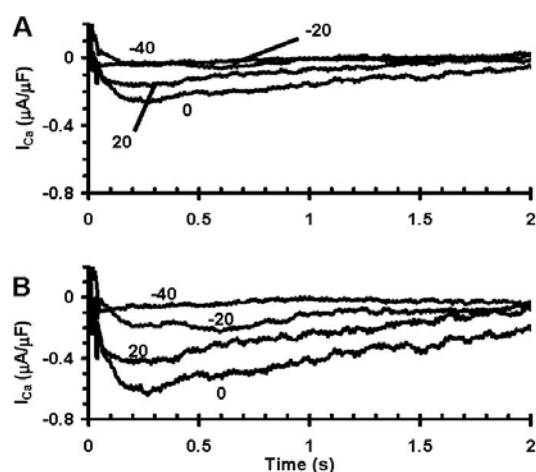


Fig. 1. Fibre stretching increased I_{Ca} size. Representative I_{Ca} traces recorded in a fibre from long-term (44 week) denervated soleus muscle. For clarity only current elicited at -40, -20, 0 and 20 mV were presented. Time course of I_{Ca} recorded at resting length (A) and in the same fibre stretched to 120% (B).

Discussion

These electrophysiological investigation showed new insights on the L-type Ca^{2+} current mechano-sensitivity in single fibres from long-term denervated Soleus muscle. The majority of the investigated fibres were unexcitable and had a great leak current except for some fibres (12%) that were less damaged. These have a reduced leak current and are excitable, according to their ability to elicit L-type Ca^{2+} currents. However, these fibres showed an atrophic state because the currents had a little amplitude respect to the innervated fibres. Interestingly, the fibre stretching increased not only the I_{Ca} size but also the number of fibres that could evoke I_{Ca} (22%).

The higher values of I_{Ca} in stretched fibres may agree with a reduced tension of actin-cytoskeleton filaments [12] that in turn reduces the stiffness of the plasma-lemma and T-tubule membrane. Such cytoskeleton alteration may determine a stronger responsiveness of L-type Ca^{2+} channels to be opened by depolarization when the muscle fibres are stretched, because of the increased compliance due to the lessened rigidity of actin filaments. These reduced rigidity may also determine a stronger stretch of T tubule membrane. Accordingly, in stretched condition I_{Ca} can be elicited in fibres that are unexcitable at resting length. Therefore, passive muscle stretch in denervated muscle may determine a stronger enhancement of the cytosolic Ca^{2+} concentration. This, in turn, yields an enhanced activation of L-type Ca^{2+} channels and, consequently of the EC coupling. Moreover, as a stronger signal to the nucleus it may participate to amplify the synthesis of new molecules

for reducing the atrophic state of muscles [8, 22, 25, 26].

The above observations are in agreement with previous works showing that mechanical stimulation induces positive effects on repairing the atrophic state of skeletal muscles [1, 22] as well as functional electrical stimulation (FES) does in denervated humans [21]. Finally, since I_{Ca} currents are enhanced by passive stretching it seems likely that the FES action may be improved by passive exercise of atrophic denervated muscles.

List of all non-standard abbreviations:

DHPR, dihydropyridine receptors; EC, excitation-contraction; RyR, ryanodine receptors; FES, Functional Electrical Stimulation; I_{Ca} , L-type Ca^{2+} current.

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