

Cytoskeleton, L-type Ca^{2+} and stretch activated channels in injured skeletal muscle

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Abstract

The extra-sarcomeric cytoskeleton (actin microfilaments and anchoring proteins) is involved in maintaining the sarco-membrane stiffness and integrity and in turn the mechanical stability and function of the intra- and sub-sarcoplasmic proteins. Accordingly, it regulates Ca^{2+} entry through the L-type Ca^{2+} channels and the mechano-sensitivity of the stretch activated channels (SACs). Moreover, being intra-sarcomeric cytoskeleton bound to costameric proteins and other proteins of the sarcoplasm by intermediate filaments, as desmin, it integrates the properties of the sarcolemma with the skeletal muscle fibres contraction. The aim of this research was to compare the cytoskeleton, SACs and the ECC alterations in two different types of injured skeletal muscle fibres: by muscle denervation and mechanical overload (eccentric contraction). Experiments on denervation were made in isolated Soleus muscle of male Wistar rats; forced eccentric-contraction (EC) injury was achieved in Extensor Digitorum Longus muscles of Swiss mice. The method employed conventional intracellular recording with microelectrodes inserted in a single fibre of an isolated skeletal muscle bundle. The state of cytoskeleton was evaluated by recording SAC currents and by evaluating the resting membrane potential (RMP) value determined in current-clamp mode. The results demonstrated that in both injured skeletal muscle conditions the functionality of L-type Ca^{2+} current, ICa , was affected. In parallel, muscle fibres showed an increase of the resting membrane permeability and of the SAC current. These issues, together with a more depolarized RMP are an index of altered cytoskeleton. In conclusion, we found a symilar alteration of ICa , SAC and cytoskeleton in both injured skeletal muscle conditions.

Key Words: cytoskeleton, L-type Ca^{2+} channel, excitation-contraction coupling, stretch activated channel, denervation, eccentric contraction, muscle injury

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In skeletal muscle Ca^{2+} is an important intracellular signaling ion and is a key factor for activating the muscle contraction [1, 3, 4]. Moreover, another role of Ca^{2+} involves the activation of Ca^{2+} -dependent signal-molecules as calcineurin [7] and the cytoskeleton myosin-actin activation and contraction.

The Ca^{2+} source is either from extracellular medium or from intracellular stores: 1. Ca^{2+} influx from extracellular medium is allowed by voltage operated dihydropyridine receptors/L-type calcium channels (DHPR/L-CaC) opening, by ligand activated cationic channels and not-voltage dependent channels; 2. Ca^{2+} release from intracellular stores occurs through the ryanodine receptors/ Ca^{2+} release channels (RyR/CRC); 3. RyR1 activation follows DHPR/L-CaC activation due to its coupling with this protein; in

contrast, RyR2,3 activation, being not coupled with DHPR, is induced by Ca^{2+} released from the surrounding RyR1.

Excitation-contraction coupling (ECC) is the process whereby depolarization of the T-tubular system triggers the Ca^{2+} release from the sarcoplasmic reticulum into the sarcomeric space [1, 14] that leads to muscle contraction. The first step in ECC is a voltage-sensing process that is the change of orientation of charged molecules within the DHPR/L-CaC of the T-tubular membrane as a result of membrane depolarization. This is detected as intramembranous charge movement (ICM) and it shows three components (Q_β , Q_γ and Q_δ) in normally polarized skeletal muscle fibres [5, 18, 2]. So, DHPR is thought to have two actions: as a voltage sensor and a voltage-operated L-CaC.

Interestingly, there is a reciprocal interaction between intracellular and sarcolemmic proteins. RyR1 activation can, in turn, act as a signal for the DHPR/L-CaC, with a sort of cross talk between RyR1/CRC and DHPR/L-CaC [19, 20]. Precisely, there is a retrograde action from RyR1/CRC that, once activated, acts as a signal for DHPR/L-CaC, causing an increase of Q_T and Q_C charge and L-CaC currents (ICa) as demonstrated by the reduced size of these charges and ICa when RyR1/CRC are blocked by ryanodine or ruthenium red [19, 20].

Other retrograde signals come from the cytoskeleton expression and activity. The extra-sarcomeric cytoskeleton (acto-miosin microfilaments and anchoring proteins) is involved in maintaining the sarco-membrane stiffness and integrity, and regulates the Ca^{2+} entry through the L-type Ca^{2+} channel and the stretch activated channels (SAC) [17, 9]. In contrast, intra-sarcomeric cytoskeleton (titin, nebulin and proteins of Z and M lines) is involved in maintaining the assembly of the sarcomeric acto-miosin proteins and the visco-elastic proprieties of the sarcomeres. Moreover, being intra-sarcomeric cytoskeleton bound by intermediate filaments, as desmin [10], to costameric proteins and other proteins of the sarcoplasm, such as receptors and ionic channels, it was expected that the passive and active electrophysiological properties as well as the mechanical ones of skeletal muscle fibres may be affected by the modified architecture and functionality of the cytoskeleton.

In skeletal muscles myoplasmic Ca^{2+} is important for activating many Ca^{2+} -sensitive signalling molecules. These are involved in fibre contraction but also in gene regulation for atrophy-hypertrophy and slow-fast balance and mitochondrial biogenesis such as Ca^{2+} /Calcineurin [3, 7], Ca^{2+} /calmodulin [13], PGC-1 α [15], the Ca^{2+} -dependent protease calpain that regulates the extra- and intramiofibrillar cytoskeleton and so the mechanical sarcomeric activity, cytoskeleton motor proteins (myosin II) and the Ca^{2+} -dependent intra-sarcomeric protein titin that probably regulates the static sarcomeric stiffness [8, 10]. Finally, cytoskeleton is involved in mechanically coupling the sarcomeric membrane to nuclear membrane and regulates the traffic of molecules and proteins synthesized by the nucleus towards the sarcolemmic membrane.

The aim of this research was to compare the cytoskeleton alterations, SACs activity and the ECC process in two different types of injured skeletal muscle fibres: by a mechanical overload (eccentric contraction) and by α -motor neurons denervation.

Materials and Methods

This research adheres to APS's Guiding Principles in the Care and Use of Animals and is approved by the Padua and Florence Universities Ethics Committees.

Experiments on denervated muscles were made in isolated *Tibialis anterior* and *Soleus* muscles removed from anesthetized male Wistar rats (weight: 150 to 200 g); forced eccentric-contraction (EC) injury experiments were made in isometric condition from *Extensor Digitorum Longus* (EDL) muscles removed from anesthetized young adult Swiss mice (25-30g).

The method we used consisted of conventional intracellular recording by microelectrodes (50-70 M Ω) inserted in a single fibre of an isolated skeletal muscle bundle. By voltage-clamp records we analyzed the passive properties of the fibres (Rm, membrane resistance, and Cm, membrane capacitance as an index of the sarcolemmic and T-tubular surface) and the voltage-dependent L-type Ca^{2+} current, ICa. These parameters allowed us to know the degree of damage of the first steps of excitation-contraction coupling, ECC, and to calculate the free myoplasmic Ca^{2+} concentration by the analysis of the reversal potential of the I-V activation curve.

The condition of the cytoskeleton was evaluated by the recording the SAC currents and from the resting membrane potential (RMP), determined in current-clamp mode.

Results and Discussion

Our results demonstrate that in skeletal muscle injured by means of cyclic eccentric contractions there is a decrease in size of L-type current, that is an index of a reduced functionality of these channel proteins. Moreover, we observed a positive shift of the voltage threshold and voltage dependence of activation as well as a negative shift of the voltage dependence of inactivation. In addition, muscle fibres showed an increase of the resting membrane permeability and of the SAC current together with a more depolarized RMP. This suggests a leaky sarcolemma and a reduced cytoskeleton expression/activity that, in turn, induces an increase of the intracellular Ca^{2+} concentration, which was confirmed by the negative shift of the L-type Ca^{2+} current reversal potential. Moreover, the alteration of the cytoskeleton may not only increase the mechano-sensitivity of SACs but also that of L-type Ca^{2+} channels [6].

The shift towards more positive potentials of L-type Ca^{2+} current activation is directly involved in ECC and was formerly observed in the slow skeletal muscle fibres of the frog by using ryanodine or ruthenium red. These treatments in fact, acting on RyRs, increased the number of L-type Ca^{2+} /DHPR receptors uncoupled to ryanodine receptors. Accordingly, blocking the interaction between DHPRs and RyRs by heptanol or octanol depressed in size L-type Ca^{2+} current, whereas the block of RYR shifted the Q_T and Q_C charge and ICa voltage-activation towards more positive potential [19, 20]. In agreement to these findings, the shift towards more positive potentials of L-type Ca^{2+} current that we observed in injured skeletal muscle fibres

could indicate an increased number of uncoupled DHPRs with RyR1. In fact, it was demonstrated that a similar voltage shift was paralleled by an increased number of DHPRs uncoupled with RyRs in a subset of skeletal muscle fibres of old mice [11, 12]. In conclusion, in injured muscle fibres the sarcoplasmic membrane, channel proteins and ECC properties are paralleled by cytoskeleton alterations. These effects share common alteration in muscle disuse and ageing with muscle injury as a consequence of denervation and eccentric contraction undergo marked atrophy and necrosis through a common set of transcriptional changes. Fibers underwent substantial damage showing severe disorganizations of the contractile apparatus and intermyofibrillar edema. Moreover, fibres exhibited signs of necrosis. At the ultrastructural level, the injured fibers displayed sarcomere disorganization, Z-disc disruptions, abnormal cisternae and tubules of sarcoplasmic reticulum, associated with marked alterations in subsarcolemmal mitochondria. Indeed, the damaged mitochondria displayed a marked swelling, disarrayed cristae and a reduced electron density of the matrix. Biochemical analysis revealed that lactate dehydrogenase (LDH) activity was significantly decreased of approximately in injured muscle compared to control [21,16]. It was postulated that the increase of DHPR/L-CaC-CRC/RyR uncoupling paralleled by cytoskeleton and sarcolemmic loss of functionality are the major determinant of weakness and fatigue in mammalian species including humans. In accord, an increased number of DHPRs unlinked with RyRs, and a fast activation kinetics of ICa were observed also in a subset of skeletal muscle fibres of atrophic old mice [11, 12].

In summary, the results demonstrated that in injured skeletal muscle conditions caused by eccentric contractions [16] and denervation [22, 23, 21] there is a symilar alteration of ICa, SACs activity and cytoskeleton functionality.

List of non-standard abbreviations

Cm: membrane capacitance; DHPR/L-CaC: dihydropyridine receptors/L-type calcium channels; EC: eccentric contraction; ECC: excitation-contraction coupling; ICa: L-type Ca²⁺ current; SAC: stretch activated channel; Rm resting membrane resistance; RMP: resting membrane potential; RYR: ryanodine receptor.

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