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Review

Reactive oxygen species and calcium signals in skeletal muscle: A crosstalk involved in both normal signaling and disease

Alejandra Espinosa[a], Carlos Henríquez-Olguín[b,c], Enrique Jaimovich[b,*]

[a] Departamento de Tecnología Médica, Facultad de Medicina, Universidad de Chile, Independencia 1027, 8380453 Santiago, Chile
[b] Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile, Chile
[c] Unidad de Fisiología Integrativa, Laboratorio Ciencias del Ejercicio, Clínica MEDS, Las Condes, Chile

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ABSTRACT

Reactive Oxygen Species (ROS) have been profusely studied as agents of potential damage to living cells and they have been related to a number of pathological processes. Increasing evidence points to a more positive role of ROS in cell signaling and the detailed mechanism that regulates the precise amount of ROS needed for cell functioning without the deleterious effects of excess ROS still needs to be resolved in detail.

In skeletal muscle the main source of ROS during normal functioning appears to be NADPH oxidase 2 (NOX2), which is activated by electrical stimuli (or exercise) through a cascade of events that include ATP release through pannexin1 channels. NOX2 is a protein complex that assembles in the T-tubule membrane before activation and ROS production by NOX2 appears to be important for muscle adaptation through gene expression and mitochondrial biogenesis as well as for improving glucose transport after insulin action.

Excess ROS production (or diminished antioxidant defenses) plays a role in a number of pathological processes in skeletal muscle. Together with increased reactive nitrogen species, an increase in ROS appears to have a deleterious role in a model of Duchenne muscular dystrophy as well as muscle wasting in other diseases such as aging sarcopenia and cancer cachexia. In addition, ROS is involved in obesity and muscle insulin resistance, both of which are causally related to type 2 diabetes.

A detailed description of the fine-tuning of ROS (including all sources of ROS) in skeletal muscle in health and disease will significantly contribute to our knowledge of both muscle adaptation and muscle related pathologies.

Contents

* Corresponding author at: Laboratorio de Fisiología Celular de Músculo, Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile, Independencia 1027, Santiago 8389100, Chile.
E-mail address: ejaimovi@med.uchile.cl (E. Jaimovich).

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1. Introduction

Skeletal muscle is a primary tissue in the response to metabolic alteration induced by physiological or pathological stimulus. The *redox* homeostasis appears to be a key modulator of skeletal muscle plasticity/dysfunction in response to exercise or metabolic diseases. Several signaling pathways in striated muscle can be activated by an increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) production.

In skeletal muscle, ROS is produced by several sub-cellular compartments under stress or metabolic conditions [1]. The best-studied ROS sources in striated muscle include mitochondria, xanthine oxidase (XO), and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs). The NOXs are oligomeric enzymes that generate O_2^- not only as a by-product of cellular metabolism but also as the main product. This process occurs in a regulated manner in response to cytokine, hormonal, and mechanical signals [2]. There is strong evidence that NOX2 and its homologues are a major source for ROS under resting and contracting conditions [3,4].

The dose-response relationship between ROS production and muscle adaptation/dysfunction has not been easy to determine. The hormesis concept, derived from the field of toxicology, describes the dose response relationship when a stressor is beneficial in moderate levels and detrimental in high levels [1]. It has been proposed that the ROS-dependent hormesis model could explain some of the responses to exercise, insulin resistance, and muscle wasting (Fig. 1). We will summarize the evidence that favors a tight regulation of ROS levels in skeletal muscle and its association to calcium signals. This relevant subject needs further studies to understand its important role in the physiology and pathophysiology of skeletal muscle.

2. ROS as second messengers in normal skeletal muscle

Skeletal muscle has a *redox* equilibrium between ROS/RNS generation and antioxidant-induced defense that are in constant rate even after contraction. Among ROS, H_2O_2 has signaling properties, because it is a molecule derived from dismutation of superoxide anion, produced by superoxide dismutase (SOD). H_2O_2 influences a range of cellular events through its kinetics properties, life span and intracellular specific generation, and plays a crucial role in signal transduction in skeletal muscle [5]. Second messenger characteristics of H_2O_2 are involved in gene expression and glucose uptake, among other cellular processes. Most of the H_2O_2 produced in skeletal muscle comes apparently from NOX2; it had been assumed for many years that mitochondria was the most important H_2O_2 source, but it was reported that superoxide production by mitochondria is about 0.15% of the total O_2 consumed [6].

2.1. Role of ROS during muscle contraction/exercise

Muscle contraction is an event characterized by activation of multiple intracellular pathways critical to skeletal muscle plasticity and adaptation [7]. Endocrine, mechanical, and metabolic signals control the muscle adaptation in response to contractile activity [8]. Excitation-contraction coupling (E–C coupling) is followed by an adaptive change in gene expression named excitation-transcription coupling [9,10]. Thus, changes in gene expression govern skeletal muscle adaptation in response to contractile activity.

The increase of ROS production during exercise was described for the first time in the eighties [11]. In skeletal muscle, ROS and RNS activate several *redox* sensitive pathways that participate in acute and chronic response to exercise [12]. For example, exercise training induces upregulation of antioxidants enzymes such as MnSOD, GPx, and catalase [12]. Interestingly, the supplementation of general ROS scavengers blunts these antioxidant upregulation induced by exercise (for review see Ref. [13]). Thus, ROS appear to be necessary to adaptive protein synthesis in response to training.

Mitochondrial biogenesis is a well-described training-induced muscle adaptation. Mitochondrial biosynthesis involves the regulated expression of mitochondrial and the nuclear genes [14]. The transcriptional coactivator, peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1α), is necessary for mitochondrial biogenesis, which improves the expression of nuclear genes encoding mitochondrial proteins [15]. Endogenous and exogenous ROS stimulation have been shown to induce upregulation of PGC-1α [16,17]. Moreover, high doses of dietary antioxidants block exercise-induced mitochondrial biogenesis [18,19], suggesting that ROS is needed for this adaptation in skeletal muscle.

A better understanding of the ROS pathways activated during exercise might be important to unveil the molecular mechanisms of muscle adaptation. Recent evidence suggests that specific circuits and localized ROS production explain the divergent response to oxidant molecules. During muscle contraction, there is a larger cytosolic ROS production with a discrete mitochondrial signal [3]. Thus, non-mitochondrial ROS sources such as xanthine oxidase (XO) and NOXs may play a major role in the contraction-induced intracellular signaling mediated by ROS. Our group has reported that NOX2 contributes to ROS production after depolarization in skeletal muscle [20,21]. Moreover, NOX2 inhibition reduces the adaptive gene expression induced by endurance exercise.

The hormesis model partially explains the role of ROS in the skeletal muscle physiology (Fig. 1); a physiological and transient ROS generation induces antioxidant genes expression and maintains *redox* balance. A decrease/increase in normal ROS levels alters *redox* homeostasis inducing muscle alterations [22–24].

2.2. Physiological role of ROS under insulin action

During the last decade, literature suggests that ROS generation in response to physiological stimuli such as insulin may also facilitate signaling by reversible protein modification and by inhibiting protein tyrosine phosphatases. For example, the glutathione peroxidase 1 KO mice ($Gpx1^{-/-}$) has higher insulin sensitivity and are resistant to high fat diet (HFD)-induced obesity, enhancing PI3 K/Akt signaling [25]. Apparently, H_2O_2 is part of the events triggered by insulin in skeletal muscle cells, acting as a second messenger; implying that both its production and its degradation occur via specific enzymes, which provide specificity and account for site-specific effects.

The first report that showed that insulin induces H_2O_2 was described in rat epididymal fat cells [26]. NOX2 appears to be the main ROS source under insulin stimulation in adipocytes. Moreover, we described that insulin induces ROS generation in skeletal myotubes [27] and adult fibers [28] in a NOX2-dependent manner. The physiological role of insulin-dependent ROS generation has been studied during the past years and ROS appears to be necessary for insulin-dependent glucose uptake [29,30] and GLUT4 translocation [31] in skeletal muscle cells.

We recently reported that insulin-dependent GLUT4 translocation to the cell surface requires intracellular Ca^{2+} release through both RyR1 and IP_3R Ca^{2+} channels [31]. For this reason, any impairment of intracellular calcium homeostasis could affect glucose uptake in skeletal muscle.

Enhanced insulin sensitivity after exercise was first described in 1982 [32]. An insulin effect in skeletal muscle acutely increases between 2 and 48 h following exercise [33,34]. Recently, Trewin et al.[35] reported that ROS attenuation blunted the post-exercise insulin sensitivity using hyperinsulinemic–euglycemic clamp in