Redox regulation in skeletal muscle during contractile activity and aging

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ABSTRACT: Skeletal muscle has the ability to adapt and remodel after functional, mechanical, and metabolic stresses by activation of different adaptation mechanisms that induce gene expression, biochemical changes, and structural remodeling. Skeletal muscle cells continuously generate reactive oxygen and nitrogen species (RONS), which can act as mediators in cellular signaling pathways that regulate the adaptation mechanisms. There is strong evidence that indicates that RONS are generated in skeletal muscle cells during contractile activity and this induces the activation of transcription factors which modulate gene expression of antioxidant and protective proteins. Thus, it has been proposed that RONS act as signals that modulate the adaptation mechanisms in skeletal muscle and other cells. Structural and functional changes occur in skeletal muscle during aging and are characterized by a reduction of muscle mass and force (sarcopenia). The causes are known, however, there is considerable support for an involvement of RONS in the process of aging and sarcopenia. Several studies indicate that adaptive responses of skeletal muscle that are activated and regulated by RONS are disrupted during aging. This reduction of skeletal muscle adaptation to contractile activity during aging might be responsible for the loss of muscle mass and function and the progressive deterioration of this organ. In summary, there is sufficient evidence that indicates that cellular redox regulation in skeletal muscle is crucial in the physiology and pathology of skeletal muscle. However, new methodologies and experimental models are required for understanding the complex biology of RONS in the cell. This will provide future interventions that mitigate pathologies and aging of skeletal muscle.

Key words: aging, reactive nitrogen species, reactive oxygen species, redox signaling, skeletal muscle

INTRODUCTION

Skeletal muscle composes approximately 40% of the body mass and can be considered the largest organ in the body (Preedy et al., 2001). Skeletal muscle is continuously subjected to functional, mechanical, and metabolic stresses that can induce different adaptation mechanisms which involve gene expression, biochemical changes, and structural remodeling to protect it from future stresses. Skeletal muscle is a postmitotic tissue that is formed mainly by fibers that continuously generate reactive oxygen and nitrogen species (RONS). This production of RONS is increased during contractile activity and aging of skeletal muscle. The net excess of RONS generation can lead to oxidative stress, and this phenomenon can induce irreversible damage in the cell. In the recent years, evidence has demonstrated that RONS act as mediators in several cellular signaling pathways that control physiological and pathological cellular processes. This has been clearly identified in skeletal muscle. However, there is still a lack of knowledge about the specificity of the different RONS and the role of the spatio-temporal generation of these molecules in the biology of skeletal muscle during contractile activity and aging. In this review, we propose to briefly describe the latest achievements and insights in the field of redox biology in the physiology and process of aging in skeletal muscle.

CELLULAR BIOLOGY OF RONS

RONS in the Cell

Reactive oxygen and nitrogen species play an important role in physiology and pathophysiology in biologi-
Regulation of RONS in the Cell

Cells are equipped with enzymatic and nonenzymatic antioxidant systems to neutralize ROS and RNS to maintain redox homeostasis and maintain cellular functions in response to different cellular environments and fates. The main antioxidant enzymes are superoxide dismutase, glutathione peroxidase, and catalase. In addition, there are accessory proteins, such as peroxiredoxin, glutaredoxin, and thioredoxin reductase, that protect cells against oxidation. The nonenzymatic antioxidant system of the cell involves compounds, such as glutathione, vitamins C and E, carotenoids, uric acid, and bilirubin. These compounds are involved in thiol-disulfide exchange reactions and play a role in maintaining the cellular redox balance. Glutathione is the most important and abundant nonenzymatic antioxidant in the cell (Powers and Jackson, 2008).

Oxidative Stress

Cells need to preserve a delicate balance between ROS and RNS generation and elimination to maintain the correct redox status necessary to carry out vital functions. Any dysfunction of the antioxidant system can lead to alterations in cellular redox status. Thus, an increase in ROS and RNS production or a decrease in the capacity of ROS and RNS scavenging by antioxidant defenses can lead to an overall increase of intracellular level of ROS and RNS that can disrupt redox homeostasis. This phenomenon is known as oxidative stress. In excess, ROS and RNS can attack cellular structures, such as lipids, proteins, and DNA, thereby inducing irreversible changes that can lead to the disruption of cellular functions and integrity. Under normal physiologic conditions, the reactive nature of ROS and RNS allows their incorporation into the structure of macromolecules in a reversible fashion. Such reversible oxidative modifications play a critical role in different signaling pathways that regulate different cellular functions and the fate of the cell (Sies and Jones, 2007; Trachootham et al., 2008).

ROS and RNS in Cellular Transduction Signaling

One of the current major research areas in redox biology is to understand the roles of ROS and RNS in cellular signaling pathways. In physiological conditions, ROS and RNS interact with proteins, lipids, and nucleic acids and induce modifications in those structures that trigger several vital cellular processes directly (i.e., posttranslational modifications followed by protein translocation) or indirectly by changing the cellular microenvironment in different cellular compartments (i.e., the reduced or oxidized microenvironment in the cell cytosol, nucleus, or mitochondrial matrix). Depending on the redox state of cellular compartments, some molecular reactions can be enhanced or blocked (e.g., the binding of some transcription factors to DNA in the nucleus requires a reduced microenvironment). Many studies have provided evidence for the pivotal role of ROS and RNS in signal transduction and recognized these molecules as second messengers (Forman et al., 2008; Powers and Jackson, 2008).

REDOX REGULATION OF SKELETAL MUSCLE DURING CONTRACTILE ACTIVITY

During contractile activity, skeletal muscle increases the production of RONS. The first evidence for this was published in the early 1980s by Davies et al. (1982) and Jackson et al. (1983). Approximately 10 yr later, the generation of specific RONS during contractile activity in skeletal muscle was identified in different studies. Reid et al. (1992a,b) reported the release of superoxide by the contracting diaphragm. The first evidence of nitric oxide (NO) production by skeletal muscle during contractile activity was reported in 2 simultaneous studies from different groups (Balon and Nadler, 1994; Kobzik et al., 1994). O’Neill et al. (1996) detected the production of hydroxyl radical during contractions of skeletal muscle. In addition to the increase in RONS during exercise, the ability of skeletal muscle to increase the enzymatic antioxidant capacity in response to exercise was recognized (Ji, 1993; Powers et al., 1994). Other studies identified the crucial role that glutathione plays in the regulation of RONS activity and redox status in exercising subjects (Ji et al., 1992; Sen et al., 1992). Subsequently, it was proposed that the key role that RONS play in modulating cellular signaling processes involved in the activation of different pathways that regulate gene expression (Sen and Packer, 1996). This initiated the interest in RONS in redox signaling that continues to this day.
Production of ROS and NO in Skeletal Muscle During Contractile Activity

During exercise RONS are produced by different organ systems. However, due to the energy demand, it is thought that skeletal muscle is the major source of RONS generation during contractile activity (Powers and Jackson, 2008). In the following section we describe cellular locations in which RONS potentially can be generated in skeletal muscle cells during contractile activity and in basal conditions. Superoxide can be generated at multiple subcellular sites in skeletal muscle fibers, and this production increases during muscle contractions. It has been reported that mitochondria are the main intracellular source for superoxide generation and superoxide is generated at complex I and III of the electron transport chain during mitochondria respiration (Powers and Jackson, 2008). It has been argued that between 2 and 5% of the total oxygen consumed by mitochondria may undergo partial reduction and formation of superoxide (Boveris and Chance, 1973). During contractile activity, there is an increase in the ATP consumption due to the ATP utilization by the contractile machinery of the cell. This situation causes intracellular ATP demand and stimulates mitochondria to increase the rate of oxidative phosphorylation to synthesize more ATP. The increase of oxidative phosphorylation involves an increase in oxygen consumption and the consequent increase in superoxide production. We have recently reported, using a physiological model of isolated single mature skeletal muscle fiber, that contractile activity induces an increase in the net intracellular production of ROS in skeletal muscle (Palomero et al., 2008b). This increase in ROS, mainly H$_2$O$_2$, is less compared with that reported in other in vitro model studies in which myotubes obtained from skeletal muscle primary cells (Vasilaki et al., 2006a) or from a skeletal muscle cell line (McArdle et al., 2005) were used. In addition, some authors have suggested that mitochondria may not be the dominant source of ROS production during contractile activity of skeletal muscle (Jackson et al., 2007). Some studies indicate that there are NAD(P)H oxidase enzymes associated with the sarcoplasmic reticulum of cardiac and skeletal muscle (Xia et al., 2003). These enzymes are responsible for generation of superoxide, which appears to modulate calcium release by the sarcoplasmic reticulum through oxidation of the ryanodine receptor (Cherednichenko et al., 2004). Transverse tubules of skeletal muscle also contain a NAD(P)H oxidase that appears to release superoxide to the cytosol of skeletal muscle cells (Esquinas et al., 2006). Many studies have revealed that skeletal muscle cells release superoxide into the extracellular space (Powers and Jackson, 2008). A NAD(P)H oxidase complex has been identified to be constitutively expressed in diaphragm and limb muscles of the rat and localized at the plasma membrane (Javesghani et al., 2002), and this NAD(P)H oxidase complex may release superoxide to the extracellular space or cytosol (Powers and Jackson, 2008). It has been reported that increased phospholipase A$_2$ activity stimulates ROS generation in muscle mitochondria (Netheyr et al., 2000) and cytosol (Gong et al., 2006) and release ROS into the extracellular space (Zuo et al., 2004). Both forms of phospholipase A$_2$, calcium-dependent and calcium-independent, are involved in the process. However, the calcium-independent form appears to be mainly involved in ROS generation under basal conditions, whereas during contractions or other processes that elevate intracellular calcium, the calcium-dependent phospholipase A$_2$ is activated and stimulates ROS production at supranormal rates (Gong et al., 2006). Some studies have reported a role for xanthine oxidase in superoxide generation by skeletal muscle (Heunks et al., 2001; Gomez-Cabrera et al., 2003). Rat skeletal muscles contain significant levels of xanthine oxidase (Apple et al., 1991), and it has been reported that this enzyme is located in endothelial cells of capillaries and smaller vessels in human cardiac and skeletal muscle (Hellsten-Westing, 1993). Although some evidence has been provided for a role of xanthine oxidase, further studies are required in this area to determine the role that xanthine oxidase plays in ROS production during contractile activity.

Skeletal muscle continuously generates NO, and this production is increased by contractions (Balon and Nadler, 1994). Nitric oxide is synthesized by the enzyme NOS. There are 3 NOS isoforms: neuronal (nNOS) or type I, inducible (iNOS) or type II, and endothelial (eNOS) or type III. Skeletal muscle mainly expresses nNOS, which is associated with the dystrophin-glycoprotein complex in the muscle sarcolemma. Endothelial nitric oxide synthase is localized in the muscle mitochondria, and iNOS is only expressed in some inflammatory conditions of skeletal muscle (Powers and Jackson, 2008). During contractile activity, skeletal muscle increases the amount of NO released to the extracellular space (Pattwell et al., 2004) or intracellular space (Pye et al., 2007). In addition, passive stretching of skeletal muscle has been reported to induce the NO release from rat skeletal muscle in vitro and increase nNOS expression (Tidball et al., 1998).

An interesting aspect of contractile activity of skeletal muscle is the involvement of ROS and RNS in force generation and muscle fatigue. Several groups have made relevant contributions to the area. For instance, Reid et al. (1992a,b) and Kobzik et al. (1994) have indicated that ROS and RNS modulate contractile function of respiratory and limb skeletal muscle. Endogenous NO exerts a tonic effect in unfatigued muscle. The force of submaximal tetanic contractions is increased by treatment with NOS inhibitors and NO scavengers. Conversely, submaximal force is depressed by NO donors. Exogenous ROS have biphasic effects on unfatigued muscle, exposure to less ROS increases force, whereas greater concentrations depress force. The net effect of endogenous ROS on unfatigued muscle to increase force is opposed by NO, which depresses force. During strenuous contractions, skeletal muscle produces ROS and...
NO derivatives at accelerated rates. This situation causes oxidative stress and contributes to the development of acute fatigue. It does not appear that NO and ROS are equally involved in this process. Pharmacological treatment with antioxidants has also been shown to inhibit fatigue, an observation that demonstrates the importance of ROS as mediators in muscle fatigue (Powers and Jackson, 2008; Reid, 2008). In summary, there is strong evidence that indicates ROS and RNS are generated in skeletal muscle cells during contractile activity and modulate key aspects of muscle function.

Activation of Cellular Adaptation Mechanisms Induced by Contractile Activity in Skeletal Muscle

It is well known that skeletal muscle adapts rapidly after exercise. These adaptations consist of structural and biochemical changes in the muscle cells that result in considerable protection against potential damage caused by later exercise (Goldspink, 1994). It has been demonstrated that activities of key antioxidant enzymes are modified by chronic and acute exercise protocols (Ji et al., 1992; Ji, 1993). In addition, we investigated the possibility that the ROS generated during contractions might be a signal for the initiation of the adaptive processes because there had been increasing data indicating that exposure of cells to ROS induced or repressed a wide variety of different genes (McArdle et al., 2001). These effects seem to be due to modification of the intracellular redox balance, which influences multiple signaling pathways and activation of key transcription factors that modulate the expression of the genes controlled by these factors. Thus, our group demonstrated, in a skeletal muscle mouse model, that contractile activity induces activation of redox transcription factors, such as nuclear factor-kappaB (NF-κB), activator protein 1 (AP-1), and heat shock transcription factor 1. These are involved in the expression of antioxidant enzymes, such as catalase and superoxide dismutase, and protective proteins, such as heat shock proteins (HSP; Vasilaki et al., 2006c). In a further study in humans, Morton et al. (2006) indicated that trained men display a selective upregulation of basal heat shock and antioxidant protein content in skeletal muscle. Subsequently, we demonstrated that stimulation of the activation of transcription factor for HSP occurs in muscles of human subjects who followed a relatively nondamaging contraction protocol (Palomero et al., 2008a). Genes that are regulated by oxidative stress have been identified in different studies. These include genes for early response, genes involved in antioxidant protection, and genes for specific stress and HSP (Ammendola et al., 1995; Storz and Polla, 1996; Jackson et al., 2002). A protocol of acute contractile activity or longer term exercise training in rodents specifically resulted in increased activities of the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase, and an increase in the muscle content of protective proteins such as HSP (Higuchi et al., 1985; Ji, 1993; McArdle et al., 2001; Broome et al., 2006). In addition, studies in humans have reported comparable results (Jenkins et al., 1984; Khassaf et al., 2001; Palomero et al., 2008a).

REDOX REGULATION OF SKELETAL MUSCLE DURING AGING

During aging, muscles become smaller and weaker such that by the age of 70 yr in humans the cross-sectional area of skeletal muscle is reduced by 25 to 30% and muscle strength is reduced by 30 to 40% (Porter et al., 1995). The structural and functional changes that occur in muscles during aging have been well characterized, and the reduction in muscle strength is attributed to a large decrease in the total number of individual muscle fibers within the muscle and an atrophy of the remaining muscle fibers (Brooks and Faulkner, 1988). The causes of age-related loss of muscle mass and function are not fully understood, but considerable support exists for a role of ROS in mediating the process, and several studies have reported increases in markers of ROS production in skeletal muscle during aging. In normal physiology, the closely regulated generation of ROS mediates some adaptive responses of muscle to contractile activity, but during aging these responses are severely blunted. This inability to adapt to contractions is important in mediating age-related muscle dysfunction (McArdle et al., 2004; Jackson, 2005; Broome et al., 2006; Vasilaki et al., 2006c).

ROS and RNS in Skeletal Muscle During Aging

It is recognized that ROS and RNS are generated throughout the lifespan, but the lack of any established technique to accurately monitor these species has prevented comparative studies of their activities throughout the lifespan. The most widely considered theory of aging is the free radical theory of aging, which was proposed by Harman in 1956. This theory proposes that ROS produced from the normal mitochondrial metabolism cause a progressive damage to cellular biomolecules (i.e., proteins, membrane lipids, and nucleic acids), resulting in a decline in their function (Harman, 2003). The free radical theory of aging is not exclusive of other aging mechanisms, such as cell senescence, telomere shortening, genomic instability, and mitochondrial DNA damage and can explain various other mechanisms of aging (Benz and Yau, 2008). This theory must now account for the recognition that ROS and RNS are important signaling molecules. This indicates that to test the free radical theory of aging, it is necessary to understand and eventually manipulate the signaling pathways controlled by ROS and RNS (Muller et al., 2007).
Mechanisms in Aged Skeletal Muscle

The processes underlying the age-related loss of muscle mass and function (also known as sarcopenia) are unclear. However, consistent evidence indicates that ROS are mediators in the aging process. Several authors have reported changes in markers of ROS production in skeletal muscle during aging (Lass et al., 1998; Vasilaki et al., 2006c). Many studies indicate that mitochondria may be the main resource for ROS generation in cells during aging [see Sanz et al. (2006) for a review]. However, current data are inconclusive in terms of demonstrating that mitochondria are a major contributor to increased ROS generation and oxidation during aging. The methodology to assess mitochondrial ROS production has relied extensively on isolation of mitochondria from tissues, and this approach may potentially lead to an artifactual increase in ROS generation from mitochondria of aged tissues due to their fragility. There are other subcellular sources of ROS generation, such as the sarcoplasmic reticulum and plasma membrane, which have not been extensively studied in skeletal muscle during aging. Some investigators have studied extracellular activities of ROS in muscle from aged rodents and found no increase in comparison with adult mice (Vasilaki et al., 2006b; Close et al., 2007). In contrast, Muller et al. (2006) used a mouse model that lacks Cu-Zn superoxide dismutase and showed accelerated age-related loss of skeletal muscle mass and function. That study provided some direct evidence that an increase in intracellular superoxide can cause aging of skeletal muscle.

Impairment of Cellular Adaptation
Mechanisms in Aged Skeletal Muscle

As we mentioned previously, ROS mediate some of the adaptive responses of skeletal muscle to contractile activity. However, these responses are severely blunted in muscles from old rodents. Thus, Vasilaki et al. (2006c) demonstrated that there is increase in the antioxidant defense enzyme activity in exercised muscles from adult mice. This increase is associated with the activation of transcription factors NF-κB and AP-1. However, in exercised muscles from old mice, the activation of transcription factors NF-κB and AP-1 is attenuated and the modulation of adaptive responses, such as induction of antioxidant enzymes and protective proteins, are disrupted in muscles from these old mice (Vasilaki et al., 2006c). Consequently, the inability of skeletal muscle to adapt to contractions may contribute to muscle dysfunction during aging (McArdle et al., 2004). An explanation for the inability of old muscle to respond to stress is a failure of redox signaling processes. Some oxidation of cysteolic thiols is essential for activation of transcription factors, such as NF-κB, AP-1, and so on, which are involved in the expression of antioxidant and protective proteins. However, a greater oxidation of all cell components or a relatively specific oxidation of nuclear thiols may prevent these adaptive responses. Some data indicate that aging is associated with a decrease in the extracellular redox potential of glutathione (Jones, 2006), but there are no data regarding with this intracellular redox potential of glutathione in skeletal muscles from old organisms. Recently, we have assessed the redox potential of glutathione in muscles from young and old mice, and our results indicate that the redox potential of glutathione decreases in muscles from old mice [J. Palomero, D. Pye (Pathophysiology Research Unit, School of Clinical Sciences, University of Liverpool, Liverpool, UK), T. Kabayo (Pathophysiology Research Unit, School of Clinical Sciences, University of Liverpool), and M. J. Jackson, unpublished results]. This indicates that muscles from old mice are exposed to a greater oxidation than muscles from young mice and the excess of oxidation might block the activation of transcription factors and lead to a subsequent impairment of gene expression of proteins associated to mechanisms of cellular protection and adaptation. Although further studies are needed, it appears that the fall of redox potential of glutathione is critical in the aging of skeletal muscle. Some indirect data support the possibility that the nuclei of muscle and other cells of aged rodents are relatively oxidized because there is increased oxidation of nuclear DNA in old mice and people (Sanz et al., 2006), but the effect on nuclear redox potential has not been examined. Other indirect evidences support the possibility that thioredoxin plays a fundamental role in aging processes because overexpression of thioredoxin in mice is reported to increase lifespan (Yoshida et al., 2003).

SUMMARY AND CONCLUSIONS

Skeletal muscle has the ability to adapt and remodel after different stresses. Reactive oxygen and nitrogen species are continuously generated in skeletal muscle and play an important role in cellular signaling. Contractile activity in single muscle fibers induces a moderate generation of ROS and NO, and these are implicated in generation of muscle force and muscle fatigue. In addition, contractile activity induces adaptive responses. During aging, there is a reduction of muscle mass and force. Several studies indicate that RONS are involved in the process of aging and sarcopenia. It appears that cellular signaling pathways, which are activated and modulated by RONS and which control the adaptation mechanisms of skeletal muscle, are impaired during aging. The inability of skeletal muscle to adapt to contractile activity during aging might be responsible for the loss of muscle mass and function and the progressive deterioration of this organ. There is sufficient experimental evidence that indicates that cellular redox regulation in skeletal muscle is crucial in the physiology and pathology of skeletal muscle. However, new methodologies and experimental models are required for understanding the complex biology of RONS in the cell. This will provide future interventions that mitigate pathologies and aging of skeletal muscle.
LITERATURE CITED


