Role of Ca\(^{2+}\) signaling in skeletal muscle hypertrophy and atrophy

Naoki Ito and Shin’ichi Takeda*

Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-higashi, Kodaira, Tokyo 187-8502, Japan

Received: February 17, 2015 / Accepted: March 6, 2015

Abstract Skeletal muscle maintains an adequate volume that is commensurate with its surrounding environment. Although intracellular signaling molecules and pathways underlying the regulation of protein synthesis/degradation and subsequent muscle hypertrophy/atrophy are well studied, upstream regulators are largely unknown. In this review, we summarize the recent advances relating to the role of Ca\(^{2+}\) signaling as an upstream regulator of intracellular signaling pathways that regulate muscle plasticity, suggesting a new therapeutic target to control muscle mass.

Keywords: skeletal muscle, muscle hypertrophy, muscle atrophy, Ca\(^{2+}\), calcium signaling

Introduction

Skeletal muscle mass is regulated by a balance of protein synthesis and degradation\(^1\)-\(^3\). Increased muscle activity, such as exercise, resistance training or mechanical load induces the activation of protein synthesis that leads to muscle hypertrophy. In contrast, prolonged periods of muscle inactivity such as limb immobilization, bed rest or microgravity, as well as several common diseases including AIDS, advanced cancer, diabetes mellitus and sepsis, lead to significant muscle atrophy and muscle weakness because of increased protein degradation\(^4\)-\(^8\). For a person of advanced age, age-related loss of muscle mass and force (sarcopenia) contributes to disability and becomes a serious health issue\(^9\). Furthermore, loss of muscle mass has a diverse effect on overall health and viability, because muscle not only contributes to the structure and movement of the body, but also to the storage and supply of nutrients, interacts with other organs (fat and liver, in particular), and plays essential role in metabolism through secretion of muscle-derived cytokines, termed myokines\(^10\)-\(^12\).

In skeletal muscle, a well-studied Ca\(^{2+}\)-dependent process is excitation-contraction (EC) coupling during muscle contraction. The α1 subunit of the L-type Ca\(^{2+}\) channel in the transverse tubules, known as the dihydropyridine receptor (DHPR), interacts with the ryanodine receptor (RyR) in the sarcoplasmic reticulum (SR) to form the voltage sensor of EC coupling. Membrane depolarization activates DHPR, and in turn induces Ca\(^{2+}\) release from the SR through the RyR. The subsequent increase in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) induces muscle contraction by activation of actin-myosin cross-bridging\(^13\)-\(^14\).

In addition to muscle contraction, as well as the other organs or cells, Ca\(^{2+}\) is a highly multi-functional intracellular signal that regulates divergent cellular processes (for details, see review by Berridge MJ et al.\(^15\)). Recent studies suggest that Ca\(^{2+}\) signaling contributes to both muscle hypertrophy and atrophy, suggesting Ca\(^{2+}\) signaling is a regulator of muscle plasticity. This bilateral character for the regulation of muscle mass might be due to the divergent response of Ca\(^{2+}\) signaling with different levels and spatiotemporal dynamics. However, the mechanisms by which Ca\(^{2+}\) signaling control muscle mass are largely unknown. In this review, we first provide a brief summary for the intracellular signaling pathways that regulate muscle hypertrophy and/or atrophy, and then summarize the role of Ca\(^{2+}\) signaling in the regulation of muscle mass.

Molecular basis of muscle hypertrophy and atrophy

IGF-1/Akt/mTOR pathway in muscle hypertrophy. The major signaling pathway that controls skeletal muscle hypertrophy is the insulin-like growth factor-1 (IGF-1)/Akt/mammalian target of rapamycin (mTOR) pathway\(^2\),\(^3\),\(^16\). IGF-1 has a similar structure to insulin. Overexpression of IGF-1 in skeletal muscle induces an increase in muscle mass and force\(^7\). Akt is a serine/threonine kinase which is downstream of IGF-1. Furthermore, activation of Akt only in adult skeletal muscle is sufficient to induce muscle hypertrophy\(^18\),\(^19\). mTOR, one of the well-known downstream targets of Akt, is activated by glucose, growth factors such as IGF-1, amino acids, and by physical activity such as exercise\(^20\),\(^21\). mTOR is pivotal for inducing protein synthesis, which is mediated by its downstream target p70S6k, and subsequent hypertrophy\(^22\). Furthermore, in addition to the IGF-1-mediated activation of mTOR, several reports show that the load-induced activation of
mTOR is not mediated by IGF-1 or Akt\(^{23-26}\). Thus, the mechanisms by which load-induced activation of mTOR occurs are still controversial.

**Ubiquitin-proteasome system and autophagy-lysosome pathway in muscle atrophy.** The major molecular systems that promote protein degradation and subsequent muscle atrophy are the ubiquitin-proteasome and autophagy-lysosome systems\(^{27-29}\). Two E3 ubiquitin ligases, MuRF1 and atrogin-1, are the major genes responsible for the ubiquitin-proteasome system in muscle atrophy\(^{30}\). MuRF-1 ubiquitinates and degrades the sarcomeric proteins such as myosin heavy chain, myosin light chain and titin\(^{31-33}\). Atrogin-1 inhibits the muscle growth process by ubiquitinating myoD and eIF3\(^*\)\(^{34,35}\). In addition to MuRF-1 and atrogin-1, Sartori et al., recently showed that BMP-Smad1/5/8 signaling is involved in muscle atrophy by regulating Fbxo30, which encodes muscle ubiquitin ligase of the SCF complex in atrophy-1 (MUSA1)\(^{36}\). However, the precise targets for MUSA1 are still unknown.

Autophagy is a highly conserved homeostatic process regulating the degradation of cytoplasmic components that engulf damaged organelles, toxic protein aggregates and intracellular pathogens for clearance\(^{37}\). Genetic disruption of autophagy-related genes in skeletal muscle causes the accumulation of abnormal organelles, indicating that autophagy is essential for turnover and recycling of the cytoplasm and organelles to maintain muscle homeostasis\(^{38,39}\). However, involvement of autophagy in muscle atrophy has been controversial, because disruption of autophagy-related genes has been shown to both prevent and have no effect on the loss of muscle mass\(^{28,29,40,41}\).

**Ca\(^2+\) signaling as an upstream regulator of muscle mass**

**Role of Ca\(^2+\) signaling in muscle hypertrophy.** The link between Ca\(^2+\) signaling and muscle growth/hypertrophy was first observed by focusing on the calcineurin/nuclear factor of activated T-cells (NFAT)-dependent pathway. Calcineurin is a protein phosphatase that is activated by NFAT-dependent activation of autophagy, suggesting a link between muscle activity, Ca\(^{2+}\) signaling and maintenance of muscle mass\(^{42}\). Interestingly, Hernandez-Ochoa et al., showed that the overexpression of FoxO1 suppresses electric stimulation-induced Ca\(^{2+}\) release by suppressing the expression of Nav1.4\(^{43}\). Although the effects of FoxO on the activation of autophagy is thought to be regulated at the transcriptional level\(^{44}\), a part of FoxO-dependent autophagy may be mediated through inactivation of muscle inexcitability and a subsequent decrease in [Ca\(^{2+}\)].

When Ca\(^{2+}\) is depleted from the SR, the plasma membrane calcium release-activated channels (CRAC) are activated, and restore the levels of Ca\(^{2+}\) in the SR\(^{45}\). Muscle-specific knockout of stromal interaction molecule 1 (STIM1), an activator of store-operated calcium release-activated calcium channel protein 1 (ORAI1) channels, results in impaired muscle growth because of the impaired activation of Akt or Erk1/2, indicating that STIM1-mediated store-operated Ca\(^{2+}\) entry is required for muscle growth\(^{46}\). Furthermore, Woo et al., shows that junctophilin-2, which contributes to the formation of junctional membrane complexes that link the transverse tubules and the SR membrane, control muscle hypertrophy by regulating store-operated Ca\(^{2+}\) entry via ORAI1 in *vitro*\(^{55,56}\). Because overexpression of IGF-1 enhances activity of store-operated Ca\(^{2+}\) channels, a part of the effects of IGF-1 on muscle hypertrophy may be mediated by the STIM1-ORAI1 pathway\(^{57}\).

We recently show that the neuronal nitric oxide synthase (nNOS)/transient receptor potential cation channel subfamily V member 1 (TRPV1)-mediated Ca\(^{2+}\) signaling acts as an upstream regulator of the load-induced activation of mTOR and subsequent muscle hypertrophy\(^{58,59}\). TRPV1 is a member of the TRP channel family that is activated by temperatures greater than 43°C, or capsaicin, a pungent component of hot red pepper\(^{49}\). Although TRPV1 is largely localized at the plasma membrane in neurons, TRPV1 is localized in the SR in muscle and the endoplasmic reticulum in neurons, and is reported to be involved in Ca\(^{2+}\) release\(^{60,61}\). We show that the load-induced activation of nNOS and subsequent production of NO/peroxynitrite causes an increase in [Ca\(^{2+}\)], through TRPV1. This increase in [Ca\(^{2+}\)] activates mTOR, promotes muscle hypertrophy and prevents denervation-or hindlimb suspension-induced muscle atrophy. Gulati
et al., showed that the amino acid-induced activation of mTOR is mediated by Ca\(^{2+}\)/calmodulin signaling in HeLa cells\(^{44}\). Considering that the activation of intracellular signal transduction, including mTOR and MAPK, by the activation of TRPV1 or mechanical stimulation are similar\(^{39}\), the load-induced and the amino acids-induced intracellular signal transduction must be regulated by a common mechanism that is mediated by Ca\(^{2+}\) signaling. Similarly, the eccentric contraction-induced activation of p70S6k is prevented by inhibition of stretch-activated channels\(^{35}\). In addition to TRPV1 and ryanodine receptors, TRPC1 and inositol triphosphate receptor (IP3R) are known to be localized at the SR\(^{390}\). Notably, Mercan et al., revealed that the amino acid-induced increases in [Ca\(^{2+}\)]\(_i\) through IP3R is responsible for the activation of mTOR, p70S6K1 and subsequent myoblast growth\(^{67}\). A more detailed role of the effect of Ca\(^{2+}\) derived from the SR on muscle hypertrophy might be revealed by analyzing these channels in the future. Furthermore, Wang et al., recently showed that orexin/hypocretin activates mTOR in an Akt-independent and Ca\(^{2+}\)-dependent manner, which is similar to the load- and amino acid-induced signaling events\(^{68}\). Although future studies using multiple experimental models and cells are required, these results suggest that the link between Ca\(^{2+}\) signaling and mTOR is important, not only for muscle hypertrophy, but also on a broad-range physiological scale.

**Role of Ca\(^{2+}\) signaling in muscle atrophy.** In contrast, several studies have shown that the increase in [Ca\(^{2+}\)]\(_i\), is a cause of muscle atrophy and/or muscle weakness. Calpains are non-lysosomal cysteine proteases that are activated by increased [Ca\(^{2+}\)], thus the sustained increase in [Ca\(^{2+}\)] leads to calpain-dependent proteolysis\(^{69}\). Indeed, calpain activity is increased in hindlimb suspension\(^{70}\). Furthermore, Salazar et al., showed that the overexpression of calpastatin, an endogenous inhibitor for calpain, prevents muscle weakness in hindlimb suspension, but does not alleviate muscle atrophy\(^{71}\). Calpain activity is also increased during sepsis, and treatment with the calpain inhibitor BN82270 results in the prevention of sepsis-induced muscle proteolysis\(^{72,73}\).

The production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is known to be increased with aging and some other pathological conditions, which causes oxidative and nitrosative stress to cells. Recent studies indicate that the leaky ryanodine receptor that is induced by oxidation or nitrosylation, and a subsequent increase in [Ca\(^{2+}\)], contributes to age-related muscle weakness and muscular dystrophy\(^{74-76}\). Interestingly, treatment with S107, which inhibits Ca\(^{2+}\) leakage from the SR by reducing the deletion of calstabin from the RyR, alleviates muscle weakness and the dystrophic phenotype. In relation to these results, treatment with nifedipine or dantrolene, an inhibitor of DHPR or the RyR, alleviates the phenotype of dystrophic muscle\(^{77,78}\). Furthermore, the overexpression of sarcoplasmic reticulum Ca\(^{2+}\) ATPase1 (SERCA1), which is responsible for Ca\(^{2+}\) uptake into the SR in δ–sarcoglycan-null mice, resulted in attenuation of the dystrophic phenotype\(^{79}\). Thus, these results suggest that the altered [Ca\(^{2+}\)], underlies not only the mechanisms of muscle atrophy, but also the pathophysiological phenotype of muscular dystrophy, and improvement of Ca\(^{2+}\) signaling would be a potential cure for muscle weakness. We previously showed that the sarcolemmal nNOS translocates to the cytosol, and subsequent production of NO causes hindlimb suspension-induced muscle atrophy\(^{80}\). Because nNOS could be a source of nitrosative stress, translocated nNOS may promote muscle atrophy through S-nitrosylation of RyRs.

### Conclusion

As summarized above, increased [Ca\(^{2+}\)], both promotes muscle growth/hypertrophy and muscle atrophy/weakness. The precise mechanisms by which Ca\(^{2+}\) signaling induces these opposite effects are largely unknown, but this may be due to the different levels of increased [Ca\(^{2+}\)], and/or its spatiotemporal regulation. Therefore, elucidation of the molecular mechanisms underlying the regulation of [Ca\(^{2+}\)], and subsequent muscle hypertrophy and atrophy may lead to the development of new therapeutic strategies to control muscle mass.

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

### Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research (B) for the Japan Society for the Promotion of Science Fellows.

### References


