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Neuromuscular Junction Degeneration In Muscle Wasting

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Abstract

**Purpose of review**—Denervation is a hallmark of age-related and other types of muscle wasting. This review focuses on recent insights and current viewpoints regarding the mechanisms and clinical relevance of maintaining the neuromuscular junction to counteract muscle wasting resulting from aging or neural disease/damage.

**Recent findings**—Activity-dependent regulation of autophagy, the agrin-MuSK-Lrp4 signaling axis, and sympathetic modulation are principal mechanisms involved in stabilizing the neuromuscular junction. These findings are derived from several animal models and were largely confirmed by human gene expression analysis as well as insights from rare neuromuscular diseases such as amyotrophic lateral sclerosis and congenital myasthenic syndromes. Based on these insights, agrin-derived fragments are currently being evaluated as biomarkers for age-related muscle wasting. Tuning of autophagy, of the agrin pathway, and of sympathetic input are being studied as clinical treatment of muscle wasting disorders.

**Summary**—Basic research has revealed that maintenance of neuromuscular junctions and a few signaling pathways are important in the context of age-dependent and other forms of muscle wasting. These findings have recently started to enter clinical practice, but further research needs to substantiate and refine our knowledge.

**Keywords**

Autophagy; atrogene; agrin; Lrp4; sympathetic agonist
Introduction

Sedentary lifestyle and aging make muscle wasting an increasing health burden. Being at the crossroad between nerve and muscle [1], neuromuscular junctions are entering center stage for basic concepts, diagnosis and therapy of muscle wasting [2]. Recent progress in the understanding of neuromuscular junction functions in muscle wasting and their application to clinical aspects are reviewed here.

Muscle wasting, the atrogene concept, and neuromuscular junction

Several conditions including aging, disuse, heart failure, diabetes, cancer, and neuromuscular transmission disorders lead to profound muscle wasting and weakness. A common set of proteins are strongly up- or down-regulated under different conditions leading to muscle wasting (also called muscle atrophy) and were termed ‘atrogenes’ [3]. Two of these proteins, namely Atrogin-1 and MuRF1 are E3 ubiquitin ligases and have been characterized as the key regulators for the proteasomal degradation of muscle tissue muscle wasting conditions. The finding that the neuromuscular junction fragments and loses functionality upon aging-related muscle wasting (sarcopenia) and other atrophic conditions and that these phenomena are partially reversible by metabolic maneuvers [4–8] have spurred interest in understanding the molecular links between muscle wasting and related neuromuscular junction phenotypes. Notably, while MuRF1 was generally considered to orchestrate sarcomeric protein degradation upon muscle wasting, it was recently found to be clearly enriched at the neuromuscular junction and to be involved in muscle wasting-induced autophagic degradation of a major neuromuscular junction component, i.e. nicotinic acetylcholine receptor (AChR) [9,10]. These studies linked the atrogene concept to neuromuscular junction maintenance and autophagy. Increased turnover of AChR, fragmentation of neuromuscular junctions, and precocious synaptic dysfunction including partial denervation were observed in mice with skeletal muscle-specific loss of Atg7, a principal component of the autophagy machinery [11]. This further supported a major role of autophagy for neuromuscular junction homeostasis. Accordingly, autophagy was found to be dysregulated in skeletal muscle of aging dogs [12] and humans [11]. It has been determined that caloric restriction and physical exercise are beneficial for rescuing neuromuscular junction fragmentation [2,13,8] and innervation status [2,14], and at the same time reduce impairment of autophagy [11] as well as sarcopenia onset or progression [15–18]. This suggests autophagy modulation to be a potential therapeutic target in neuromuscular transmission disorders (Fig. 1).

Agrin signaling – diagnostic and therapeutic toolbox for muscle wasting

While autophagy and other vesicular trafficking processes are key to the delivery, distribution, and degradation of neuromuscular junction components, the agrin-MuSK-Lrp4 signaling axis mediates crosstalk between nerve and muscle [2] (Fig. 1). Indeed, neuronal agrin is released by the motor neuron into the synaptic cleft where it binds to the Lrp4 co-receptor to activate the receptor tyrosine kinase MuSK. This leads to stabilization and clustering of AChR via Dok-7 and further mechanisms that involve also the 43 kDa-protein rapsyn. The agrin-MuSK-Lrp4 signaling pathway is of principal importance for the establishment and maintenance of neuromuscular junctions. Mutants in any of its
components are either not viable or develop severe neuromuscular transmission disorders termed congenital myasthenic syndromes (CMS) [2,19]. Also, neuromuscular junction remodeling that entails reorganization of AChR patches for maintaining a tight pre-to-postsynaptic matching appears to require local cleavage of agrin. This is executed by the protease neurotrypsin and leaves a C-terminal agrin fragment (CAF, Fig. 1), which becomes soluble and circulates in the blood. Given massive neuromuscular junction remodeling in the course of some forms of muscle wasting and particularly during sarcopenia, the use of circulating CAF levels as a diagnostic tool of neuromuscular junction disintegration has become an attractive concept [20]. Indeed, recent clinical investigations supported the efficacy of CAF as a biomarker for sarcopenia primarily in men [21–23]. However, sample sizes in these studies are still small asking for larger trials.

Apart from the diagnostic relevance of CAF, a neurotrypsin-resistant and soluble N-terminal fragment of agrin (Fig. 1) was tested with respect to its therapeutic efficacy [24]. Subcutaneous injection of the compound into neurotrypsinoverexpressing mice largely rescued their sarcopenic phenotype, which includes severe precocious muscle wasting, weakness, and neuromuscular junction degeneration. Another study investigated the therapeutic efficacy of modulating Dok-7 abundance and revealed that adeno-associated virus-mediated gene therapy to overexpress Dok-7 was able to significantly reduce the symptoms of two different CMS models, including a prolongation of lifespan in an Emery-Dreyfus CMS model and a complete rescue in Dok-7 knockout mice [25]. Although the therapeutic activity of both the N-terminal agrin fragment as well as the Dok-7 have been explored only in rodents so far, these studies suggest that neuromuscular junction-related muscle wasting might in the future be amenable for pharmacological intervention.

Lrp4 and Slit2 – bidirectional communication at the nerve-muscle interface

As mentioned before, Lrp4 is a major mediator of postsynaptic stability at the neuromuscular junction [2] and its persistent activity is necessary to maintain synaptic integrity and function throughout adulthood as shown by inducible Lrp4 knockout mice [26]. While Lrp4 is now an established causative gene for some forms of CMS [27,28] it has recently also been implicated in the pathogenesis of a motor neuron disease, namely amyotrophic lateral sclerosis (ALS). Indeed, Lrp4 autoantibodies were found in a high proportion of ALS patients, while they were absent in other neurological disorders [29]. Some studies now consider ALS, which is characterized by late onset and fast progression, as a disease that originates at the neuromuscular junction followed by progressive ‘dying-back’ of the rest of the neuron. Indeed, alterations of synaptic function [30], of the presynaptic terminal [31], and of terminal Schwann cells [32], which shield the neuromuscular junction, were found to precede symptom onset in mouse models of ALS and Charcot-Marie-Tooth disease. As recently reviewed, Lrp4 may act as a retrograde signal orchestrating presynaptic organization at the neuromuscular junction [2], thus potentially explaining these phenomena. However, given that Lrp4 is present in cis and in trans it remains open, if the initial neuromuscular junction damage occurred on the pre- or postsynaptic side. Evidence for a muscle-dependent retrograde signaling that determines neuromuscular junction presynaptic integrity comes from a recent report on the function of the releasable axonal guidance factor Slit2 [33]. Slit2 expression was reduced in mice...
lacking muscle β-catenin, an effector of wnt signaling. Overexpression of Slit2 in these mice rescued all their phenotypes including impairment of terminal differentiation of the neuromuscular junction presynapse, axonal pathfinding, and synapse function [33]. A recent bioinformatics screen of aging-related alterations of gene expression in humans identified Slit2 to be significantly associated with physical capacity across 116 samples [34]. Furthermore, these data highlight a well established [2] but complex role [35,36] of wnt signaling in neuromuscular junction formation and maintenance as well as after traumatic injury [37]. The earlier mentioned screen of aging related alterations of gene expression in humans [34] also identified Amotl2. Together with other proteins, Amotl2 is a component of so-called podosomes which play a role in neuromuscular junction remodeling [38].

**Enigmatic treatment of CMS**

CMS are rare hereditary neuromuscular transmission disorders that are caused by mutations in several principal components of the neuromuscular junction, including AChR, MuSK, Lrp4, Dok-7 and others [28]. Generally, neuromuscular junctions of CMS muscles are severely affected in terms of morphology and function, leading to muscle wasting and weakness. In clinical practice, many CMS patients have been found to respond well to treatment with β-adrenergic agonists, such as albuterol, salbutamol, or ephedrine [19,27,28,39] (Fig. 1). Fittingly, neuromuscular junctions have been recently identified as target of direct sympathetic innervation and this appears to be crucial for the homeostasis of neuromuscular junctions, for example by modulating AChR expression [40]. The mechanisms underlying the therapeutic efficacy of β-adrenergic agonists are likely pleiotropic in nature and might involve enhancement of gene expression [40], protein metabolism, inhibition of proteolysis, improvement of mitochondrial function, effects on the release of trophic factors and the modulation of immune cells [41].

**Conclusion**

Several forms of muscle wasting, including sarcopenia, have an important denervation component. Although muscle wasting can be observed also in the absence of neuromuscular junction derangements [42], strong links between neuromuscular junction impairment and repair of muscle wasting in particular upon aging and neural damage have been observed. Accordingly, measures to maintain and enforce neuromuscular junctions are increasingly becoming a focus in clinical research.

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Key points

- Degeneration of neuromuscular junction is important in the context of some types of muscle wasting, particularly those resulting from aging or neural damage/disease.

- Degeneration of neuromuscular junctions is modulated by autophagy, the agrin-MuSK-Lrp4 and Wnt signaling axis, and sympathetic input.

- C-terminal fragment of agrin is being evaluated as biomarker for muscle wasting.

- Modulation of autophagy, N-terminal agrin fragments, modulation of Dok-7 expression, and sympathicomimetics are being investigated for their therapeutic function in neuromuscular junction-related muscle wasting.
Figure 1. Major pathways involved in maintenance of neuromuscular junction and their links to clinical practice

Modulation of autophagy, of the agrin-Lrp4-MuSK signaling axis and of sympathetic activity have shown good results for treating sarcopenia and other forms of muscle wasting in rodent models. Thus, the therapeutic efficacies of stimulating autophagy, applying agrin N-terminal fragments, modulating Dok-7 expression and the use of sympathicomimetics are being explored. C-terminal agrin fragment (CAF) is being evaluated as a biomarker for muscle wasting. The upper panel schematically depicts therapeutic options (italic font) and biomarkers (regular font) for muscle wasting and their putative targets as discussed in the text. The lower panel indicates putative targets of therapeutic interventions, without being comprehensive.