Alteration of skeletal muscle calcium homeostasis in an animal model of cachexia and restoration by growth hormone secretagogues

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Cachexia is a complex, multifactorial syndrome involving progressive body weight loss, leading to loss of lean body mass and fat stores, combined with functional impairment, reduction in food intake, and altered metabolism (1). It is a devastingform of muscle wasting that is commonly associated with cancer in which skeletal muscle loss is the main characteristic and the primary cause of function impairment, fatigue and respiratory complications. Among the mechanisms underlying cancer or chemotherapy-induced muscle atrophy, it has been proposed an increase in muscle proteolysis and a decrease in protein synthesis driven by activation of various enzymatic pathways such as the ubiquitin-proteasome, mitogen-activated protein (MAP) kinases or myostatin (2-4). Skeletal muscle function is highly dependent on calcium signaling for contraction and myofiber protein processing. It is known that intracellular free Ca^{2+} abnormality causes disruption of normal excitation-contraction (EC) coupling as well as can stimulate activity of various enzymes (5-7). Calcium-dependent signaling pathways are believed to play an important role in skeletal muscle decline observed in cachexia, but whether intracellular calcium homeostasis is affected in this situation remains uncertain. There are currently no approved treatments for cachexia and the development of new therapies is strongly required. At this regard, growth hormone secretagogues (GHS), ghrelin mimetics, are capable to interfere also directly with muscle function and represent promising therapeutic compounds. By a multidisciplinary approach, here we characterized the calcium homeostasis in fast-twitch EDL muscle of adult rats with cisplatin-induced cachexia and established the potential beneficial effects of two GHS (hexarelin, JMV2894). We show that, besides a significant reduction of the muscle weight and fiber diameter, EDL muscles of cisplatin treated rats showed an upregulation of *atrogin1* and *Murf-1* genes, compared to control animals. Furthermore, *Pgc1-a* expression is decreased, although not significantly. Additionally, we show that the resting $[Ca^{2+}]_i$ of EDL muscle after cisplatin administration was 2-fold increased in comparison with control rats. Importantly, changes of some calcium-dependent functional outcomes, such as an increase of the latency of the action potential and a decrease of resting chloride conductance, also occurred in cachetic EDL muscles, thus indicating that the cachexia-induced alteration of calcium homeostasis influences muscle functionality. Furthermore, we demonstrate that the cisplatin-induced dysfunction of skeletal muscle calcium homeostasis is characterized by a reduced response to the application of depolarizing solution or of caffeine as well as by a reduced SOCE. The changes of some calcium-dependent functional outcomes support the impact of calcium homeostasis alteration on muscle functionality in cachetic animals. GHS efficaciously prevents cisplatin-induced muscle weight loss and $[Ca^{2+}]_i$ increase and improve the functional parameters. Our findings provide the first direct evidence of a calcium homeostasis dysregulation in cachexia and the demonstration that GHS administration efficaciously prevents cisplatin-induced calcium homeostasis alteration in skeletal muscle point out to a new molecular mechanism to counteract chemotherapy-associated cachexia finally contributing to gain insight into the therapeutic mechanisms mediated by GHS.