Al collegio docenti del Dottorato in Medicina Molecolare

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Attività scientifica svolta nel 1° anno di Dottorato, Anno Accademico 2019/20

INTRODUCTION

Tubular aggregates myopathy (TAM) is a rare variant or myopathy that can have dominant or recessive transmission. It is a progressive disease with symptoms like loss of strength, muscle pains, cramps and at cellular levels the presence of tubular aggregates (TAs) inside muscle fibers. TAMs usually are caused by defects in SOCE activation. SOCE (Store-Operated Calcium Entry) is an essential pathway for the uptake of calcium from the extracellular space. The mechanism is of notable importance in muscle cells (and fibers) to fill sarcoplasmic reticulum of Ca^{2+}, needed for the signalling and muscular contraction. SOCE is composed mainly of 2 proteins: stromal interaction molecule 1 (STIM1) and Calcium release-activated calcium channel protein 1 (ORAI1). STIM1 is sensible to Ca^{2+} level inside the SR thanks to EF-hand luminal domain, and has the role of initiator of SOCE mechanism following the depletion of calcium storage. When calcium ions no longer bind STIM1 luminal domain this protein lengthen the cytoplasmic domain and take contact with ORAI1 on the cellular membrane, this conformation with STIM1 extended towards the membrane and ORAI1 recruited and activated is called “puncta”. The results of this activation is the influx of calcium from the extracellular space inside the cytoplasm. Calsequestrin (CASQ) is another crucial protein in calcium regulation and homeostasis, weighing 45 kDa is localized inside the lumen of SR. His role is to acts as a buffer of calcium, binding it with low affinity but high avidity, allowing the storage in high quantity. CASQ has a role in the regulation of SOCE, interacting with STIM1 when calcium concentration decrease.

METODS

Muscle fibers were isolated from FDB muscles of mice and plated on Lab-Tek™ II previously incubated with laminin. To evaluate calcium fluxes and SOCE activation intra-reticular storage of calcium was depleted using different molecules and concentration. Caffeine, Chloro-meta-creosol, Thapsigargin, Ionomycin and Cyclopiazonic acid were all tested, alone and in combination, to asses the best cocktail for a reliable and complete depletion of SR. SOCE activation was tested using a calcium rich buffer after the depletion. Intracellular calcium concentration was visualized with the ratiometric fluorescent calcium indicator Fura-2AM using a fluorescence microscope.

RESULTS

The fibers stay vital and responsive until 24h after being plated. The best cocktail both for complete calcium depletion and avoidance of fibers damage is composed of 1mM CmC and 0.004mM of Thapsigargin in a calcium free medium with 100mM of EGTA. The depletion phase can vary from 20 to 30 minutes, SOCE must be tested as soon as intracellular calcium level are back at equilibrium to avoid damage due to calcium starving. Using this protocol SOCE activation was visualized in the majority of tested fibers.

• Partecipazione a congressi e corsi:

F. Zappulla, L. Ricceri, R. Lorenzini, F. Manti, V. Raffa, A. Ahluwalia, M. Mele, A.Gazzano, A. Sbrana, A. Coli; Corso di formazione “Protezione degli animali impiegati nella ricerca: aspetti scientifici, etici e applicativi” – 8, 15 e 22 ottobre 2019.

Soft Skills for PhD, 11 e 13 maggio, 3,8,9,11,15,16,17,18,22,23,25,26 e 29 giugno, 10 luglio, 8 e 9 settembre 2020.