SKELETAL MUSCLE CLC-1 CHANNEL: FROM GENE TO PROTEIN, FROM BIRTH TO AGING TOWARD A PERSONALIZED MEDICINE

1)Conte E. 2)Fonzino A. 3)Cibelli A. 4)De luca A. 5)Pierno S. 6)Nicchia GP. 7)Camerino GM.

University of Bari

Voltage-gated ClC-1 chloride channels play a critical role in controlling the membrane excitability of skeletal muscles (Pedersen et al.,2016). Loss of function mutations in human ClC-1 channels have been linked to the hereditary muscle disorders myotonia congenita (MC) (Poroca et al.,2017). A reduction in membrane resting chloride conductance (gCl) primarily determined by ClC-1 channels together with a decrease of the genes encoding for these channels have been reported in the muscles of over 24-months aged rats (Camerino et al.,2016) moreover in muscle disuse (Pierno et al.,2013) has been associated to a phenotype dependent alteration of gCl. Finally a decrease in membrane gCl paralleled by a decrease of ClC-1 gene expression represents a severe iatrogenic effect of drug treatment such as statins (Camerino et al.,2016). Little is known of the changes occurring in gene and protein expression of ClC-1 channel during the lifespan and in particular of its cellular localization. Previous studies (Conte Camerino et al.,1989) have shown that 7 days after birth, the gCl of rat extensor digitorum longus (EDL) is very low and increases rapidly with age and ClC-1 gene expression shows the same trend (Steinmeyer et al.,1991). During aging a decrease of gCl and ClC-1 mRNA expression was also found with respect to adulthood (Lueck et al.,2007). To better investigate on the modification of ClC-1 expression during skeletal muscle development, we performed a systematic study of ClC1 protein and mRNA, in slow-twitch soleus (SOL) and fast-twitch (EDL) rat muscles from birth to old age by western blot and qPCR analysis. Our preliminary data confirm that ClC-1 protein content is lower in rat SOL muscle with respect to EDL muscle. Furthermore, protein ClC-1 expression gradually increases 10-fold from birth to 8 months of age and decreases by 50% in 27-months aged rats. Since ClC-1 channel can be inactivated by protein kinase C (PKC) (Camerino et al.,2014), we evaluated the PKC activity in EDL and SOL muscles during development by using ELISA analysis:PKC activity is maximal at birth and progressively decreases until 8 months of life, then slightly increasing in aged EDL and SOL muscles. Although the importance of ClC-1 channel activity in maintaining muscle excitability is well appreciated, its subcellular location remains controversial (Lueck et al.,2010). Using double-immunofluorescence analysis on EDL and SOL muscles cross sections, we determined ClC-1 fiber localization chosing sarcolemmal beta-dystroglycan as reference (Williams et al.,1999). The ClC-1 channel was found to be present either in sarcolemma that in cytoplasm from birth to 12-days while was present only in sarcolemma of adult and old rats in both muscles. To confirm this result we conducted high resolution confocal microscopy of ClC-1 and beta-dystroglycan immunofluorescence at 12 days and 2 months. Images confirm a strictly membrane ClC-1 localization in EDL and SOL muscles of 2 months-old rats; whereas ClC-1 channel was found to be present either in membrane or in spot near the membrane at 12 days of age. Our findings provide the direct evidence that ClC-1 expression and localization varies during skeletal muscle development offering a point of comparison for all the situations in which ClC-1 protein is altered and paving the way for the

identification of new personalized medicines (such as chaperons) for restoring normal ClC-1 activity in various pathological situations.

Pedersen et al.(2016) The Journal of General Physiology 147:291-308

Poroca et al.(2017), Frontiers Review

Camerino GM et al.(2016) Toxicol Appl Pharmacol. 306:36-46

Conte Camerino D. et al.(1989), Pflugers Arch 413:568

Steinmeyer et al.(1991), Nature 354:301

Lueck JD et al.(2007), Am J Physiol Cell Physiol 292: C1291–C1297

Camerino et al.(2014), Pflugers Arch 466:2215

Lueck JD et al.(2010), J Gen Physiol. 136:597-613

Williams MW et al.(1999), J Muscle Res Cell Motil. 383-93

Pierno S.et al.(2013), PLoS One 8:e65167