Definition of a specific role of PKC-theta in the expression and modulation of ClC-1 chloride channel in fast and slow skeletal muscles by using a mouse model lacking PKC-theta

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Protein Kinase C (PKC) is a family of serine/threonine protein kinases that regulate various cellular functions. This family is classified into three groups on the basis of the arrangement of their regulatory domains. It is still unclear whether different PKC isoforms may play distinct and specific roles. Different PKC isoforms are expressed in skeletal muscle, including the novel isoform PKC theta, where it mediates various cellular responses, involving muscle growth and regeneration (Serra et al., 2003). Skeletal muscle function is sustained by the resting chloride conductance (gCl) due to the ClC-1 chloride channel activity. Resting gCl controls the sarcolemma electrical stability since its reduction may produce myotonia-like symptoms. We previously showed that gCl is negatively regulated by PKC, as its activation close the ClC-1 channel in fast- and in slow-twitch muscles (Pierno et al., 2007). Slow-twitch muscles are characterized by lower value of gCl compared to fast-twitch muscles, due in part to a higher basic activity of PKC and in part to reduced ClC-1 expression. Here we better investigate on the role of PKCθ in the regulation of ClC-1 channel expression and activity in extensor digitorum longus (EDL) and in soleus (Sol) muscles, using two different models of PKCθ-null mice: a PKCθ knockout model, in which the PKCθ gene was inactivated in all cells (Sun et al., 2000) and the mPKCθK/R transgenic model, in which a dominant-negative mutant form of PKCθ is expressed under the control of a muscle-specific promoter (Serra et al., 2003). We found that the expression of ClC-1 channel, evaluated by real time-PCR, was not modified either in EDL or in Sol of PKC KO mice with respect to wild-type mice, demonstrating that PKCθ does not control the ClC-1 expression. In parallel, the electrophysiological studies, done by using the two intracellular microelectrode technique, showed a significant 38% increase of gCl in Sol muscle of KO mice with respect to wild-type. A lesser increase of gCl (23%) was found in EDL muscle. Muscle excitability was reduced accordingly to gCl increase. Similar effects, although less marked, were observed in K/R mice. Chelerythrine, a non-specific PKC inhibitor, further increase gCl (by 25%) in Sol muscle showing that other PKC isoforms are involved in the control of gCl. Chelerythrine have slight effect on gCl of EDL in PKC theta KO mice. Fluvastatin, a hypocholesterolemic drug, stimulate PKC activity (Pierno et al., 2009) and significantly reduced gCl in EDL muscle of PKC KO mice by 30% confirming that also in EDL other PKC isoforms contribute to ClC-1 channel modulation. A cytofluorimetric analysis (Fraysse et al., 2003) demonstrate a significant reduction of cytosolic calcium in both muscle types of PKC theta KO and K/R mice as compared to the wild-type. Interestingly, we demonstrate a decreased calcium sarcolemmal permeability in KO mice. The evaluation of the expression of ClC-1 and other genes in skeletal muscle of K/R mice is in progress. Those results indicate that PKC theta partially contribute to the regulation of ClC-1 channel activity in both muscle types and that this isoform can also modulate calcium homeostasis likely by interacting with calcium channels. Although other PKC isoforms seems to be responsible for the regulation of the ClC-1 channel in skeletal muscle, targeting PKCθ can be a valuable therapeutic strategy for chloride channel related disease. (Supported by ASI-OSMA)

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