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INTRINSIC ACTIVITY

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MEETING ABSTRACT

A2.31

ERC1 increases membrane and functional expression of the voltage sensor of excitation–contraction-coupling $Ca_V1.1$

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Background: ERC1, a member of the family of CAST/ELKS scaffold proteins, is responsible for supporting the structure of presynaptic active zones. ERC1 directly interacts with the Ca_V β subunit of voltage-gated Ca^{2+} channels (VGCC) through the guanylate kinase-like (GK) domain [1]. Importantly, this interaction affects VGCC activity, as ERC1 deletion leads to reduced calcium influx at inhibitory synapses in the hippocampus, the calyx of Held, rod photoreceptors, and pancreatic β cells [2,3,4]. Here, we hypothesized that ERC1, which is endogenously expressed in skeletal muscle, might also influence the membrane and functional expression of $Ca_V1.1$, as well as voltage-induced Ca^{2+} release from the sarcoplasmic reticulum.
Methods: We investigated the effect of ERC1 overexpression or deletion on $Ca_V1.1$ and the ryanodine receptor 1 (RyR1) membrane and functional expression in different cell types (skeletal muscle C2C12 wild-type and ERC1 knockout and HEK-TetOn-STAC3 cells), utilizing immunocytochemistry and electrophysiology analyses.
Results: First, we examined the impact of ERC1 overexpression on $Ca_V1.1$ and RyR1 levels in skeletal muscle C2C12 cells. Whereas $Ca_V1.1$ cluster intensity was enhanced by 15.6%, RyR1 expression remained unchanged. Additionally, we generated an ERC1 knockout C2C12 cell line (clone C3) with CRISPR/Cas9, in which we analysed the effect of ERC1 deletion or reconstitution on $Ca_V1.1$ and RyR1 expression levels. Similarly to the overexpression experiments, ERC1 enhanced $Ca_V1.1$ membrane expression by 15.5% while the RyR1 expression remained unaltered. In addition, to analyse the effect of ERC1 on $Ca_V1.1$ functional expression, we performed patch-clamp experiments in HEK cells and found that ERC1 increased $Ca_V1.1$ current density by 44%.
Discussion: Our results demonstrate that ERC1 increases the number of $Ca_V1.1$ channels in the membrane of skeletal muscle cells and $Ca_V1.1$ current density in HEK cells. In order to investigate the effect on endogenous $Ca_V1.1$, we are currently analysing the $Ca_V1.1$ currents and excitation–contraction coupling in muscle cells upon ERC1 overexpresssion.
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Keywords: ERC1 – $Ca_V1.1$ channels – voltage-gated calcium channels – skeletal muscle
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