Tuinte *et al.*: *Intrinsic Activity*, 2023; **11**(Suppl.1):A1.5
doi:10.25006/IA.11.S1-A1.5
published online: 22 September 2023

INTRINSIC ACTIVITY

27th Scientific Symposium of the Austrian Pharmacological Society

Vienna, 29–30 September 2023

MEETING ABSTRACT

A1.5

Dissecting the functions of multiple interactions of STAC3 in skeletal muscle excitation–contraction coupling

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Background: The adaptor protein STAC3 was discovered to be an essential protein for skeletal muscle excitation–contraction (EC) coupling and exerts three distinct functions: (i) it facilitates membrane expression of $Ca_V1.1$; (ii) it is crucial for $Ca_V1.1$ function as the voltage sensor of EC coupling; (iii) lastly, it is essential for the conformational coupling between $Ca_V1.1$ and the ryanodine receptor 1 (RyR1). Previously, two distinct interactions between STAC3 and $Ca_V1.1$ were identified: the one between the SH3-1 domain of STAC3 and the II–III intracellular loop of $Ca_V1.1$, and the one between the C1-linker region of STAC3 and the proximal C-terminus of $Ca_V1.1$.
Methods: To determine which interaction is important for each function, two STAC3 fragments, each containing the domain responsible for one interaction, were reconstituted in a double $Ca_V1.1$/STAC3 knockout skeletal muscle cell line. With electrophysiological recordings using the fluorescent calcium indicator Fluo-4, both calcium currents and the calcium release from the SR could be measured simultaneously. Charge movement was measured using the non-conductive $Ca_V1.1$ channel.
Results: Electrophysiological recordings revealed that the STAC3 C1-linker fragment expression rescued $Ca_V1.1$ charge movement and calcium currents. However, the calcium release from the sarcoplasmic reticulum was severely reduced. Conversely, reconstitution of only the STAC3-SH3s domains did not rescue any function. Simultaneous reconstitution of both fragments also did not fully rescue EC coupling, suggesting that the isolated SH3 domains interact with low affinity. To increase the local concentration, we linked the SH3 domains to the Ca_V ß1a subunit. This fragment alone rescued minimal EC coupling, but no calcium currents. However, when co-expressed with the other STAC3 fragment, full currents and EC coupling were reconstituted.
Discussion: These results demonstrate that the C1-linker/C-terminus interaction is responsible for STAC3-targeting to the EC-coupling machinery and $Ca_V1.1$ functional expression, while the low-affinity SH3s/II–III loop interaction merely enhances EC coupling.
Acknowledgements: This study was supported by the Austrian Science Fund FWF (grant ZFP337760).
Keywords: excitation–contraction coupling – STAC3 – voltage-gated calcium channels – $Ca_V1.1$ channels

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