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Down regulation of the cardiac sodium channel Nav1.5 by the MAGUK protein CASK: role of its functional domains

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Proteins of the MAGUK (Membrane-Associated Guanylate Kinase) family are implicated in trafficking, targeting, and signaling of ion channels in cardiac myocytes (CM). We identified in the heart a member of these scaffolding proteins, CASK (Calcium/Calmodulin-dependent Serine protein Kinase) which directly interacts with NaV1.5 channels and negatively regulates the corresponding current INa at the lateral membrane of CM. At the moment, the mechanisms underlying this regulation are only partly elucidated. As other MAGUKs, CASK contains multiple protein-protein interaction domains such as a N-terminal CAMKII-like domain, a single PDZ domain and a C-terminal GUK-like domain. Our aim was to investigate which functional domain(s) of CASK is/are responsible for interaction with NaV1.5 channels and for the negative regulation of INa. We designed seven adenoviral CASK constructs with a single functional domain truncated at the time (CASK?CAMKII, CASK?L27A, CASK?L27B, CASK?PDZ, CASK?SH3, CASK?HOOK and CASK?GUK) to overexpress the protein in adult rat CM. GFP and the WT CASK protein served as controls. The effect of the overexpression of the different constructs were investigated using patch clamp electrophysiology, RT-qPCR, biochemistry and high resolution 3-dimensional deconvolution microscopy. Whole-cell patch-clamp experiments showed that CASK WT overexpression reduced INa whereas CASK?L27B and CASK?GUK constructs completely or partially restored INa. Immunostaining experiments revealed that the deletion of either L27B or GUK domain enhanced the expression of NaV1.5 at the membrane of CM compared to CASK WT overexpression. Finally, RT-qPCR and western blot experiments showed that mRNA and protein levels of NaV1.5 were not increased by either CASK?L27B or CASK?GUK overexpression. These results indicate that the rescue of INa upon CASK?L27B or CASK?GUK overexpression was not transcriptional nor translational and rather due to an increased surface expression of NaV1.5. A likely explanation is that CASK impedes anterograde trafficking and/or stabilization of NaV1.5 channels at the sarcolemma through interactions with either L27B or GUK domain. This hypothesis will be investigated using time lapse experiments to follow NaV1.5 trafficking in CM overexpressing CASK, CASK?L27B or CASK?GUK. GST pull-down assays will be performed to investigate which domain of CASK is involved in the interaction with the channel.