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Abstract 5309: beta-Arrestin 1 (bArr1) and Ankyrin 2 (ANK2) Regulate Cav1.2 Trafficking through Complex G Protein Mediated Signaling

Maria A Diverse-Pierluissi, Rachele Lipsky, Akil A Puckerin, Joanne Stocks, Vivek Patel, and Fadi G Akar
Mount Sinai Sch of Medicine, New York, NY

Originally published 22 Mar 2018 | Circulation. 2018;118:S_524

Abstract

Mechanisms governing the trafficking of cardiac voltage dependent calcium channels ($Ca_V1.2$) remain largely unknown. We have previously shown that neuronal calcium channels are internalized into clathrin coated vesicles upon G protein coupled receptor (GPCR) activation. Here, we hypothesize that GPCR mediated signaling regulate $Ca_V1.2$ trafficking and surface expression through interaction with cytoskeletal and signaling molecules.

Methods: Sequential immunoprecipitation in adult rat cardiomyocytes was used to investigate the interaction between $Ca_V1.2$, cytoskeletal and signaling molecules. Live cell imaging and immunofluorescence microscopy were used to measure changes in the cellular localization of $Ca_V1.2$ and these proteins upon activation of GPCR signaling.

Results: Cav1.2 interacts with ANK2, βArr1 and spectrin. Sustained β adrenergic receptor (βAR) activation increases Cav1.2/spectrin and decreases $Ca_V1.2$/ANK2 and $Ca_V1.2$/βArr1 interactions. Sustained βAR activation induces rapid (<5 min) Cav1.2 internalization and dissociation from ANK2 as $Ca_V1.2$/ANK2 co-localization is markedly reduced (−58% $p<0.01$) in isoproterenol treated myocytes. A cell permeant peptide (1.4 μg/ml) that disrupts $Ca_V1.2$/ANK2 interaction decreases (−40±12%, $p<0.01$) Cav1.2 cell surface expression. Pretreatment with pertussis toxin prevents $Ca_V1.2$ internalization suggesting that $G_{i/o}$ mediates this response. Similarly, Src kinase inhibition reduces (by 90%) $Ca_V1.2$ internalization. Differential labeling of pre-existing $Ca_V1.2$ at the cell membrane and newly inserted $Ca_V1.2$ using fluorophore conjugated dihydropyridines reveal that activation of $G_{i/o}$ proteins underlies $Ca_V1.2$ internalization and insertion. In contrast, activation of the muscarinic M2 receptor (10μM carbachol) causes only insertion of new channels ($n=14$) and fails to alter the association of $Ca_V1.2$ with ANK2 and spectrin. Finally PI3 kinase inhibition (10nM wortmannin) prevents $Ca_V1.2$ membrane insertion.

Conclusions: Our findings reveal novel signaling mechanisms that regulate $Ca_v1.2$ trafficking, internalization and membrane insertion in a receptor subtype dependent manner. These mechanisms may be central to pathologies associated with a hyperadrenergic state.

This research has received full or partial funding support from the American Heart Association, AHA National Center.

Footnotes

Back to top