Characterizing the Interaction Sites Between the Cardiac Sodium Channel $Na_v1.5$ and Calcium Channel $Ca_v1.2$

Biomedical Sciences, HF

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1. Abstract

Cardiac excitability relies on the coordinated activity of ion channels such as the sodium channel $Na_v1.5$ and the calcium channel $Ca_v1.2$ [1]. Dysfunctions contribute to arrhythmogenic disorders, including Brugada syndrome. While a functional interplay of $Na_v1.5$ and $Ca_v1.2$ is suspected [2], their exact binding sites remain unclear. This study used Co-Immunoprecipitation, Proximity Ligation Assay, and Bioluminescence Resonance Energy Transfer to map these interactions. TsA-201 cells have been transfected with the corresponding DNA sequences. $Ca_v1.2$ was found to associate with three $Na_v1.5$ regions: the N-terminus, DI–II linker, and DII–III linker. PLA confirmed close proximity (<40 nm), and BRET suggested a possible direct interaction between the C-terminus of full-length $Na_v1.5$ and the N-terminus of full-length $Ca_v1.2$ at a proximity of <10nm. These findings support the concept of ion channel macromolecular complexes in cardiomyocytes and provide a basis for future studies on sodium–calcium channel cross-talk in arrhythmogenesis.

2. Introduction

Ion channels are essential for cardiac excitability and contraction [2]. $Na_v1.5$ initiates the action potential by mediating sodium influx, while $Ca_v1.2$ sustains the plateau phase through calcium entry [3]. Dysfunction of either channel contributes to arrhythmias and sudden cardiac death. Evidence suggests that $Na_v1.5$ and $Ca_v1.2$ interact, yet the specific binding regions remain undefined. Clarifying these sites is important for understanding ion channel macromolecular complexes.

3. Aims and Leading Questions

Aim 1: Detection of specific $Na_v1.5$ intracellular interdomain linkers interacting with $Ca_v1.2$ in transfected cells.

Aim 2: Investigation of direct or indirect interaction between $Na_v1.5$ and $Ca_v1.2$ in vivo.

Leading Questions:

- Which intracellular interdomain linkers of $Na_v1.5$ are responsible for the interaction with $Ca_v1.2$?
- How can different detection methods be interpreted and compared to confirm and support direct interactions in vivo?

4. Material and Methods

TsA-201 cells were cultured under standard conditions and transfected with $Ca_v1.2$ and $Na_v1.5$ constructs using the liposome-based reagent LipoD293. Plasmid DNA was prepared through cloning and MaxiPrep purification. Protein expression was validated by SDS-PAGE and Western blotting. To map specific binding regions of $Na_v1.5$, co-immunoprecipitation (Co-IP) with HA-tagged fragments was performed, followed by Western blot detection. To confirm these findings in situ, Proximity Ligation Assay (PLA) was used to visualize interactions within <40 nm in fixed cells. Finally, Bioluminescence Resonance Energy Transfer (NanoBiT system) was applied to assess direct interactions (<10 nm) between $Na_v1.5$ and $Ca_v1.2$ in live cells.

5. Results

The interaction between $Na_v1.5$ and $Ca_v1.2$ was analyzed using complementary approaches, yielding the following key findings:

Co-Immunoprecipitation (Co-IP): $Ca_v1.2$ interacted with three intracellular regions of $Na_v1.5$ – the N-terminus, DI–II linker, and DII–III linker. No interaction was observed with the DIII–IV linker or the C-terminal region. **Proximity Ligation Assay (PLA):** Confirmed close subcellular proximity (<40 nm) of $Na_v1.5$ and $Ca_v1.2$ in cells, validating the physiological relevance of the interactions identified by Co-IP. **BRET (live-cell assay):** Suggested a possible direct interaction between the C-terminus of $Na_v1.5$ and the N-terminus of $Ca_v1.2$, while other probe orientations showed weaker or no signals. **Summary:** The combined approaches demonstrate that $Na_v1.5$ interacts with $Ca_v1.2$ at multiple intracellular regions, supporting the concept of ion channel macromolecular complexes in cardiomyocytes.

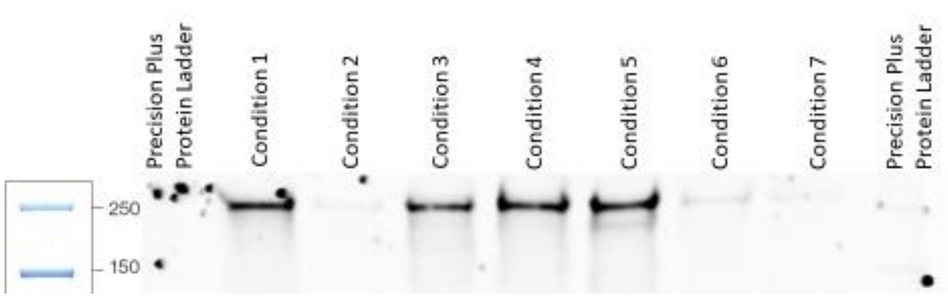


Figure 1: Co-immunoprecipitation of $Ca_v1.2$ with HA-tagged $Na_v1.5$ fragments detected by chemiluminescence. $Ca_v1.2$ co-precipitated with the N-terminal, DI–II, and DII–III linkers (Condition 3-5), but not with the DIII–IV linker or C-terminus. Full-length $Na_v1.5$ -HA served as positive control (Condition 1); untagged $Na_v1.5$ confirmed specificity (Condition 2).

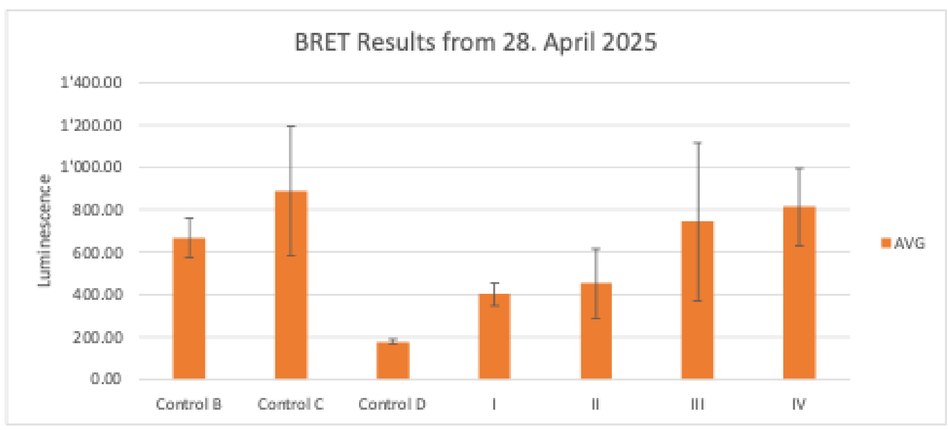


Figure 3: BRET assay results from 25. April 2025 (Run 2) with optimized control conditions. BRET luminescence values for conditions I–IV and refined controls B–D. Control A was excluded due to excessive signal intensity as in previous runs. Control B (SmBiT control vector + $Na_v1.5$-LgBiT N-terminal) and Control C (SmBiT control vector + $Na_v1.5$-LgBiT C-terminal) produced moderate to high signal intensities, indicating functional NanoBiT probe assembly. Control D (empty vector) shows background luminescence. Samples I–IV showed elevated signals compared to Control D, with conditions III and IV yielding the highest intensities, suggesting efficient reconstitution of NanoBiT components and close proximity between $Na_v1.5$ and $Ca_v1.2$ in live cells. Signal variation reflects differences in probe orientation and expression efficiency.

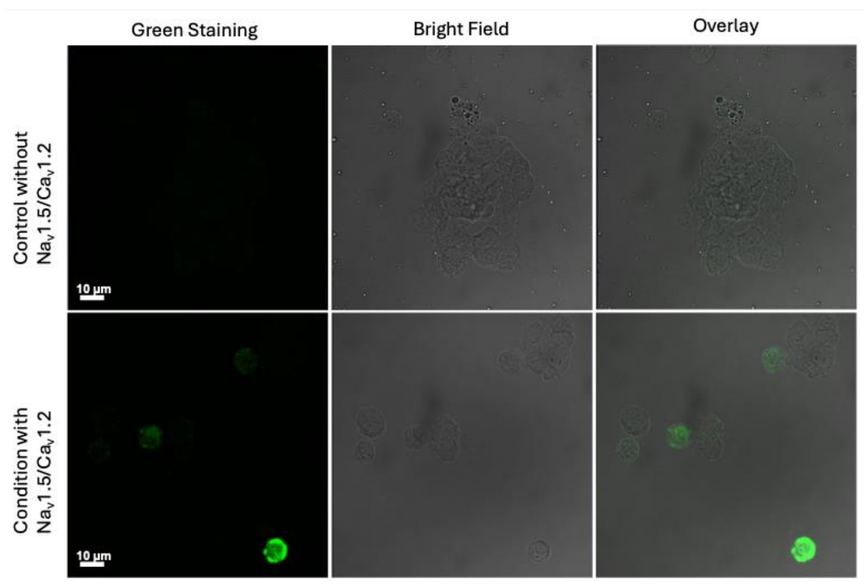


Figure 2: Representative Proximity Ligation Assay (PLA) of $Na_v1.5$–Cav1.2 interaction in TsA-201 cells. Controls lacking one or both channels showed no PLA signal. Co-expression of both channels produced distinct green spots (<40 nm), indicating specific interaction. Columns display bright-field, fluorescence, and merged images.

References

[1] Radwański, P. B., Lyu, Y., Payne, S. L., Trousdale, J., & Efimov, I. R. (2021). Sodium–calcium crosstalk in cardiac electrophysiology and arrhythmia. *Front. Physiol., 12,* 667284. https://doi.org/10.3389/fphys.2021.667284

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[3] Fu, Y., Westenbroek, R. E., Scheuer, T., & Catterall, W. A. (2016). Basal and β-adrenergic regulation of Cav1.2 requires phosphorylation of serine 1700. *PNAS, 113*(13), 3426–3431. https://doi.org/10.1073/pnas.1600733113

Figures

Figure 1. Co-immunoprecipitation (chemiluminescence) of Cav1.2 with HA-tagged Nav1.5 fragments – own data.

Figure 2. Representative Proximity Ligation Assay (PLA) of Nav1.5–Cav1.2 interaction in TsA-201 cells – own data.

Figure 3. Bioluminescence Resonance Energy Transfer (BRET) of full-length Nav1.5 and Cav1.2 in live cells – own data.

6. Discussion

This study demonstrates that $Ca_v1.2$ interacts with multiple intracellular domains of $Na_v1.5$, supporting the concept of ion channel macromolecular complexes. The convergence of Co-IP, PLA, and BRET strengthens the evidence that these interactions are not artifacts but occur under near-physiological conditions. Such interactions may play a role in arrhythmogenesis, as dysregulation of one channel could directly influence the other. Further studies in cardiomyocytes and functional assays are needed to define the physiological consequences, but these findings provide a molecular basis for future investigations into cardiac channelopathies such as Brugada syndrome.