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研究課題名（和文）Investigation on the arrhythmogenecity of Nav1.5-TRPM4 channel complex

研究課題名（英文）Investigation on the arrhythmogenecity of Nav1.5-TRPM4 channel complex

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研究成果の概要（和文）：心筋における重要なイオンチャネルであるNav1.5は、多様なタンパク質と相互作用し、チャネルの発現と機能を調整するという確かな証拠が存在する。本研究では、心筋におけるNav1.5とTRPM4のタンパク質複合体が実験的に確認された。さらに、ゲノム解析で特定された不整脈に関連するTRPM4チャネルの変異について、電気生理学的実験と数理モデルシミュレーションを通じて調査した。ゲーティング解析から得られたデータをプルキンエ線維のモデルに適用しシミュレーションを行ったところ、TRPM4変異チャネルの活性化と組織の不均一性が増すと、Nav1.5チャネルによる興奮伝導の異常が引き起こされる可能性が示された。

研究成果の学術的意義や社会的意義
これらの研究結果は、心筋細胞においてTRPM4とNav1.5がタンパク質複合体として存在することを示しており、膜タンパク質TRPM4チャネルの輸送障害や機能不全がNav1.5の機能にどのような影響を及ぼすか、つまり正常な心臓の興奮や伝導に乱れが生じる可能性について、興味深い示唆を与えている。

研究成果の概要（英文）：Compelling evidence suggests that the critical cardiac Nav1.5 channel interacts with various proteins, which regulate its expression and functionality. This study has shown a protein complex formation between Nav1.5 and TRPM4 within the myocardium. Additionally, we explored mutations in the TRPM4 channel, identified through genomic analysis of familial atrioventricular conduction block, using both experimental and numerical simulation approaches. By integrating the results from gating analysis into a Purkinje fiber cardiomyocyte model for simulations, we noted that the augmented activity of TRPM4 mutant channels, along with tissue heterogeneity, significantly modified Nav1.5 function, causing complex abnormalities in excitation propagation. These results offer valuable insights into how TRPM4 anomalies or malfunctions might affect Nav1.5 activity in cardiomyocytes, potentially leading to disturbances in normal cardiac excitation and conduction patterns.

研究分野：Cardiac electrophysiology

キーワード：TRPM4　Nav1.5　Arrhythmogenicity　Conduction disturbance

１．研究開始当初の背景

Despite significant advancements in understanding cardiac channelopathies, these conditions continue to pose challenges as complex genetic disorders with substantial morbidity and severe complications, both within Japan and worldwide. The pivotal role of mutations and malfunction of the Nav1.5 channel in conduction block disorders, long QT syndrome (LQTS), and Brugada syndrome (BrS) is widely acknowledged. Dysfunctions in Nav1.5 have the potential to disrupt the cardiac electrical system, contributing to the onset of severe arrhythmias. Furthermore, there is compelling evidence indicating that the cardiac Nav1.5 channel interacts with various proteins, regulating channel expression and function.
Emerging research suggests that the Ca^{2+}-activated cation channel TRPM4 may also contribute to cardiac arrhythmogenesis. Mutations in TRPM4 have been associated with a range of conduction abnormalities, including LQTS, BrS, and progressive familial heart block. Activation of the TRPM4 channel is believed to prolong action potentials (AP) or the QT interval, eliciting broader changes in cardiac electrophysiology observed in TRPM4 transgenic mice.
These findings raise intriguing possibilities regarding the potential impact of impaired TRPM4 trafficking or function in cardiomyocytes on Nav1.5 performance, potentially disrupting normal cardiac excitation and conduction.

２．研究の目的

In this study, building upon the aforementioned considerations, our objective is to delve deeper into the importance of the interplay between Nav1.5 and TRPM4 channels. Additionally, we seek to elucidate their respective roles in the development of cardiac arrhythmias.

３．研究の方法

Immuno-fluorescence, Duolink proximity and Co-immunoprecipitation experiment

HL-1 cells were cultured in Claycomb medium with 10% FBS on pre-coated cover slips. Following fixation with 4% para-formaldehyde and permeabilization, cells were immunolabeled and analyzed using confocal microscopy. The Duolink proximity ligation assay (PLA) was performed according to the manufacturer's instructions. Briefly, cells were fixed and incubated with primary antibodies, followed by addition of Duolink secondary antibodies conjugated to PLA probes. After rolling circle amplification, samples were mounted with DAPI-containing mounting medium and analyzed using confocal microscopy. Negative controls were included without primary antibodies. Protein extraction from cells was performed using IP lysis buffer supplemented with a protease inhibitor cocktail. Lysates were incubated with a Nav1.5 antibody, followed by pull-down with Dynabeads™. Finally, eluted proteins were analyzed via western blot.

Electrophysiology

In patch clamp experiments, borosilicate glass electrodes (4-6MΩ) were utilized alongside a high impedance, low noise patch clamp amplifier, controlled by the commercial software 'Patchmaster'. Data analysis and visualization were conducted using commercial software packages such as Origin 9 and Clampfit v.10.

Numerical model simulation

For single-cell action potential (AP) simulation, we utilized the latest human Purkinje fiber model code from the CELLML repository, run using OpenCOR. Corrections were made for unit definition inconsistencies. To incorporate TRPM4 channel kinetics and closely mimic the Trovato 2020 model AP time course, 40% of the original model's background Na conductance (I_{Nab}) was replaced by TRPM4 channel conductance and its mutants. For 1D-cable simulations, we employed the 'Chaste' simulation package with a 3 cm-long cable. In 2D-sheet (1x1cm, 0.02cm internodal space) simulations, evoked and spontaneously active HL-1 models were utilized, integrating TRPM4 channel kinetics and its mutants. Fibroblasts (CF) were randomly arranged at specified densities.

４．研究成果

Close Localization of TRPM4 and Nav1.5 Proteins in Cardiomyocytes

The co-localization of TRPM4 and Nav1.5 proteins was investigated using confocal immunofluorescence microscopy in HL-1 cardiomyocytes. To obtain more precise information about their interaction, the Duolink PLA assay was conducted. This assay allows for the

detection of protein targets with single-molecule resolution in cells and tissues. The Duolink dot signals indicate that TRPM4 and Nav1.5 proteins are located in close proximity, within a distance of less than 40 nm. Fluorescent images of HL-1 cells demonstrate the close proximity of endogenous TRPM4 and Nav1.5 proteins. Additionally, TRPM4 was detected in immunoprecipitates when pulled down with Nav1.5 antibody.

Gating Analysis Reveals That E7K and Q854R Mutation Facilitates TRPM4 Channel Opening

TRPM4 WT, E7K and Q854R mutant-mediated currents were recorded using Ion-C/A recording of heterologously expressed HEK293 cells, wherein a 5 μM ionomycin-containing Ca^{2+}-free solution was applied to permeabilize the cell membrane immediately after giga-seal formation. Subsequently, the membrane was exposed to solutions with varying concentrations of Ca^{2+} to activate the current.

To better understand the altered gating caused by the mutations, we calculated the rate constants of opening (α) and closing (β) based on the voltage- and Ca^{2+}-dependent Po-Vm and τ -Vm relationships using a two-state C-O transition model. α and β were empirically fitted using exponentials with first- or second-order Vm polynomials, respectively.

Reconstructed relationships of Po, α , and β versus Vm and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) indicate that the E7K and Q854R mutations render the channel more prone to opening at more negative membrane potentials and at lower $[Ca^{2+}]_i$ by enhancing the voltage and Ca^{2+} dependencies of α and decreasing those of β. In other words, these mutations accelerate the closed-to-open state (C-O) transition and simultaneously decelerate the reverse (O-C) transition of TRPM4 channels by facilitating voltage-dependent gating and increasing Ca^{2+} sensitivity.

Numerical Simulations Show That Preferred Opening by TRPM4 Mutation Causes Conduction Block

Computer simulations of single-cell action potentials (APs) demonstrate that the enhanced opening of E7K and Q854R-mutant TRPM4 channels more readily leads to AP prolongation as cell-membrane density increases, compared to the wild-type TRPM4 channel. When a 1D-cable model was utilized to simulate AP conduction, varying effects of the mutations were observed. The results depicted in the bottom left panel clearly show that while the conduction velocity (CV) is only minimally affected by 1- to 5-fold increases in wild-type TRPM4 channel density, the same degrees of density increase result in progressive reductions in CV for the E7K and Q854R mutants, ultimately leading to complete conduction block. This effect is more pronounced in Q854R than in E7K, particularly with faster pacing.

In 2D sheet simulations, augmenting the density of fibroblasts (CF) facilitates the fission, meander and fusion of excitation fronts, leading to heightened activity in the pacemaker region, as well as complete and partial blocks, and ectopic activities in the neighboring area.

Coupled with the findings on the proximity of TRPM4 and Nav1.5 proteins in cardiomyocytes, the aforementioned results shed light on the functional interplay, particularly the anomalous alterations induced by TRPM4 mutations. Integrating the modified gating of the E7K and Q854R mutant into a single-cell Purkinje fiber model illustrated that augmenting the channel density by 1 to 5-fold results in density-dependent AP prolongation, eventually leading to partial depolarization. Conversely, similar increases have minimal impact on the shape, duration of AP, and the resting membrane potential (RMP) in the wild-type TRPM4 channel. These divergent effects on AP and RMP are distinctly manifested in AP conduction. While the velocity of AP conduction along the 1D-cable remains relatively stable in the wild-type, it progressively diminishes with increasing density (maximal activity) of the E7K and Q854R mutant. The decrease in conduction velocity (CV reduction) correlates well with the reduction in dV/dtmax, which reflects the magnitude of the voltage-dependent Na channel (i.e. Nav1.5) current primarily contributing to AP upstroke. Although tissue-level complexity arises from local loading effects and structural discontinuities, this observation aligns with the general principle that the velocity of AP propagation correlates with the magnitude of local Nav1.5 current flow originating from the excited region. Additionally, reductions in dV/dtmax and CV coincide with depolarizing shifts of the membrane potential before AP upstroke, i.e., RMP. The level of RMP crucially determines Nav1.5 availability just before AP generation, and indeed, the extent of the observed RMP shift reasonably explains the decrease in Nav1.5 availability at AP upstroke, as estimated from its voltage-dependent inactivation curve. Overall, these findings offer compelling evidence supporting previous speculation that excessive gain-of-function mutation of TRPM4 channel activities under resting conditions would promote Nav1.5 inactivation during diastole, thereby slowing the generation and subsequent propagation of AP.

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掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/ijms241411798	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

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掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/ijms22168513	査読の有無 有
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〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関