NOVEL T-TYPE CALCIUM CURRENT PHARMACOLOGY SHOWS PROMISE IN THE STUDY OF ATRIAL FIBRILLATION

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Introduction The T-type calcium current (ICaT) has been postulated to contribute to the mechanism of pathological remodelling in atrial fibrillation. Studies targeting the T-type current pharmacologically have yielded disappointing results, most likely due to the poor specificity of these compounds. Furthermore, lack of specific pharmacology has limited progress in identifying the specific role that the T-type current plays in arrhythmogenesis. We report data demonstrating the pharmacology of novel compounds with relation to the T-type current in a heterologous expression system as well as primary cardiomyocytes. These compounds may have potential value in the investigation and/or treatment of atrial fibrillation.

Methods The human CACNA1G gene encoding ICaT mediating CaV3.1 was expressed in HEK 293 cells by plasmid transfection. The calcium current was measured by whole-cell patch clamp. The effects of the novel T-type antagonist Z944 and the agonists ST-101 and SAK3 were evaluated. Furthermore, primary atrial myocytes were obtained from murine atrial appendages. Combined calcium currents were recorded and the effects of the novel compounds above were evaluated. All statistical calculations were performed in the R statistical package and IV curves were fitted with MatLab 2020b (MathWorks Inc).

Results CaV3.1 facilitated a voltage-activated current that activated at -60 mV and peaked at -25 mV. The peak median current was 2.03 pA/pF (inter-quartile range 1.79). The current exhibited voltage inactivation with a time constant of 17.11±2.21 ms. 1 uM Z944 significantly attenuated the current by greater than 80% (p<0.001). Both agonists ST-101 (1 nM) and SAK3 (1 nM) left shifted-the voltage activation of the current and there was a trend towards increased peak current. In murine primary atrial myocytes, Z944 significantly attenuated the T-type component of the composite current-voltage (IV) curve whilst leaving the L-type component intact.

Conclusions The novel ICaT antagonist Z944 effectively blocks the T-type current in HEK293 cells and shows specific block with regards to the L-type current in murine atrial cells when measured by whole-cell voltage clamp. The agonists, ST-101 and SAK3 both left-shift the voltage-activation curve of CaV3.1. All three compounds likely have further utility in investigating the role of the T-type calcium current in relation to arrhythmia and may demonstrate therapeutic potential given their improved specificity when compared with previously used compounds.

CALMODULIN MUTATIONS ASSOCIATED WITH LONG QT SYNDROME IMPAIR L-TYPE CALCIUM CHANNEL REGULATION

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Introduction Long QT syndrome (LQTS) is an arrhythmic disorder with a prevalence of 1 in 2000, and is a leading cause of sudden cardiac death. Whilst many mutations associated with LQTS are found in cardiac ion channels, genetic screening of clinical populations has recently identified mutations in the Ca2+ sensing protein, calmodulin (CaM), however the molecular mechanism of CaM-mediated LQTS is not yet fully understood. CaM plays a key role in the regulation of multiple components of cardiac excitation-contraction coupling, including the L-type Ca2+ channel Cav1.2, where it mediates Ca2+-dependent inactivation (CDI) of the channel through interaction with its binding domains on the N- and C-termini. Ergo, CaM mutations that affect this interaction may result in a disease phenotype.

Methods Structural characterisation of CaM and two LQTS-associated CaM mutants was conducted through circular dichroism and 15N HSQC NMR to give insight into secondary structure in Ca2+-free and Ca2+-saturating conditions. Isothermal titration calorimetry was utilised to determine Ca2+-bound CaM binding affinity for peptides representing the CaM binding domains NSCaTE and IQ. Whole-cell configuration patch clamp electrophysiology of cells stably expressing Cav1.2 and transiently transfected with CaM produced insight
into the effects on regulation of L-type Ca2+ currents and CDI.

**Results**

LQTS-associated CaM mutants differentially altered Ca2+-bound, but not Ca2+-free, CaM structure, with an up to 30% reduction in \( \alpha \)-helical content. Interaction with NSCaTE and IQ peptides were also affected, with mutant A increasing affinity for the IQ domain by almost 2-fold, whilst mutant B exhibited a greater than 3-fold weaker binding to NSCaTE. Electrophysiological examination of Cav1.2 function revealed that CaM mutations dramatically impaired channel CDI, without significantly affecting the voltage dependence of activation and inactivation.

**Conclusions**

Together, these results demonstrate that disease-associated CaM mutations severely impact the structure-function relationship of CaM and its regulation of Cav1.2, through mechanisms unique to each CaM variant. This provides a crucial insight in to the molecular factors contributing to CaM-mediated arrhythmias. This work is funded by the Wellcome Trust PhD Studentship (to NG) and the British Heart Foundation Intermediate Basic Science Research Fellowship FS/17/56/32925 (to NH).

**GENOME-WIDE ASSOCIATION STUDY COUPLED WITH PROMOTER INTERACTOMIC DATA IDENTIFIES NEUROPLASTIN (NPTN) AS A POTENTIAL NOVEL GENE REGULATING HEART RATE**

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**Introduction**

Long-range chromosomal interactions bring distal regulatory elements to gene promoters to influence gene expression. Previously, we mapped the promoter interactome of cardiomyocytes derived from human embryonic stem cells (hESC-CMs) and contrasted these with undifferentiated hESCs. The promoter interacting regions in hESC-CMs (PIRs) overlapped significantly with GWAS signals associated with heart rate. One such locus located upstream of the HCN4 gene was identified (Figure 1). However this risk locus was found to maintain a promoter interaction with neuroplastin (NPTN) gene ~200kb away, rather than with HCN4, which is a key cardiac ion channel. In this study, we investigate the possible role of this NPTN promoter interacting region (NPTN-PIR) in cardiac rhythm.

**Methods**

First, we conducted data-mining using publicly available databases to identify the gene expression pattern of NPTN, cardiac expression quantitative trait loci (eQTLs) in the region, and phenotypes of NPTN-knock out mice. Second, we deleted the promoter interacting region (NPTN-PIR) in human embryonic stem cells (hESCs) to assess its effect on gene expression using CRISPR.

**Results**

Although NPTN is predominantly expressed in the brain, the gp55 (2.2kb) transcript of NPTN was expressed in the heart. Specifically, atrial appendages tend to have higher expression of NPTN than ventricular tissue. Cardiac eQTLs in the NPTN-PIR were significantly associated with NPTN but not HCN4 expression, supporting the occurrence of promoter interaction between the NPTN gene and the NPTN-PIR. Phenotypically, mice with NPTN gene knockout showed a significant difference in QT dispersion, an indication of arrhythmia risk. By deleting the NPTN-PIR in hESCs, we observed that the expression of NPTN was downregulated but HCN4 was unchanged (Figure 2). Upon cardiomyocyte differentiation, marker genes for atrial cardiomyocytes, such as NPPA, were downregulated in the differentiated cells without the NPTN-PIR, suggesting that atrial differentiation may be perturbed when the NPTN-PIR is absent and NPTN is downregulated.