Restoring the Function of hERG LQT2 N-terminal Mutants with Non-Canonical Amino Acids

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ABSTRACT

The human Ether-á-go-go potassium (hERG) channels produce the I_{Kr} current in the heart. This current drives the repolarization of membrane potential and terminates the action potential. Point mutations that introduce a TAG stop codon in hERG channels have been found in patients of LQT syndrome type 2. LQT2 syndrome patients have an increased risk of cardiac arrhythmias that may result in sudden cardiac arrest. We propose that the increased action potential duration (APD) that characterizes LQT2 can be explained by the loss of hERG function and thus a decreased I_{Kr}.

This work studies the effect of such mutations in the expression and electrical properties of hERG channels and the rescue of function by incorporating non-canonical amino acids (ncAAs) in hERG TAG LQT2 mutants. When transfected in HEK293 cells, hERG1a Q81X produces a functional channel with a faster deactivation rate and smaller currents than the wild-type channel, while S182X, E229X and W398X transfected cells do not produce measurable current. Then, with the use of an ortholog set of aaRS and tRNA, we incorporated L-ANAP, a ncAA in some of the hERG LQT2 N-terminal TAG mutants. The incorporation of the ncAA into hERG Q81X restores the wild-type phenotype of hERG currents and produces a functional hERG channel in cells transfected with hERG S182X and E229X, but not with W398X. We propose that the role of hERG TAG mutants in LQT2 can be due to a lack of channel expression, but also due to an effect in the channel kinetics and that the WT channel expression and function can be rescued by the incorporation of an ncAA.

hERG is a voltage activated K^+ Channel

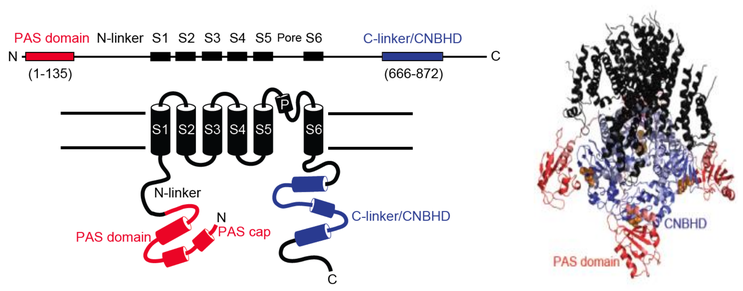

Figure 1. hERG channels are voltage-activated K^+ channels. They are tetramers where each subunit has six transmembrane (S1-S6) domains, a pore (P) domain which forms the ion conduction pathway and intracellular N- and C- terminal regions. hERG channels have a distinctive N- terminal PAS domain and a C-terminal CNBDH domain.

hERG has a characteristic slow deactivation that require the interaction of the PAS and CNBDH domains

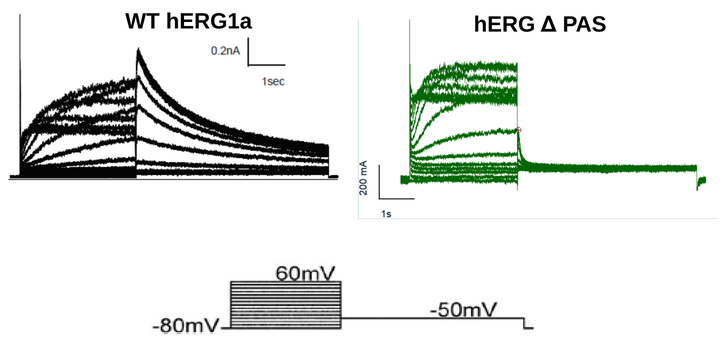

Figure 2. Representative family of currents elicited by series of voltage steps in HEK293 cells transfected with WT hERG1a or hERG Δ PAS. The voltage protocol applied in whole cell configuration is depicted below. WT hERG channels present a characteristic slow deactivation, regulated by direct interactions between the PAS and CNBDH domains. Deletion of the 135 amino acids corresponding to the PAS domain results in fast inactivation of the channel.

Long QT syndrome type 2

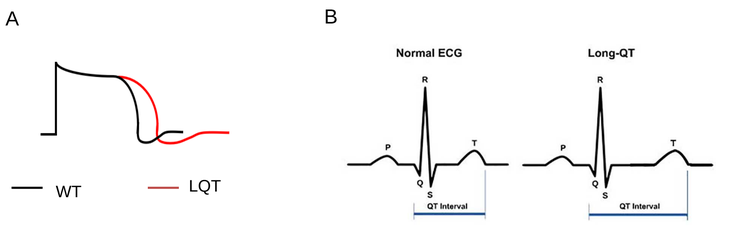

Figure 3. A. Schematic of the ventricular action potential (AP) between WT (black) and LQT (red) phenotypes. B. Schematic of electrocardiograms in WT (left) and LQT (right), showing the elongated QT interval.

hERG TAG mutants associated with LQT2 syndrome

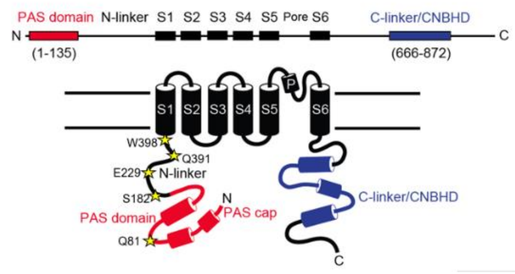

Figure 4. Point mutations in hERG channels that introduce a TAG stop codon have been linked to long QT syndrome type 2. The yellow stars in the hERG1a subunit diagram indicate the localization of TAG mutations located in the N-terminal: Q81X, S182X, E229X and W398X.

L-ANAP, a non-canonical aminoacid

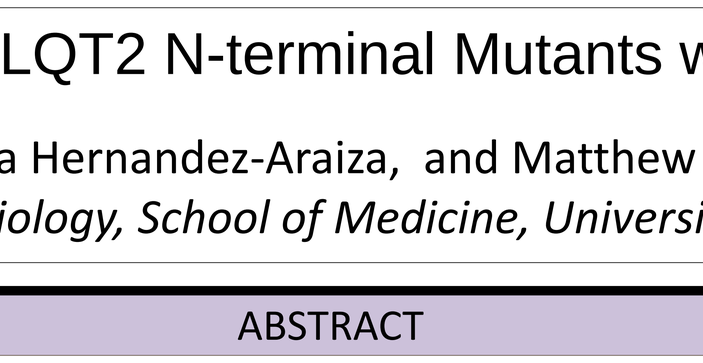

Figure 5. Schematic of the ortholog set of tRNA, and aminoacyl tRNA synthetase (RS) modified to introduce a ncAA using the amber stop codon (TAG). By transfecting HEK293 cells with the plasmids containing the ortholog set and the hERG TAG LQT2 mutants and exposing the cells to L-ANAP in the bath we can test the incorporation of the ncAA in the mutant channels and its effect on hERG currents.

hERG Q81X produces a truncated channel by iniciating translation at M124

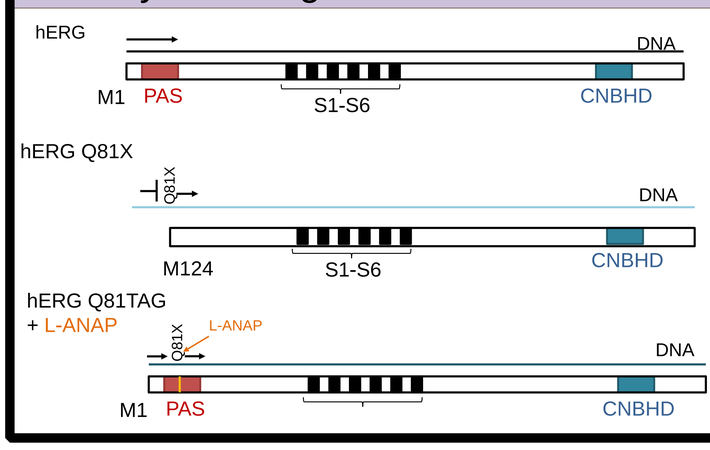

Figure 6. Scheme of DNA and proteins encoding (top) wild-type hERG (center) hERG Q81 TAG and (lower) hERG Q81 +L-ANAP. hERG can produce a truncated channel that starts at M124 and has fast deactivation similar to hERG ΔPAS. M1= initiating Met start site, M124= putative alternative Met start site.

Hypothesis

We hypothesize that LQT2-linked hERG TAG mutants present loss of function due to lack of protein translation or abnormal deactivation and that the WT current can be restored with the incorporation of ncAA such as L-ANAP.

L-ANAP incorporation rescues WT slow deactivation in **hERG Q81X**

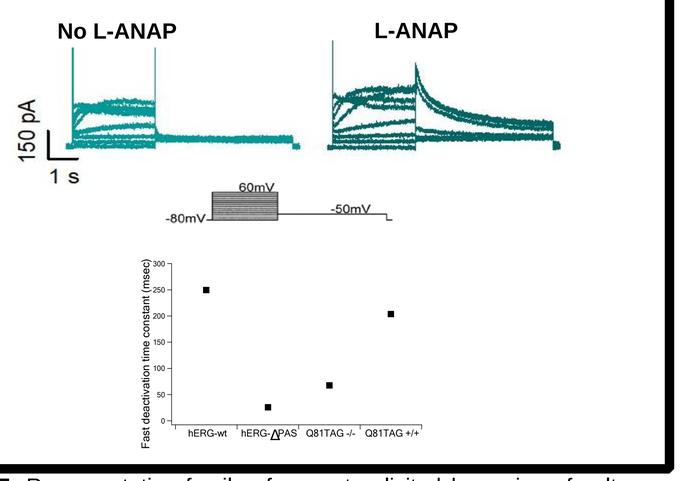

Figure 7. Representative family of currents elicited by series of voltage steps in HEK293 cells transfected with WT hERG Q81X. On the left, currents from cells incubated without L-ANAP, on the right, currents from cells incubated with L-ANAP in the cell culture media. In the absence of L-ANAP, hERG Q81X produces a channel with fast deactivation similar to that of hERG Δ PAS. When the HEK293 cells are incubated in the presence of L-ANAP the channel presents the fast deactivation typical of WT hERG.

L-ANAP incorporation rescues expression of some hERG TAG LQT2 mutants

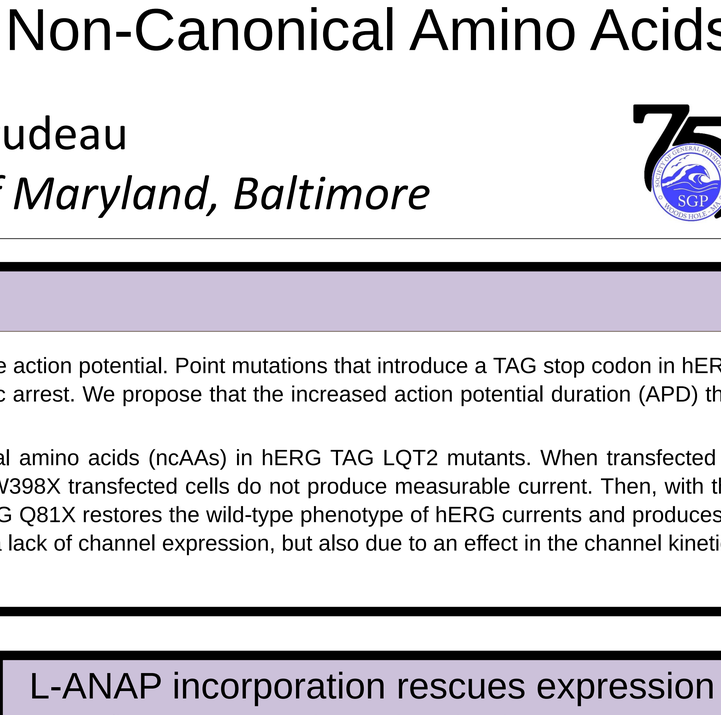

Figure 8. Representative family of currents elicited by series of voltage steps in HEK293 cells transfected with different hERG TAG LQT2 mutants and incubated with (right) or without (left) L-ANAP in the bath. L-ANAP incorporation produces S182X and E229X currents, but not W289X.

Rescue of hERG currents by incorporation of L-ANAP

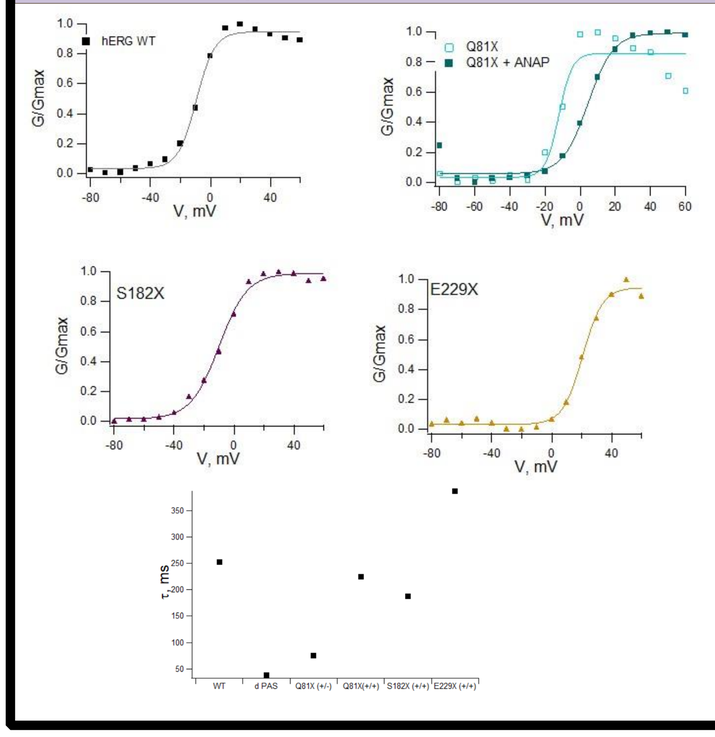

Figure 9. Comparison of GV curves and fast deactivation constants between WT hERG.and the hERG TAG LQT2 mutants that showed incorporation of L-ANAP.

Conclusions and further direction

The incorporation of a ncAA such as L-ANAP can rescue the WT current of hERG TAG LQT2 mutant channels in mammal cells. Further work will explore the possible incorporation of other ncAA such as BZF in the cases where L-ANAP did not incorporate.

A next step will be to test the incorporation of ncAA in cardiomyocytes derived from human induced pluripotent stem cells (CM-hiPSC) in which LQT2 TAG mutations have been incorporated by CRISPR. This model will allow us to determine if the recue of hERG WT currents correlate with a rescue of action potential duration.

References

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Acknowledgements

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