INVESTIGATING THE PATHOPHYSIOLOGY OF VARIOUS $NA_V1.4$ MUTATIONS IN PARAMYOTONIA CONGENITA AND THEIR SENSITIVITY TO DIFFERENT BLOCKERS

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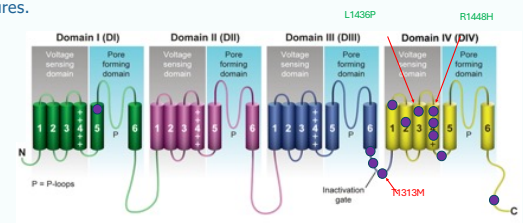
INTRODUCTION

- Myotonia is defined as a reduced ability to relax skeletal muscle after its contraction.
- Nondystrophic myotonias are a diverse group of rare neuromuscular disorders (incidence ~1:100.000) due to mutations in the genes coding for the muscle voltage-gated Na_v ($Na_v1.4$ encoded by *SCN4A*) or Cl- (ClC-1 encoded by *CLCN1*) channels. They result in various diseases, such as myotonia congenita **, paramyotonia congenita(PMC)**, hyperkalemic periodic paralysis with myotonia, and other rarer disorders.
- The term "para" in PMC reflects the fact that circumstances leading to myotonic episodes are different from those in classical myotonias.
- PMC patients have usually more severe myotonic episodes during exercise and cold temperatures.

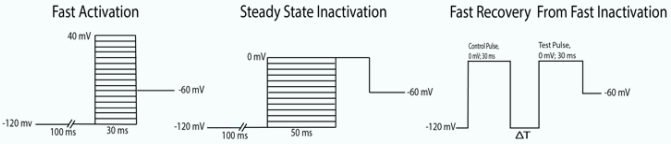


AIM

The main goal of our research was a full characterization of 2 (L1436P and R1448H) Nav1.4 mutants *in vitro*, including the temperature-dependence of the defect, and the evaluation of various Na_v blockers to find the drug best suited to reduce the consequences of the inactivation defect.

METHODS

- HEK293 cells were transiently or permanently transfected with human $Na_v1.4$ channel.
- A CsF based intracellular solution was used during whole cell patch clamping.
- Three protocols were used to characterize the channels as shown below.



RESULTS

1. Comparison of the biophysical properties of WT, L1436P and R1448H mutants

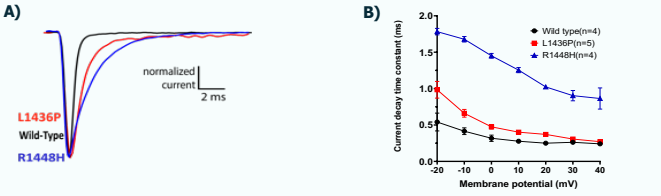


Figure 1: Na_v currents were recorded in HEK293T cells expressing WT(wild-type) or mutant hNav1.4 channels. **1.A** 1 representative cell is shown for each subtype. A step from -120 to -10 mV evokes a rapidly inactivating current with slower kinetics in the mutants. **1.B** Time constant of decay of Na_v currents elicited between −20 and +40 mV. Fit was mono-exponential. Note that the inactivation was less slowed down in L1436P than in R1448H.

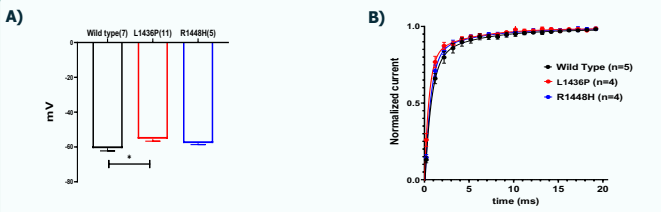


Figure 2.A: Voltage-dependence of short steady-state inactivation protocol (see above) shows a significant depolarizing shift of $V_{1/2}$ for the Nav1.4/L1436P mutation.
Figure 2.B: Fast recovery from fast inactivation for the different channel types (see protocol above). Although both mutants tended to recover faster, the difference did not seem to reach significance.

2. A cold temperature affects inactivation kinetics to a larger extent in the mutants

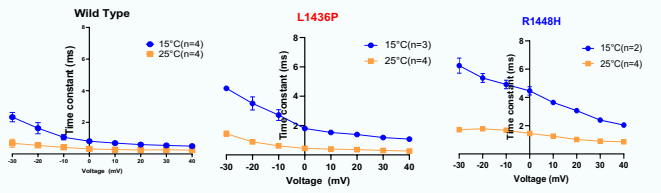


Figure 3: Temperature dependence of fast inactivation kinetics in wild-type and different mutations.

3. Flecainide and Lidocaine have different effects on the WT and mutants.

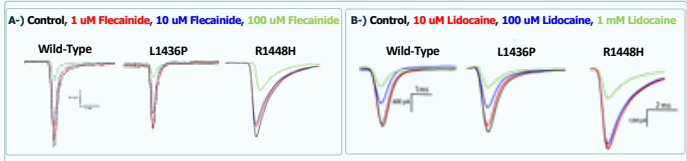


Figure 3: Raw Na_v current traces were recorded at -10 mV during the activation protocol in WT and mutant channels. Drugs were applied by superfusion during 3 minutes.

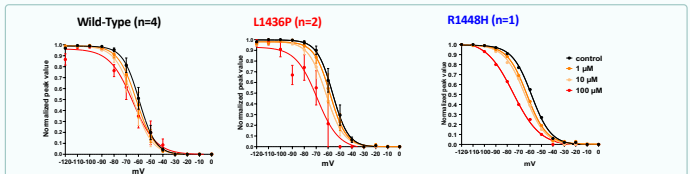


Figure 4: Concentration-response curves for **flecainide** on steady-state inactivation in the three subtypes. Data were fitted using a Boltzmann equation and $V_{1/2}$ were extracted (see table below).

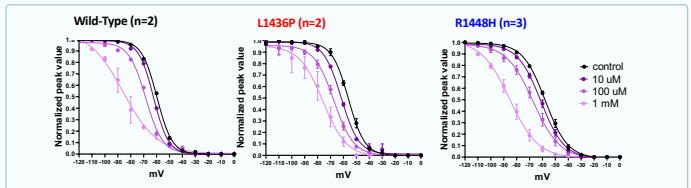


Figure 5: Concentration-response for **lidocaine** on steady-state inactivation. Note that this drug has a larger effect on this parameter than flecainide, as expected.

Flecainide	control	1 µM	10 µM	100 µM
Wild Type-fV50 (mV)	-59,4	-61,6	-64,0	-64,6
L1436P -fV50 (mV)	-56,2	-58,2	-61,6	-69,2
R1448H -fV50 (mV)	-58,6	-62,7	-64,3	-74,7

Flecainide seemed to have a larger effect on $V_{1/2}$ in mutants than in the WT.

Lidocaine	control	10 µM	100 µM	1 mM
Wild Type-fV50 (mV)	-59,6	-62,3	-68,3	-85,8
L1436P -fV50 (mV)	-56,1	-61,6	-67,4	-75,8
R1448H -fV50 (mV)	-57,7	-61,2	-67,2	-85,4

Lidocaine seemed more effective on the R1448H mutation than on L1436P at the highest concentration tested.

CONCLUSIONS

- The present study reveals the biophysical properties of the Nav1.4 L1436P mutant for the first time.
- We have started to assess the effectiveness of Na_v blockers on this mutant in comparison with WT and more common mutants.

PERSPECTIVES

- ❑ More work is needed to establish the efficiency of the different $Na_v1.4$ blockers in different mutations found in PMC patients.
- ❑ We plan to perform similar experiments with mexiletine, lacosamide and other drugs to find the most suitable drug for each mutation.
- ❑ The experiments will be extended to at least one other common mutation (T1313M).

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