Abstract: P265

Functional characterization of a LQT3 mutation located in the PY motif of the cardiac sodium channel associated with altered channel ubiquitylation

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Background: Congenital long QT syndrome type 3 (LQT3) is associated with gain-of-function mutations in SCN5A, the gene encoding the cardiac sodium channel Nav1.5, leading to an increased late sodium current (INa) and consequent action potential (AP) prolongation and arrhythmia. Ubiquitylation by the ubiquitin ligase Nedd4-2, which binds to the PY motif of Nav1.5, is known to regulate the internalization and degradation of the channel protein. We here investigated the biophysical properties of a LQT3-associated SCN5A mutation (p.Y1977N) located in the PY motif of Nav1.5.

Methods and results: HEK293 cells were transfected with wild type (WT) or p.Y1977N Nav1.5 in the presence of Nedd4-2. Pull-down experiments revealed that the interaction of Nav1.5 with Nedd4-2 was abolished by the mutation. Nedd4-2 decreased INa when co-expressed with WT Nav1.5 but had no effect on Y1977N-based INa. The in vivo relevance of this regulatory mechanism was assessed by generating a knock-in mouse line harbouring the mouse homolog p.Y1981N mutation homozygously. In hearts of adult Y1981N mice, total Nav1.5 protein levels were increased by 21±10% and ubiquitylation of Nav1.5 was reduced by 18±9% as compared to WT littermates. Nevertheless, Y1981N mice showed no alterations in ECG parameters in vivo or ventricular repolarization ex vivo. Patch clamp experiments performed in isolated ventricular cardiomyocytes revealed no differences in peak INa, late INa, AP upstroke velocity or AP duration between Y1981N and WT mice.

Conclusions: The LQT3-associated SCN5A mutation p.Y1977N located in the PY motif of Nav1.5 disrupts Nedd4-2 binding and consequent ubiquitylation. Nevertheless, mice carrying the murine homologous p.Y1981N mutation show no electrophysiological alterations or changes in INa, suggesting the presence of compensatory mechanisms. Hence, additional factors may be required to reduce the "ubiquitylation reserve" of Nav1.5 and unmask alterations in p.Y1981N channel degradation and (gain-of-)function. Future identification of these modulatory factors may identify potential triggers for arrhythmias and sudden cardiac death in the setting of this LQT3 mutation.