Abstract: P470

Microtubule plus-end tracking protein complex: a novel pharmacological target for modulating Nav1.5 trafficking and function

Authors:
GA Marchal¹, V Portero¹, S Casini¹, AO Verkerk¹, N Galjart², CA Remme¹, ¹Academic Medical Center of Amsterdam, Experimental Cardiology - Amsterdam - Netherlands, ²Erasmus Medical Center, Cell Biology and Genetics - Rotterdam - Netherlands,

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Background and purpose:
The cardiac sodium channel Nav1.5, is responsible for the fast upstroke of the action potential in cardiomyocytes and proper cardiac conduction. Nav1.5 is known to be distributed within cardiomyocytes in distinct subcellular domains, and is highly present at the intercalated disc. Our group previously showed that trafficking of Nav1.5 in cardiomyocytes to the membrane is mediated by the microtubule network. The microtubule plus-end binding protein CLIP-associating protein 2 (CLASP2) and End binding 1 (EB1) which are both mainly localized at the intercalated discs in adult cardiomyocytes, are important for microtubule recruitment. We here investigated the functional relevance of this microtubule plus-end tracking protein complex on Nav1.5 trafficking and function.

Methods and results:
We first studied the effect of overexpression of EB1 on sodium current in HEK293 cells transiently expressing human Nav1.5 (h-Nav1.5). EB1 overexpression significantly increased sodium current without affecting current kinetics. We next investigated the effect of pharmacological GSK3β inhibition, which is known to enhance interactions between microtubule plus-end binding proteins CLASP2 and EB1. HEK293 stably expressing h-Nav1.5 and adult murine cardiomyocytes were incubated with either 5μM of the GSK3β inhibitor SB216763 (SB2) or DMSO as a control. Overnight incubation of HEK293 cells stably expressing Nav1.5 with SB2 significantly increased sodium current density (DMSO: 487±32 pA/pF, SB2: 849±104 pA/pF) without affecting steady state activation and inactivation kinetic properties of the channel. In adult mouse cardiomyocytes, an increased sodium current density was observed after acute (2 hour) incubation with the inhibitor, as compared with DMSO treated cells (DMSO: 63±5 pA/pF, SB2: 78±7 pA/pF). Sodium channel kinetics were unaffected by SB2 treatment, suggesting that GSK3β inhibition modulates Nav1.5 trafficking.

Conclusion:
In this study we show that an increased expression and/or recruitment of the microtubule plus-end binding protein complex enhances sodium current. Pharmacological modulation of this complex by for instance GSK3β inhibition constitutes a promising approach for improving cardiac conduction.