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Title: Exploring the extracellular access pathway for charged local anaesthetics in voltage-gated sodium channels

Abstract in Englisch: Background
Voltage-gated ion channels are essential proteins for cellular excitability as they are necessary for generation of action potentials in neurons, as well as in cardiac and muscle cells. Action potentials trigger synaptic transmission in neurons, and contraction in muscle cells. These proteins belong to the family of p-loop channels, and most of the family members are important pharmaceutical targets for different classes of drugs. Although 3D crystal structures of some members of this family are available, for the physiologically and pharmaceutically important eukaryotic voltage-gated sodium channels the structure can only be predicted by homology models.
Our aim was to test if the traditionally used bacterial potassium channels, or the more recently described prokaryotic sodium channels provide better results in predicting the ion conducting pore structure of the mammalian skeletal muscle type sodium channel NaV1.4. To answer this question, we have used an indirect experimental approach. QX222 is a permanently charged, membrane impermeable quaternary amine analogue of lidocaine.
While the frequently used local anaesthetic lidocaine is believed to reach its binding site, which is located in the internal vestibule of the channel, through the cell membrane, QX222 can only access this binding site via a hydrophilic route across the channel protein. This pathway exists in the cardiac isoform (NaV1.5), but not in other isoforms. In addition, mutations have been shown to open such an external access pathway (EAP) in NaV1.2 and NaV1.4 e.g. (I1575A) (Ragsdale, McPhee, Scheuer, & Catterall, 1994; Sunami, Glaaser, & Fozzard, 2001), which is insensitive to extracellular QX222 application. Amino acids surrounding this pathway were mapped and modelled in several publications e.g. (Bruhova, Tikhonov, & Zhorov, 2008). Previous data from our lab suggested that two residues along this EAP directly contact each other (Zarrabi et al., 2010). One of them - I1575 – is located on the upper S6 transmembrane segment of domain IV, while the other – K1237 - is in on the p-loop of domain III and is important for the ion selectivity of the channel. In the first crystal structure of a prokaryotic sodium channel, NaVAb(Payandeh, Scheuer, Zheng, & Catterall, 2011), a tryptophan residue - W1531 - of the DIII p-loop is located between these two EAP lining residues. Therefore, we tested the hypothesis that mutations at site W1531 create an EAP.
Results: Whole-cell patch clamp measurements were performed on transiently transfected tsA201 cells. Extracellular QX222 (500 µM) blocked currents through W1531A and W1531G by 18±3% and 15±2%, respectively. In both constructs block development was extremely fast (time constants: ~3s and ~2s for W1531A and W1531G), i.e. ~10-20 fold more rapid than I1575A (~40s). Thus, mutations at site 1531 open an access pathway allowing for rapid block by QX222, as predicted from the crystal structure of NaVAb.
This new EAP was tested for its size and permeability for different ion species with electrophysiological and computational methods. The cavity created by the mutations was large enough to accommodate QX222 in the simulations, and experimentally the large tetra-methyl-ammonium ion was conducted by the EAP. The unique features of the novel mutations opening this EAP may be useful in the future for better understanding of binding of local anaesthetic drugs to the sodium channels.
Summary: We conclude that the use of crystal structures of prokaryotic sodium channels allows for better predictions of the pore structure of eukaryotic sodium channels than homology models based on crystallized potassium channels.