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MEETING ABSTRACT

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A structural motif that may serve as sealing of the outer vestibule of voltage-gated Na^+ and Ca^{2+} channels

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Background: In voltage-gated Na^+ and Ca^{2+} channels the extracellular part of the pore is lined by infolding loops connecting transmembrane segments V and VI of each of the four domains ("P-loops"). A highly conserved part of the P-loops is a ring of four tryptophan residues, each contributed by one of the four domains. The crystal structure of the bacterial voltage-gated Na^+ channel Na_VAb suggests that these residues establish hydrogen bonds with amino acids of a neighbouring subunit thereby stapling together adjacent subunits at the selectivity filter. Recently, we have shown that replacement of the domain-IV tryptophane in the rat skeletal muscle Na^+ channel $rNa_V1.4$, W1531, by glycine produces an external access pathway for charged local anesthetics to the receptor site located at the internal cavity. This pathway is conductive both for ions and for larger organic molecules [1]. This finding supports a role of the ring of tryptophans in structural stabilization of the external vestibule as suggested by the Na_VAb crystal structure [2]. However, as opposed to the homotetrameric assembly of Na_VAb, eukaryotic Na^+ channels are composed of four non-identical domains. Thus, we wanted to explore whether mutations of the tryptophans homologous to $rNa_V1.4$-W1531 in domains I–III had similar effects as $rNa_V1.4$-W1531G.

Methods: To this end we expressed the mutations W402G, W756G and W1239G in tsA201 cells using transient transfection, and tested the mutations by means of the whole-cell patch clamp technique.

Results: Unlike wild-type channels, all tested mutations were blocked by external application of the membrane-impermeable derivative of lidocaine, QX222. This suggests that the tested mutations created an external access pathway for QX222 to its binding site in the internal cavity of the channel.

Discussion: Thus, similar to the prokaryotic Na_VAb the ring of tryptophans may serve to stabilize the structure of the external vestibule in eukaryotic Na^+ channels.

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