Spider peptide toxin HwTx-IV engineered to bind to lipid membranes has an increased inhibitory potency at human voltage-gated sodium channel $Na_V1.7$

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The human voltage-gated sodium channel subtype 1.7 ($hNa_V1.7$) is emerging as an attractive target for the development of potent and subtype selective novel analgesics with increased potency and fewer side effects than existing therapeutics. HwTx-IV, a spider derived peptide toxin, inhibits $hNa_V1.7$ with high potency and is therefore of great interest as an analgesic lead. In the current study we characterized the membrane binding properties of HwTx-IV to determine whether increasing its interaction with lipid membranes would lead to an increase in the inhibitory potency of the peptide at $hNa_V1.7$. HwTx-IV analogues [E1PyrE]HwTx-IV (mHwTx-IV) and [E1G,E4G,F6W,Y30W]HwTx-IV (gHwTx-IV) were studied, and their ability to bind to model membranes binding was compared to that of HwTx-IV. Whereas HwTx-IV and mHwTx-IV exhibited weak binding affinity for lipid membranes, gHwTx-IV showed improved affinity for the model membranes studied regardless of the overall charge or fluidity of the lipids used. Furthermore, our results suggest that gHwTx-IV binds superficially to the lipid bilayer surface through electrostatic and hydrophobic interactions. In addition, activity assays using SH-SY5Y neuroblastoma cells expressing $hNa_V1.7$ showed that gHwTx-IV has increased activity at $hNa_V1.7$ compared to HwTx-IV revealing that an increase in the affinity for lipid membranes improves HwTx-IV potency. The correlation between membrane interactions and $hNa_V1.7$ inhibition was explored using a range of biophysical techniques including computational analysis, surface plasmon resonance, fluorescence spectroscopy and fluorescence imaging plate reader activity assays on HwTx-IV and the two analogues. Our results suggest that gHwTx-IV interacts with the lipid membrane *via* electrostatic interactions before binding to the channel and we hypothesize that increasing the affinity of gating modifier toxins to lipid bilayers is a strategy that may be useful for improving their potency at $hNa_V1.7$.