IDENTIFICATION AND CHARACTERIZATION OF THE NAV1.7 INHIBITORY PEPTIDE GPTX-1 FROM TARANTULA VENOM

Bryan D. Moyer, Dong Liu, Joseph Ligutti, Anruo Zou, Justin K. Murray, Les P. Miranda and Stefan I. McDonough

Amgen Inc., Thousand Oaks, CA

Peptides isolated from the venom of spiders, snakes, scorpions, and insects have provided important insights into many facets of ion channel biology including gating, function, and structure. To identify novel inhibitors of the voltage-gated sodium channel Nav1.7, an ion channel important for action potential generation in nociceptors and pain signalling, venom collected from the Chilean rose tarantula (*Grammastola porteri*) was fractionated and an active peptide, termed GpTx-1, was discovered. This presentation will describe the identification and characterization of GpTx-1 using automated and manual patch clamp electrophysiology approaches. GpTx-1 is a member of the inhibitory cysteine knot family of peptide toxins that rapidly inhibited human Nav1.7 and endogenous tetrodotoxin-sensitive (TTX-S) Nav currents recorded from mouse dorsal root ganglion neurons (IC50 ~ 5 nM). Using a panel of human Nav channels to evaluate the selectivity of peptide block, GpTx-1 was more potent on Nav1.3 (IC50 ~ 20 nM) and Nav1.4 (IC50 ~300 nM) but less potent on Nav1.5 (IC50 > 4 uM) and Nav1.8 (IC50 > 10 uM). Peptide SAR identified GpTx-1 analogs with increased selectivity against Nav1.4 (IC50 > 5 uM) that maintained Nav1.7 potency. Interrogation of a panel of Nav1.7 - Nav1.5 chimeric channels demonstrated that the voltage sensor region of domain II, comprising transmembrane domains 1-4, was required for GpTx-1 inhibition. High frequency depolarizations to +100 mV partially relieved hNav1.7 inhibition, suggesting lower peptide affinity for channel open states. GpTx-1 was not more potent when tested on partially inactivated Nav1.7 channels, suggesting interaction with a closed state. Voltage dependence of Nav1.7 activation was shifted ~10 mV in the depolarized direction with a subsaturating concentration of GpTx-1, indicating reduced Nav1.7 voltage sensitivity in the presence of peptide. In conclusion, the tarantula-derived GpTx-1 peptide is a potent Nav1.7 inhibitor that can be used to advance Nav1.7 ion channel biology.