Identification of Nav1.8 channel domains responsible for μO-conotoxin MrVIB binding and channel biophysical properties

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Voltage-gated sodium channels (VGSCs) are expressed primarily in excitable cells, such as central and peripheral neurons and muscle, and play a pivotal role in the initiation and propagation of action potentials. To date, nine subtypes of the pore-forming α subunit have been identified, each with a distinct tissue distribution, biophysical property and sensitivity to the neurotoxin tetrodotoxin (TTX). The 260 kDa α-subunits exhibit intracellular N- and C-termini and consist of four domains, each containing six membrane-spanning segments. $Na_v1.8$, a TTX-resistant subtype, is predominantly expressed in sensory neurons and plays a pathophysiological role in neuropathic pain. In contrast to TTX-sensitive α-subtypes, $Na_v1.8$ exhibits slower activation and inactivation kinetics and is inhibited by μO-conotoxin MrVIB from *Conus marmoreus* (Ekberg *et al.*, 2006). To determine which domain confers $Na_v1.8$ α-subunit its biophysical properties and MrVIB binding, we constructed various chimeric channels between $Na_v1.8$ and a TTX-sensitive $Na_v1.2$ that is expressed in the central nervous sytem. Wild type and chimeric channels were expressed in *Xenopus* oocytes and depolarization-induced Na^+ currents were recorded using the two-electrode voltage clamp technique. Slow inactivation kinetics of $Na_v1.8$ was changed to fast kinetics when domain 1 and 2 was replaced by the corresponding domains of $Na_v1.2$; slow activation kinetics remained unaltered. MrVIB (1 μM) inhibits $Na_v1.8$ currents by 80% whereas no significant effect was observed on $Na_v1.2$ currents. A similar sensitivity to MrVIB was observed for $Na_v1.2$ /1.8 chimeras containing $Na_v1.8$ domain 2. In contrast, $Na_v1.2$ /1.8 chimeras containing $Na_v1.2$ domain 2 were insensitive to MrVIB. Taken together, these results suggest that domain 2 of Nav1.8 is critical for MrVIB binding and activity. A previous study on $Na_v1.4$ reported that MrVIB hinders the voltage sensor in domain 2 from activating and, hence, the channel from opening (Leipold *et al.*, 2007). The binding of TTX may rely on a comparative mechanism since the presence of one or two binding sites within VGSCs $Na_v1.8/1.2$ chimeras had no effect compared to the wild type VGSC subtype $Na_v1.8$. All results were confirmed by a set of at least 10 cells/experiments.

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