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Invited review

Block of voltage-gated calcium channels by peptide toxins

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ABSTRACT

Venoms from various predatory species, such as fish hunting molluscs scorpions, snakes and arachnids contain a large spectrum of toxins that include blockers of voltage-gated calcium channels. These peptide blockers act by two principal manners - physical occlusion of the pore and prevention of activation gating. Many of the calcium channel-blocking peptides have evolved to tightly occupy their binding pocket on the principal pore forming subunit of the channel, often rendering block poorly reversible. Moreover, several of the best characterized blocking peptides have developed a high degree of channel subtype selectivity. Here we give an overview of different types of calcium channel-blocking toxins, their mechanism of action, channel subtype specificity, and potential use as therapeutic agents.

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1. Overview of voltage-gated calcium channels

Voltage-gated calcium channels are the major source of depolarization-evoked calcium entry into excitable cells of brain, heart, and muscle (Zamponi et al., 2015). This in turn supports many critical physiological functions that range from muscle contraction, to the release of neurotransmitters and calcium-dependent gene transcription, among many others. The mammalian genome encodes as many as ten different genes that lead to different types of Cav subunits, the principal pore forming subunit that forms the core of the calcium permeable ion channel (Simms and Zamponi, 2014). They have been grouped into three major classes (Cav1, Cav2 and Cav3) which in turn correspond to different types of calcium currents that have been identified in native tissues (for review, see Simms and Zamponi, 2014). The Cav1 family encodes four different types of L-type channels. Among the Cav2 family, Cav2.1, Cav2.2 and Cav2.3 correspond, respectively, to P/Q-type, N-type and R-type currents. The Cav3 family represents three different types of T-type calcium channels (also known as low-voltage activated channels due to their hyperpolarized voltage range of activation) (Catterall et al., 2005). Members of the Cav3 family are thought to be monomers whereas Cav1 and Cav2

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channels are multimeric complexes that also include a cytoplasmic Cavβ and an extracellular Cavα2δ subunit (Simms and Zamponi, 2014; Catterall et al., 2005). These ancillary subunits serve primarily to regulate calcium channel trafficking and function, but have also been shown to alter the pharmacological characteristics of the channels (Dolphin, 2016). All Cav subunits share a common transmembrane topology of four homologous transmembrane domains that each contain six membrane spanning helices plus a reentrant p-loop motif that forms the pore of the channel and imparts calcium selectivity (Catterall et al., 2005). The fourth transmembrane helix in each domain contains a positively charged amino acid residue in every third position and forms the voltage sensor of the channel (Catterall, 2010). The major domains are connected by large cytoplasmic regions, in addition to being flanked by intracellular N- and C-terminal regions. The domain I-II linker is the locus for Cavβ subunit interactions (Pragnell et al., 1994). The recent breakthrough resolution of the Cav1.1 channel structure at a nearly atomic level brings us a step closer to the understanding of the interface between the Cav subunit and ancillary subunits (Wu et al., 2015, 2016). Notably these studies revealed that Cavα2δ has a docking site formed by extracellular segments of domains I, II, and III, all organized with other extracellular loops into a dome structure above the selectivity filter. Whether this organization is common to other Cav subtypes remains to be determined.

The Cav subunit is the target for pharmacological agents that block (and in some cases enhance) calcium channel activity (Zamponi et al., 2015). This is relevant in the context of therapeutics, as calcium channel inhibitors have been used to treat disorders such as hypertension, pain and epilepsy (for review, see Khosravani and Zamponi, 2006; Waxman and Zamponi, 2014; Bourinet et al., 2014; Zamponi et al., 2015; Zamponi, 2016). Calcium channel blockers include small inorganic ions such as cadmium which act by occluding the permeation pathway (Lansman et al., 1986), small organic molecules such as dihydropyridines which have been used as a tool to identify native L-type calcium currents (Randall and Tsien, 1995), and larger peptide-based toxins that are isolated from a wide variety of venomous animal species. Here, we provide an overview of different peptide toxins that act on various members of the calcium channel superfamily, and touch on their therapeutic potential.

2. ω-conotoxins

The family of ω-conotoxins is derived from the venoms of a variety of different marine molluscs that use their venom to hunt fish. They are typically between twenty and thirty amino acids in size and display a rigid backbone structure that is spatially constrained by the formation of disulfide bonds formed between six conserved cysteine residues (Olivera et al., 1986, 1991; Lewis et al., 2012). Blocking affinity and channel subtype selectivity vary with amino acid sequence in the various loops between the cysteine bonds. In general, ω-conotoxins act by physically occluding the pore of the channel, thus preventing calcium influx. In many cases, binding is very tight, leading to slow dissociation rates and hence poorly reversible block (for example, Mintz et al., 1992; Boland et al., 1994; Ellinor et al., 1994).

One of the defining characteristics of Cav2.2 (N-type) calcium channels is their inhibition by ω-conotoxin GVIA, a 27 amino acid peptide isolated from the fish hunting mollusc *Conus geographus* (Olivera et al., 1994; McCleskey et al., 1987). GVIA block is exquisitely selective for Cav2.2 channels and virtually irreversible (Boland et al., 1994; McDonough et al., 2002), however, strong membrane hyperpolarization has been shown to accelerate the dissociation of this peptide from the channel (Stocker et al., 1997; Feng et al., 2003). Structure-function analysis based on chimeric calcium channel constructs has revealed that GVIA interacts with the outer vestibule of the pore comprised of the extracellular domain III S5–S6 (p-loop) region (Ellinor et al., 1994). Moreover, mutations in this region (especially a Glycine residue at position 1326) have been shown to affect not only the blocking rate constant, but also to have the capability to render the block reversible (Feng et al., 2001). These data fit with a structural homology model of Cav2.2 based on the structure of bacterial sodium channels (Lewis et al., 2012). This picture is likely to be refined in light of the recent structural data of the Cav1.1 channel in complex with a Cavα2δ subunit that is docked near the top of the channel pore (Wu et al., 2016). Such structural information may offer some mechanistic insights into why the Cavα2δ subunit affects blocking and unblocking rate constants for certain types of ω-conotoxins (Mould et al., 2004). The venom of *Conus geographus* also contains other related ω-conotoxins, such as GVIB, GVIC, GVIIA, and GVIIA, however they are not as well characterized at the electrophysiological level as GVIA (Olivera et al., 1994).

A number of calcium channel blocking peptides have been isolated from the venom of *Conus magus*, another fish hunting snail. This includes ω-conotoxins MVIIA, MVIIB, MVIIC, and MVIID (Hillyard, 1992; Olivera et al., 1994), which for the most part preferentially target Cav2.2 channels (Olivera et al., 1985; Monje et al., 1993; Fox, 1995). However, MVIIC also blocks Cav2.1 calcium channels and its blocking effects are reversible (Woppmann et al., 1994; Grantham et al., 1994), underscoring the point that small changes in amino acid composition between MVIIA and MVIIC are sufficient to alter channel subtype selectivity. The mode of action of these peptides is similar to that of GIVA, and they compete for a common interaction site on the channel (Olivera et al., 1994), although subtle differences in the way these toxins interact with the channel have been suggested (Woppmann et al., 1994).

MVIIA has received particular attention, as this peptide can be synthesized such that it retains its native conformation (Xiao and Bennett, 1995). This has allowed the exploration of this peptide as a therapeutic agent for pain, based on the underlying principle that the Cav2.2 channel plays a major role in the transmission of pain signals in the spinal dorsal horn (Bourinet et al., 2014). Intrathecal delivery of MVIIA (a.k.a. ziconotide or Prialt) mediates analgesic effects in both animals and humans. However, although Prialt is approved for treating intractable cancer pain in humans it has a narrow therapeutic window and the potential for causing severe CNS side effects (Antanassoff et al., 2000; Penn and Paice, 2000; Miljanich, 2004; Staats et al., 2004; Thompson et al., 2006; Wallace et al., 2006; Ver et al., 2008). The toxicity of MVIIA has recently been attributed to a methionine residue at position 12 of the toxin molecule, which is known to dock to a hydrophobic binding pocket comprised of residues I300, F302, L305 of the Cav2.2 subunit (Wang et al., 2016).

A number of additional ω-conotoxins have been identified in the venoms of *Conus fulman* and *Conus catus* and shown to mediate potent inhibition of Cav2.2 channels and to exhibit analgesic effects. This includes ω-conotoxins FVIA (a reversible N-type channel blocker; Lee et al., 2010), as well as CVIB (Motin et al., 2007), CVID (Lewis et al., 2000; Scott et al., 2002; Adams et al., 2003), CVIE and CVIF (Berecki et al., 2010). Interestingly, CVID and CVIE (but not CVIF) mediate analgesia in mice even after systemic administration (Sadeghi et al., 2013). Of further note, CVID has been advanced to human clinical trials where a larger therapeutic window was observed compared with Prialt (Schroeder et al., 2006), however, this peptide did to our knowledge not advance beyond phase II. Nonetheless, the larger therapeutic window has been attributed to the fact that CVID shows greater selectivity for Cav2.2 channels over Cav2.1.

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