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Invited review

Block of voltage-gated calcium channels by peptide toxins

Emmanuel Bourineta, Gerald W. Zamponib, * 

a Institute for Functional Genomics, CNRS UMR5203, INSERM U1191, University of Montpellier, LABEX I3ST, Montpellier, France 
b Department of Physiology and Pharmacology, Hotchkiss Brain Institute and Alberta Children’s Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary, Canada

Article info

Article history:
Received 8 September 2016
Received in revised form 14 October 2016
Accepted 15 October 2016
Available online xxx

Keywords:
Calcium channels
Conotoxin
Agatoxin
Protoxin
Spiders
Venom
Pain

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.

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

* Corresponding author.
E-mail address: zamponi@ucalgary.ca (G.W. Zamponi).
channels are multimeric complexes that also include a cytoplasmic Cavβ and an extracellular Cav2.2 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 Cav2.2 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 blockers 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; McCluskey et al., 1987). GVIA block is, virtually irreversibly for Cav2.2 channels (Berecki et al., 2010). Interestingly, CVID and CVIE (but not CVIF) mediate analgesia in both animals and humans. However, although Prialt has been 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 (Wall 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 PVIA (a reversible N-type channel blocker; Lee et al., 2010), as well as CVID (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 CVID) 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 (Schoeder et al., 2006), however, this peptide did 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.
The ω-conotoxins SIA, SVIA and SVIB have been isolated from Conus striatus venom. Although they are pore blockers, it has been suggested that their sites of action are distinct from those of GVIA (Ramilio et al., 1992). Moreover, similar to MVIC, SVIB reversibly blocks Cav2.2 and Cav2.1 channels (Woppmann et al., 1994; Nielsen et al., 1996).

The venoms of Conus Victoriae and Conus regis, respectively, contain α-conotoxins Vc1.1 and Rg1A. These peptides were classified as α-conotoxins in line with their action on nicotinic acetylcholine receptors (Clark et al., 2006; Nevin et al., 2007). Subsequently, potent inhibition by these toxins of Cav2.2 calcium channels was reported. However, both Vc1.1 and Rg1A do not act directly on the channel, but instead by activating GABA_b receptors (Callaghan et al., 2008; Callaghan and Adams, 2010; Cuny et al., 2012; Huynh et al., 2015). This is thought to occur by interference with the receptor dimer ectodomain interface (Adams and Berrecl, 2013), which then triggers inhibition of Cav2.2 channels via a G protein pathway. By doing so, the Vc1.1 peptide produces analgesia in rodent models of neuropathic and gastrointestinal pain, and reduces the excitability of human dorsal root ganglion neurons (Klimis et al., 2011; Castro et al., 2016). Vc1.1 is being explored as a possible pain therapeutic for humans, and this has been facilitated through the generation of a cyclized version of the peptide that remarkably allows oral delivery of Vc1.1 (Carstens et al., 2011).

While the majority of ω-conotoxins appear to target Cav2.2 and Cav2.1 calcium channels, there are some examples of L-type calcium channel blocking ω-conotoxins. For example, ω-conotoxin TxVII from Conus textile blocks L-type calcium channels from a freshwater pond snail, Lymnaea stagnalis (Fainzilber et al., 1996). Glaccontryphan-M, an unusual peptide isolated from the venom of Conus marmoreus contains γ-carboxyglutamyl (Gla) residues in positions 2 and 4 that bestow calcium binding ability onto this toxin. It has been shown to block L-type currents in mouse pancreatic beta cells (Hansson et al., 2004). Block, however, was found to be incomplete with ~35% inhibition at saturating concentrations. Substitution of the Gla residues with glutamate abolished block, as did removal of extracellular calcium.

3. ω-Agatoxins

The venom of the American funnel-web spider Agenepolipsis aperta contains a number of different neurotoxins, including the calcium channel blocking ω-agatoxins (Adams et al., 1993; Olivera et al., 1994). Among these, ω-agatoxin IVA has been used as an experimental tool to identify and distinguish native P- and Q-type currents (Adams et al., 1993; Llinas et al., 1986; Turner et al., 1992), with P-type currents showing an approximately ten-fold greater sensitivity to this toxin. Both P-type and Q-type currents are supported by different variants of Cav2.1 channels (i.e., different splice isoforms), and differential assembly with different types of ancillary Cavβ subunits (Bourinet et al., 1999; Meremelstein et al., 1999), with alternate splicing of an asparagine-proline motif in the domain IV S3–S4 linker greatly affecting blocking affinity (Bourinet et al., 1999). This region is implicated further in toxin block by a study that examined the role of a specific glutamate residue that appears to be critical for toxin block (Winterfield and Swartz, 2000). ω-agatoxin IVA is somewhat larger than the family of ω-conotoxins (i.e., 48 amino acids), but also has constrained backbone structure through formation of as many as four disulfide bonds (Olivera et al., 1994).

Unlike the ω-conotoxins, ω-agatoxin IVA does not cause pore block, but instead acts as a gating inhibitor (Fig. 1). Binding of the toxin antagonizes the activation of the channel by preventing the movement of the voltage sensor domains (Mintz et al., 1992; McDonough et al., 1997a; Bourinet et al., 1999), which fits the notion that toxin binding occurs at a region adjacent to the voltage sensor in domain IV. This inhibition can be overcome by application of strong depolarizing voltage pulses (Mintz et al., 1992). The voltage-induced reversibility of blocking action is also reflected in a massive (i.e., many tens of millivolts) shift in the apparent half-activation potential of the channel that is and likely due to disruption of the toxin from its binding site by virtue of outward voltage sensor movement (Mintz et al., 1992). Although ω-agatoxin IVA is selective for Cav2.1 channels, higher concentrations of this toxin also have the propensity to affect other members of the Cav2 channel family, including Cav2.2 (Sidach and Mintz, 2000) and Cav2.3 (Williams et al., 1994).

A much larger member of the ω-agatoxin family is ω-agatoxin IIIA (Venema et al., 1992; Olivera et al., 1994). It is nearly double in size compared to IVA (78 amino acid residues) and contains as many as 12 cysteine residues that are capable of six disulfide bonds (Olivera et al., 1994). This toxin has poor selectivity among the family of high-voltage activated channels, blocking L-type, P/Q-type and N-type channels, whereas T-type calcium channels appear to be spared (Mintz et al., 1991; Mintz, 1994). This toxin acts as a pore blocker, however, a residual current remains even at saturating concentrations. In Cav2.2 channels, this residual current is insensitive to ω-conotoxin GVIA, indicating that ω-agatoxin IIIA may act via incomplete pore occlusion (Mintz, 1994). In contrast, in P-type channels, ω-agatoxin IVA blocks this residual current, consistent with the idea that ω-agatoxin IVA does not require access to the pore of the channel (Mintz, 1994).

The American funnel web spider also produces two other ω-agatoxins, namely ω-agatoxin IA and II. The former appears to be specific for insect calcium channels, whereas the latter blocks both mammalian and invertebrate channels (Olivera et al., 1994). The two toxins differ in structure, with ω-agatoxin IA being a heterodimer with five disulfide bonds (Santos et al., 1992), and ω-agatoxin II displaying only three disulfide bonds.

4. Other toxins from spiders and scorpions

The venom of the tarantula Grammostola spatulata contains a calcium channel blocking peptide termed ω-grammotoxin SIA. This toxin acts on Cav2.1, Cav2.2 and Cav2.3 channels with micromolar affinity (Lampe et al., 1993; Piser et al., 1994, 1995; McDonough et al., 1997b; Turner et al., 1995). ω-grammotoxin SIA is a gating modifier whose action is reminiscent of that of ω-agatoxin IVA (McDonough et al., 1997a), such that it produces a massive shift in the half-activation potential of the channel. Interestingly, the effects of ω-grammotoxin SIA and ω-agatoxin IVA on Cav2.2 channels are additive, such that the apparent half activation voltage of the channels is shifted by as much as +150 mV when both toxins are present (McDonough et al., 1997b). This finding implies that the two toxins, although both acting by preventing voltage-sensor movement, target distinct sites or domains of the Cav2.2 subunit. Binding studies reveal that ω-conotoxin GVIA cannot displace ω-grammotoxin SIA from its binding site (Lampe et al., 1993), consistent with the notion that the latter is not a pore blocker.

Huwenotoxin-1 is a 33 amino acid peptide isolated from the spider Selenocosmia huwena. It has been shown to block N-type currents in NG108–15 cells with an affinity of approximately 100 nM (Peng et al., 2001). L-type calcium channels were also shown to be targeted by this peptide, but with much lower affinity. Low-voltage activated channels were not affected (Peng et al., 2001). Based on its folding pattern, Huwenotoxin-1 likely acts through a pore blocking mechanism similar to that of ω-conotoxin MVIIA.

Venom from the tarantula Hysterocrates gigas contains a calcium channel blocking peptide termed SNX-482 which is a potent inhibitor of Cav2.3 calcium channels (Newcomb et al., 1998). Like ω-
grammotoxin SIA and ω-agatoxin IVA, this toxin triggers a shift in half activation potential of Cav2.3 channels by approximately 70 mV, indicating that it too is an activation gating modifier. This peptide also blocks Cav1.2 calcium channels, albeit incompletely (Bourinet et al., 2001). Although its precise site of action on Cav2.3 channels is unknown, block appears to involve domains III and IV of the channel as revealed by chimeras between Cav2.3 and Cav1.2. These findings indicate that despite their high affinity for calcium channels, peptides trapping voltage-sensing domains have promiscuous effects on distinct channels. This has been well documented for voltage-gated sodium channels (Bosmans and Swartz, 2010). Along these lines, SNX482 was recently shown to potently affect Kv4.2 and 4.3 potassium channels, suggesting that caution is warranted in the interpretation of neurophysiological observations obtained with this peptide (Kimm and Ben, 2014). Nonetheless, SNX-482 was demonstrated to have interesting effects on the descending pathways controlling opiate analgesia and on reducing morphine tolerance to pain, an effect that is mirrored in the Cav2.3 KO mice (Yokoyama et al., 2004).

SNX-325 is a 31 amino acid long peptide found in the venom of the spider Segestria florentina that contains 6 cysteine residues (Newcomb et al., 1995). It preferentially blocks Cav2.2 calcium channels, but can target other members of the high-voltage activated calcium channel family at higher concentrations. Since it is able to displace ω-conotoxin MVIIA from its binding site (Newcomb et al., 1995) this toxin likely acts as a pore blocker.

The venom of the scorpion Parabuthus transvaalicus contains kurotoxin, a 63 amino acid peptide that was the first published example of a toxin acting on a member of the T-type calcium channel family (Chuang et al., 1998). However, subsequent studies have revealed that this peptide also targets members of the Cav1 and Cav2 channel families (Sidach and Mintz, 2002), in addition to voltage-gated sodium channels (Zhu et al., 2009). At least for Cav3 channels, kurotoxin acts as a gating modifier (Sidach and Mintz, 2002). The solution structure of kurotoxin reveals striking similarities to z-scorpion toxins, but somewhat different surface properties that may explain why this toxin acts on Cav3 channels in addition to sodium channels (Lee et al., 2012).

Finally, protoxins I and II, found in the venom of the Tarantula Theriopelma pruriens, are potent sodium channel blockers (Middleton et al., 2002; Schmalhofer et al., 2008; Xiao et al., 2010), however, they have also been shown to potently block Cav3 calcium channels (Edgerton et al., 2010; Bladen et al., 2014). Protoxin I preferentially blocks Cav3.1 over Cav3.3 channels with about one order of magnitude higher affinity (Ohkubo et al., 2010; Bladen et al., 2014). This toxin appears to interact with residues in the domain IV region with some contribution from sites located in domain II of the Cav3.1 channels. In contrast, Cav3.2 channels are blocked with about 100-fold lower affinity. There appears to be some conservation in the binding sites of this toxin on Cav3 and Nav channels (Bladen et al., 2014). In contrast, protoxin II acts preferentially on Cav3.2 calcium channels and causes a curious shift in the half-inactivation potential of the channel (Bladen et al., 2014) that remains to be further explored. Recent structure function experiments in which the voltage sensing domains of Cav3.1 channels were transferred individually onto a Kv2.1 backbone have revealed that protoxin II may preferentially target the voltage sensor in domain III of Cav3 channels (Salari et al., 2016).

Additional toxins have been isolated from the Parabuthus transvaalicus scorpion (i.e., KL1 and KLII) and the tarantula Theraphosidae (PsPTx3). The former two peptides block T-type channels and sodium channels (Olamendi-Portugal et al., 2002), whereas the latter preferentially targets T-type calcium channels, with some selectivity for Cav3.2 (E. Bourinet, unpublished observations, patent WO 2010081971 A1).

In summary, a number of calcium channel blocking peptides from various arachnids have been shown to block voltage-gated calcium channels. Many of these act as gating modifiers, however, the evolutionary significance of gating block as opposed to pore occlusion in peptide toxins from spiders versus cone snails remains to be determined.

5. Calcium channel blocking toxins from snakes

Black mamba (Dendroaspis polylepis) venom contains calciseptine, a 60 amino acid peptide (de Weille et al., 1991). This
Peptide has been shown to selectively block L-type calcium channels from brain and heart (de Weille et al., 1991), and was subsequently shown to compete with dihydropyridine binding to synthaposomes (Yasuda et al., 1993). Interestingly, this peptide appears to have an agonist function on Cav1.1 channels in skeletal muscle (García et al., 2001). FS2 is a related 60 amino acid peptide, also from black mamba venom, that differs from calciceptine in three amino acids. Like calciceptine, it potently blocks L-type calcium channels in smooth muscle (Yasuda et al., 1994).

Calcidecin is a 60 amino acid peptide isolated from the venom of the green mamba Dendroaspis angusticeps. It has been shown to block high-voltage activated calcium channels in cerebellar granule cells with high affinity (Schweitz et al., 1994), with L-type channels being most effectively inhibited. The effects of this toxin were also studied on heterologously expressed channels (Stotz et al., 2000), revealing several interesting features. First, block of Cav1.2, Cav2.1, Cav2.2 and Cav2.3 channels was found to be irreversible and incomplete at saturating concentrations, with Cav1.2 channels exhibiting the greatest degree of inhibition. Second, for Cav2 channels, the toxin induced a small hyperpolarizing shift in half-activation potential, leading to a current enhancement at negative membrane potentials. Finally, experiments with chimeric calcium channels revealed that multiple membrane domains are involved in toxin block. Calcidecin binding was subsequently shown to mediate allosteric coupling to the dihydropyridine binding site (Wang et al., 2007) in line with experiments involving calciceptine (Yasuda et al., 1993).

Altogether, mamba venom contains at least three related peptide toxins that remarkable all are 60 amino acids in length, and all act by inhibiting L-type channel activity.

6. How useful are calcium channel peptide toxins as therapeutics?

Peptide toxins isolated from predatory species such as cone snails have long been explored as possible therapeutics, and is exemplified by the fact that entire research conferences have been dedicated to this subject (for example, Venoms to Drugs). With regard to calcium channel blocking peptides, most effort has been focused on their utility as potential analgesics. This is due to the fact that these peptides, which do not normally cross the blood brain barrier and can thus typically not be administered systemically, can be delivered to key sites of action in the pain pathway intrathecally. The only clinically approved calcium channel blocking peptide so far is Prialt (ω-conotoxin MVIIA) (Staats et al., 2004). However, it can hardly be described as a blockbuster drug, and both the need for intrathecal delivery via an implanted mini pump and the limited therapeutic index is severely limiting (Ver et al., 2008). Modification of the peptide structure into cyclized versions may provide a means for oral delivery, and thus the potential for much wider application for clinical use in humans. However, although there is now proof of concept with Vc1.1, producing such orally available peptides without loss of specificity and affinity remains challenging. Nonetheless, in addition to having been established as important and selective tools for isolation of native calcium currents (Randanell and Tsiens, 1995), peptide toxins retain promise as possible therapeutic agents, as either drugs, or templates for small organic mimetics. The resolution of the Cav1.1 structure may help advance the discovery of such peptide mimetics.

Acknowledgments

Work in the Zamponi laboratory is supported by a Foundation grant from the Canadian Institutes of Health Research, and a Canada Research Chairs award. The Bourinet laboratory is supported by grants from the Fondation pour la Recherche Médicale and the Agence Nationale de la Recherche.

References


