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Spider-venom peptides that target voltage-gated sodium channels: Pharmacological tools and potential therapeutic leads

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ABSTRACT

Voltage-gated sodium (Na_V) channels play a central role in the propagation of action potentials in excitable cells in both humans and insects. Many venomous animals have therefore evolved toxins that modulate the activity of Na_V channels in order to subdue their prey and deter predators. Spider venoms in particular are rich in Na_V channel modulators, with one-third of all known ion channel toxins from spider venoms acting on Na_V channels. Here we review the landscape of spider-venom peptides that have so far been described to target vertebrate or invertebrate Na_V channels. These peptides fall into 12 distinct families based on their primary structure and cysteine scaffold. Some of these peptides have become useful pharmacological tools, while others have potential as therapeutic leads because they target specific Na_V channel subtypes that are considered to be important analgesic targets. Spider venoms are conservatively predicted to contain more than 10 million bioactive peptides and so far only 0.01% of this diversity been characterised. Thus, it is likely that future research will reveal additional structural classes of spider-venom peptides that target Na_V channels.

1. Introduction

More than 42,700 extant species of spiders have been described (Platnick, 1997), with an even greater number remaining to be characterised (Coddington and Levi, 1991), making them the largest group of terrestrial predators. One of the major contributors to the evolutionary success of spiders is their ability to produce complex venoms for predation and predator deterrence (King, 2004). Spider venoms are complex chemical cocktails but the major components are small, disulfide-rich peptides. Since a single venom can contain as many as 1000 peptides, it has been conservatively estimated that >10 million bioactive peptides are likely to be present in the venoms of spiders (Escoubas et al., 2006), with only 0.01% of this diversity having been characterised. The spider-venom peptides that have been described to date are detailed in ArachnoServer, a manually curated database that provides detailed information about proteinaceous toxins from spiders (Wood et al., 2009; Herzig et al., 2011).

Voltage-gated sodium (Na_V) channels play a key role in the rising phase of the action potential in excitable cells (Catterall et al., 2005). In contrast with vertebrates, insects express only a single Na_V channel subtype (King et al., 2008a) and consequently they are extremely sensitive to Na_V channel modulators, as underlined by the fact that several of the most successful classes of chemical insecticides are Na_V channel modulators (e.g., pyrethroids, indoxacarb, dihydropyrazoles, *N*-alkylamides, and DDT) (Soderlund and Knipple, 1995; Bloomquist et al., 1996; Zlotkin, 1999). One-third of the 186 ion channel modulators listed in ArachnoServer target Na_V channels (Fig. 1), which is perhaps not surprising as these toxins allow spiders to

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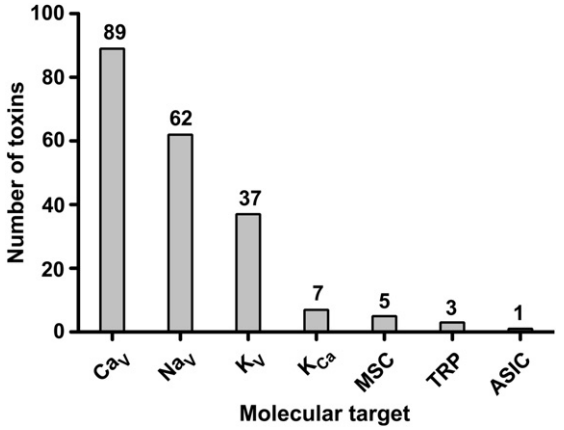

Fig. 1. Ion channel modulators from spider venoms. Molecular targets of the 186 ion channel toxins listed in ArachnoServer (www.arachnoserver.org; accessed February 12, 2012) (Herzig et al., 2011). The majority of toxins target voltage-gated ion channels (Ca_V, Na_V, and K_V), followed by calcium-activated potassium (K_{Ca}) channels, mechanosensitive ion channels (MSC), transient receptor potential (TRP) channels, and acid-sensing ion channels (ASICs). Note that some toxins target more than one type of channel, and therefore the cumulative number of toxins in the histogram is >186.

rapidly incapacitate their prey. Na_V channel toxins are found in a taxonomically diverse range of spiders, which suggests that this pharmacology was recruited at a very early stage in venom gland evolution. Moreover, the ubiquity of Na_V channel toxins in spider venoms suggests that these peptides might be useful insecticides for the control of arthropod pests (Maggio et al., 2010; Windley et al., 2012).

In addition to prey capture, spiders also use their venom to deter predators, which can include vertebrates. Moreover, even though the vast majority of spiders prey primarily on invertebrates, there is no evolutionary selection pressure to prevent spider toxins acting on vertebrate ion channels. It is therefore not surprising that many spider-venom peptides have been found to modulate the activity of vertebrate Na_V channels (Nicholson and Little, 2005; Escoubas and Bosmans, 2007; King et al., 2008a).

2. Na_V channels

In both vertebrates and invertebrates, functional Na_V channels are composed primarily of a large pore-forming α subunit (220–260 kDa) whose gating and kinetics are modified via association with one of four smaller β subunits (33–36 kDa) in the case of vertebrates or the unrelated TipE accessory subunit (65 kDa) in the case of invertebrates (King et al., 2008a). The α subunit is composed of four homologous but non-identical domains (designated I–IV) connected by intracellular linkers (Fig. 2). Each of these domains contains six transmembrane (TM) segments (S1–S6) joined by intracellular or extracellular loops. Membrane re-entrant loops between TM segments S5 and S6 dip into the TM region of the protein and form the narrow ion-selectivity filter at the extracellular end of the pore (Fig. 2). During action potential generation, the channel cycles through three states: closed, open, and inactivated. The S4 segments contain a positively charged amino acid (Arg or Lys) at every third position; this segment serves as a 'voltage sensor' that initiates voltage-dependent activation (i.e., the transition from the closed to open state) by responding to membrane depolarization and causing the channel to undergo a conformational change that allows selective influx of Na^+ ions through the pore. Sodium channel inactivation (i.e., the transition from the open to inactivated state) is mediated by a short intracellular loop termed the "inactivation gate" that connects domains III and IV (Fig. 2). In a major breakthrough, the three-dimensional structure of a bacterial Na_V channel in the closed state was recently determined (Payandeh et al., 2011), which should allow molecular modelling of the interaction between Na_V channels and ligands that modulate their activity.

In vertebrates, the α subunits are classified into nine different subtypes, denoted $Na_V1.1$ to $Na_V1.9$, and they are further characterised by their sensitivity to tetrodotoxin (TTX). $Na_V1.5$, $Na_V1.8$ and $Na_V1.9$ are TTX-resistant, whereas all other subtypes are TTX-sensitive (Catterall et al., 2005; King et al., 2008a). A combination of site-directed mutagenesis, binding studies, and electrophysiological approaches have been used to identify at least seven distinct ligand binding sites in or around the pore region of the Na_V channel α subunit, as shown in Fig. 2. The majority of these sites have been identified using venom molecules and alkaloids as pharmacological probes (King et al., 2008a). Venom peptides modulate channel activity by two main mechanisms: they either physically occlude the channel pore or they bind to an allosteric site that induces a conformational change in the channel that alters the equilibrium between the open, closed, and inactivated states (Ekberg et al., 2008).

All known Na_V channel toxins from spider venoms are allosteric modulators, also known as "gating modifiers". However, they modulate channel activity in quite different ways. For example, the lethal toxin from the Sydney funnel-web spider *Atrax robustus* (δ-HXTX-Ar1a) inhibits the *inactivation* of both insect and vertebrate Na_V channels (Nicholson et al., 1998; Grolleau et al., 2001), whereas the μ-agatoxins from unrelated American funnel spiders shift the voltage for channel *activation* to more negative potentials, increasing the probability of channel opening at the resting membrane potential (Adams, 2004).

3. Spider toxins that target Na_V channels

The primary aim of this review is to survey the range of peptide sequences and three-dimensional scaffolds that have been evolved by spiders for targeting Na_V channels, with an emphasis on those peptides that might have therapeutic potential. The 'Browse by Molecular Targets' ontology in ArachnoServer (Wood et al., 2009; Herzig et al., 2011) was used to extract all known spider-venom peptides with activity on Na_V channels (both vertebrate and invertebrate). Homologues of these toxins were found by using the BLAST function within