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Sea anemone toxins affecting voltage-gated sodium channels – molecular and evolutionary features

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ABSTRACT

The venom of sea anemones is rich in low molecular weight proteinaceous neurotoxins that vary greatly in structure, site of action, and phyletic (insect, crustacean or vertebrate) preference. This toxic versatility likely contributes to the ability of these sessile animals to inhabit marine environments co-habited by a variety of mobile predators. Among these toxins, those that show prominent activity at voltage-gated sodium channels and are critical in predation and defense, have been extensively studied for more than three decades. These studies initially focused on the discovery of new toxins, determination of their covalent and folded structures, understanding of their mechanisms of action on different sodium channels, and identification of the primary sites of interaction of the toxins with their channel receptors. The channel binding site for Type I and the structurally unrelated Type III sea anemone toxins was identified as neurotoxin receptor site 3, a site previously shown to be targeted by scorpion α-toxins. The bioactive surfaces of toxin representatives from these two sea anemone types have been characterized by mutagenesis. These analyses pointed to heterogeneity of receptor site 3 at various sodium channels. A turning point in evolutionary studies of sea anemone toxins was the recent release of the genome sequence of *Nematostella vectensis*, which enabled analysis of the genomic organization of the corresponding genes. This analysis demonstrated that Type I toxins in *Nematostella* and other species are encoded by gene families and suggested that these genes developed by concerted evolution. The current review provides a brief historical description of the discovery and characterization of sea anemone toxins that affect voltage-gated sodium channels and delineates recent advances in the study of their structure-activity relationship and evolution.

1. Introduction

Sea anemones (Actinaria, Cnidaria) are ancient sessile predators (Chen et al., 2002) that heavily depend on their venom for survival (Ruppert and Barnes, 1994). They immobilize their prey or deter their foe by using cells called nematocytes for stinging and delivery of venom. Analysis of the venom in many sea anemone species uncovered a rich repertoire of low molecular weight compounds such as serotonin and histamine (Beress, 1982), ~20 kDa pore-forming polypeptide toxins (Kem, 1988; Anderluh and Macek, 2002), 3.5–6.5 kDa polypeptide toxins active on voltage-gated potassium channels (Castaneda et al., 1995; Schweitz et al., 1995; Gendeh et al., 1997; Yeung et al., 2005) and 3–5 kDa polypeptide toxins active on voltage-gated sodium channels (Beress et al., 1975; Rathmayer and Beress, 1976; Honma and Shiomi, 2006). The combined effects of these compounds has apparently been successful over hundreds of millions of years as is evident by the ability of sea anemones to colonize and thrive in a wide variety of ecological niches. Moreover, the ever changing environment and appearance of new species has probably enforced diversification of toxins in sea anemones. Indeed, not only can a variety of toxin configurations be found in their venom

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(Honma and Shiomi, 2006), but it has been shown that each toxin is encoded by a gene family (Moran et al., 2008a).

The toxins active on voltage-gated sodium channels are abundant in all sea anemone venoms assayed to date, and are present even in rare and highly unique species (Ishida et al., 1997; Moran and Gurevitz, 2006). The abundance of these toxins and the fact they constitute a major fraction of the proteinaceous content of the venom (Beress et al., 1975) points to their major role in predation and defense.

2. The voltage-gated sodium channel as a prime target for sea anemone toxins

Voltage-gated sodium channels (Na_vs) have a pivotal role in excitability of most animals as they enable the initiation and propagation of action potentials. These channels are transmembrane complexes composed of a highly conserved pore-forming α-subunit and auxiliary subunits such as β-subunits in vertebrates (Catterall, 2000) and TipE subunits in insects (Feng et al., 1995). The ~260 kDa α-subunit protein is composed of four homologous domains (D1–D4), each comprising six transmembrane segments (S1–S6; Fig. 1). Nine genes encoding the channel α-subunit exist in the human genome. These genes share >50% identity and in many instances their expression is temporally and spatially regulated in tissues or cells (Goldin, 2002).

Due to their crucial role in excitability Na_vs have become prime targets for a wide variety of toxins. Seven neurotoxin receptor sites have been elucidated thus far on Na_v α-subunits (reviewed in Gordon et al., 2007; Blumenthal and Seibert, 2003). Four of these sites, formed by the extracellular loops that connect the transmembrane segments of the α-subunit, are recognized by water-soluble peptide toxins. Receptor site 1 is targeted by μ-conotoxins from venomous cone snails (Terlau and Olivera, 2004); receptor site 3 is targeted by toxins from sea anemones, scorpions and spiders (Catterall and Beress, 1978; Gordon and Zlotkin, 1993; Rogers et al., 1996; Little et al., 1998); receptor site 4 is targeted by toxins from scorpions and spiders (Cestèle and Catterall, 2000; Corzo et al., 2005); and receptor site 6 is targeted by δ-conotoxins from venomous cone snails (Fainzilber et al., 1994; Terlau and Olivera, 2004). Aside from μ-conotoxins, all these toxins affect the gating properties of the Na_v, either activation (receptor site 4; Cestèle and Catterall, 2000) or inactivation (receptor sites 3 and 6; Ulbricht, 2005), and therefore are called 'gating modifiers'. The classification of toxins to pharmacological groups is based on their mode of action and ability to displace one another in binding competition assays (Jover et al., 1978; Catterall, 2000; Gordon, 1997).

3. The discovery of sea anemone toxins active on voltage-gated sodium channels

Crude extracts from the tentacles of the sea anemone *Anemonia sulcata* (today named *Anemonia viridis*) were first prepared in 1902 and shown to cause anaphylaxis in dogs (Richet, 1902). The paralytic and lethal activities of a sea anemone venom were investigated in more detail by

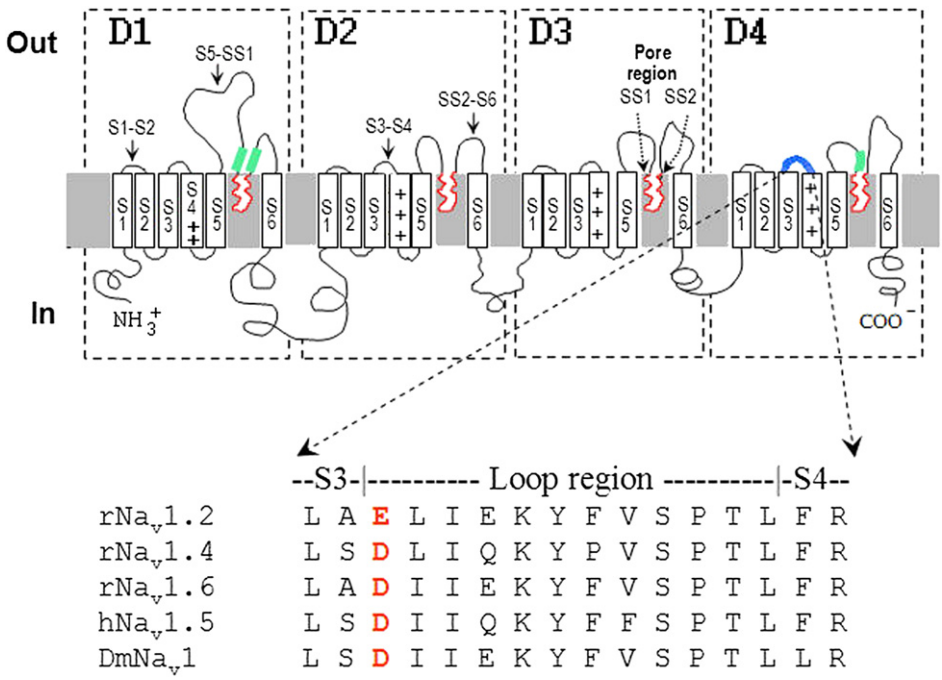

Fig. 1. Schematic illustration of the sodium channel α-subunit and regions that are putatively associated with receptor site 3. Segments SS1 and SS2 form the pore region (in red), around which the four homologous domains (D1–D4) assemble. The main region identified as affecting site 3 binding is loop S3–S4 in D4 (Rogers et al., 1996; Benzinger et al., 1998) marked in blue. Channel regions (D1/S5-SS1, D1/SS2-S6, D4/S5-SS1) whose blocking by antibodies affected Lqq5 binding (Thomsen and Catterall, 1989) are marked in light green. Multiple sequence alignment of D4/S3–S4 from $DmNa_v1$ of *Drosophila melanogaster* and several mammalian sodium channel subtypes is presented at the lower panel. The negatively charged residue equivalent to Glu-1613 in $rNa_v1.2a$ (Rogers et al., 1996) is in bold red.