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Toxicon 49 (2007) 181–193

TOXICON

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Site-3 toxins and cardiac sodium channels

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Available online 27 September 2006

Abstract

Site-3 toxins are small polypeptide venoms from scorpions, sea anemones, and spiders that bind with a high specificity to the extracellular surface of voltage-gated Na channels. After binding to a site near the S4 segment in domain IV the toxin causes disruption of the normal fast inactivation transition resulting in a marked prolongation of the action potentials of excitable tissues including those of cardiac and skeletal muscle and nerve. In this review we discuss the specific binding interactions between residues of the toxin and those of the Na channel, and the specific modification of Na channel kinetic behavior leading to a change in fast inactivation focusing on interactions deduced primarily from the study of sea anemone toxins and the cardiac Na channel ($Na_V1.5$). We also illustrate the usefulness of site-3 toxins in the study of altered Na channel behavior by drug-modification.

Keywords: Na channel; Gating currents; Inactivation

1. Introduction

An extremely interesting set of toxins, named site-3 toxins (Catterall, 1980), bind to the extracellular surface of voltage-gated Na channels and produce profound slowing of current decay. Site-3 toxins have been particularly useful in helping understand the structural basis of Na channel gating, and we will review them with particular attention to their effects on the cardiac isoform ($Na_V1.5$). Members include the 60–70 residue α-scorpion toxins (from *Leiurus quinquestriatus, Centruroides sculpturatus, Tityus serulatus*, and *Androctonus australis* and others for which a database is available (Srinivasan et al., 2002)) as well as several peptide toxins isolated from sea anenome, including Anthopleurin A (Ap-A) and Anthopleurin B (Ap-B), which were isolated from *Anemonia xanthogrammica* (49 aa), and the *Anemonia sulcata* toxins (ATXI [46 aa], ATXII [47 aa], and ATXIII [27 aa]) (Norton, 1991) as well as a several toxins isolated more recently from spiders, including several from funnel spiders, e.g. *Hadronyche versuta* (Little et al., 1998a, b) and *H. versuta*, (Fletcher et al., 1997), as well as from *Plectreurys tristis* (Corzo et al., 2003) and *Phyllodiscus semoni* (Xiao et al., 2005) (for a review see Nicholson and Graudins, 2002). In addition to an excellent summary published in 1991 (Norton, 1991), two recent reviews are also noteworthy (Blumenthal and Seibert, 2003; Honma and Shiomi, 2006). Also, a recent brief review gives additional details of the various identified spider toxins (Nicholson and Graudins, 2002).

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0041-0101/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2006.09.017

2. Early investigations

The anemone toxins were first identified in 1976: (1) by noting skeletal muscle cramps and paralysis after injection of venom in crabs (Beress et al., 1976); (2) after superfusion onto isolated cardiac muscle strips with resultant increase in inotrophy associated with action potential prolongation (Shibata et al., 1976); (3) by noting extracellular but not intracellular application of toxin-caused inhibition of I_{Na} decay in frog node of Ranvier (Bergman et al., 1976b). Initially, the idea that Na channels were represented by multiple isoforms was not appreciated, although it was noted that several of the site-3 toxins exhibited specificity for cardiac preparations. These included ATXII from *Anemonia sulcata* (Beress and Beress, 1975) and Ap-A, isolated from *Anemonia xanthogrammica* (Norton et al., 1976). In addition, several others have more recently been identified, including Jinghaotoxin I (Xiao et al., 2005) while others have been described that specifically target insect/crustacean channels (Salgado and Kem, 1992).

Early data not only reported very different apparent affinities between preparations but even within a single preparation, which depended on the technique used for the assay. For example, ED_{50}'s for ATXII based on binding/flux in rat brain synaptosomes were estimated to be 150–240 nM, (Vincent et al., 1980) while voltage clamp affinities in myelinated nerve were estimated to be 30–100 fold lower, i.e. ED_{50}'s at 5 μM (Schmidtmayer et al., 1982) and 20 μM. (Bergman et al., 1976a). It was then recognized that the action of the toxin was specific for Na channels, and that these toxins have a low affinity for the inactivated state of the channel because of voltage-dependent binding (Wang and Strichartz, 1985; Strichartz and Wang, 1986; Warashina et al., 1988). In binding and flux studies site-3 toxins were used in combination with toxins that promoted activation (usually veratridine) where binding and/or flux was augmented (Lawrence and Catterall, 1981; Frelin et al., 1984). Such data were interpreted to indicate a synergistic effect of the two agents (Corbett and Krueger, 1989), or more often a high affinity of the open state (or an open-like state) for toxin, a conclusion also suggested by early patch clamp studies (Schreibmayer et al., 1987b), but questioned by others in which other promoters of the Na channel open state were unable to mimic the effect of scorpion toxin (Rando et al., 1986).

3. Determinants of the specific interactions between site-3 toxins and Na channels

Here we focus on the major toxins from *Anemonia xanthogrammica* and their kinetic actions principally on cardiac Na channels. Extensive structure-function data are available for Ap-B, one toxin from the venom of *Anemonia xanthogrammica*, which when assayed electrophysiologically exhibits a high affinity for multiple Na channel isoforms with a 50-fold preference for rat cardiac channels over neuronal isoforms (0.1 versus 5 nM), although when assayed by flux the affinities were similar and appeared to be high affinity (9 versus 22 nM) (Gallagher and Blumenthal, 1992; Khera et al., 1995). Ap-A, the major toxin from *Anemonia xanthogrammica*, in which only seven of the 49 amino acids are different from Ap-B, was the first noted to have cardiac effects and to exhibit a 50-fold preference for cardiac over neuronal channels (2.5 versus 120 nM). Ap-A toxin also exhibited isoform preference by flux assay of approximately 30-fold (14–400 nM) (Khera et al., 1995). Other studies also reported data to support the idea that these toxins display a 20–40-fold greater affinity for cardiac over neuronal channels (Vincent et al., 1980; Lawrence and Catterall, 1981; Nagy, 1988; Gallagher and Blumenthal, 1994). It should be noted that Ap-B is generally described as a high-affinity variant, but it can also discriminate between isoforms. Cationic residues on these anemone toxins have been identified as underlying the affinity differences (Khera et al., 1995), with Arg12 being the most important, although in combination with Lys-49 it accounts for most of the cardiac specificity of Ap-A over Ap-B for cardiac channels. Although these toxins have a quite high affinity, i.e.ED_{50}'s ~1 nM, their on-rates are extremely slow. Ap-B toxin, for example, which has an ED_{50} of 0.1 nM estimated by electrophysiology for rat $Na_V1.5$, has an on-rate of only $1.4 \times 10^6\,s^{-1}\,M^{-1}$, 2–3 orders of magnitude less than predicted for diffusion of small molecules (Khera et al., 1995). Very slow rates of modification and unmodification require that affinities be measured after exposing preparations to toxin for long periods of time or be estimated from kinetic analyses of washin and washout experiments at toxin concentrations many times the EC_{50} in order to promote channel modification over a time frame achievable in a typical voltage clamp experiment. This method is illustrated in Fig. 1.