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Site-3 sea anemone toxins: Molecular probes of gating mechanisms in voltage-dependent sodium channels

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Abstract

Sea anemone toxins, whose biological function is the capture of marine prey, are invaluable tools for studying the structure and function of mammalian voltage-gated sodium channels. Their high degree of specificity and selectivity have allowed for detailed analysis of inactivation gating and assignment of molecular entities responsible for this process. Because of their ability to discriminate among channel isoforms, and their high degree of structural conservation, these toxins could serve as important lead compounds for future pharmaceutical design.

Keywords: Anemone toxin; Scorpion toxin; Sodium Channel inactivation; Voltage sensing; Positive inotropic agents

1. Introduction

Voltage-sensitive ion channels mediate a wide variety of critical biological processes, including generation of action potentials by excitable tissues. Highly specific neurotoxins which bind with high affinity and channel isoform selectivity have played a key role in identification and purification of the channel proteins, in establishing their tissue-specific expression patterns, and in defining specific residues and/or regions of the channels which are important determinants of ion selectivity and voltage sensing. This review focuses on the family of sea anemone toxins which interact with voltage-sensitive sodium channels to inhibit their inactivation via interaction at channel site 3. In the following pages, we describe the biochemistry, physiology, and molecular pharmacology of these toxins, and summarize present knowledge of their structure and function.

Anemones comprise a large and diverse population of coastal marine organisms whose habitat ranges from the tidal zone to deep water, and from tropical to cold water. Anemones are typically sessile, and when submerged usually have their tentacles and bodies expanded. They feed mostly on mollusks and small crustaceans and fish. The tentacles contain specialized structures called nematocysts, which represent the site of toxin synthesis and storage. Nematocysts are organs which contain a venom storage capsule connected by a tubular structure to the cnidocil, a needle-like surface structure which delivers the venom to prey. The cnidocil itself is contact sensitive, and upon excitation rapidly delivers its toxic contents to organisms with which it comes into contact (Halsted, 1989).

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1.1. Voltage-gated sodium channels: Currents to molecules to mechanisms:

Hodgkin and Huxley, (1952) first recorded currents from squid giant axon using a two-electrode voltage-clamp protocol, thereby demonstrating that depolarization gives rise to two transient and sequential changes in permeability. The first phase resulted from an increase in sodium permeability and the second correlated with potassium permeability (1952). These sodium currents displayed three major properties: (1) voltage-dependent activation (2) fast inactivation and (3) a high degree of selectivity. The authors interpreted the data as suggesting the existence of membrane protein complexes responsible for the generation of sodium currents.

Biochemical characterization of sodium channels was facilitated by the discovery of toxins that modify these channels with high affinity. Narahashi et al. (1964) described the purification of the poison tetrodotoxin (TTX) from Japanese pufferfish and demonstrated its ability to block sodium currents in lobster giant axons at nanomolar concentrations (1964). Blockade was highly specific, preventing sodium-dependent depolarization while leaving potassium currents intact. In 1972, Benzer and Raftery (1972) demonstrated the presence of a protease-sensitive, saturable, TTX binding component in nerve membrane. Polypeptides of MW 250 and 32 kDa from neuroblastoma cells were subsequently shown to cross link to a sodium channel-specific toxin from *Leiurus quinquestriatus* venom (Beneski and Catterall, 1980).

Purification of functional Na^+ channels relied upon the ability of solubilized channels to retain high affinity TTX binding. The TTX-binding complex solubilized from rat brain contained three subunits of MW 270, 39, and 37 kDa (Hartshorne et al., 1982), consistent with the cross-linking studies cited above. Purification of homogenous channels from detergent extracts of rat brain was accomplished by successive lectin affinity and hydroxyapatite chromatography followed by sucrose gradient centrifugation (Hartshorne and Catterall, 1984). The purified complex contained 3 polypeptides of MW 260, 39 and 37 kDa, and bound TTX with high affinity. Reconstitution of this complex into planar lipid bilayers yielded TTX-sensitive channels whose opening was favored by depolarization (Hartshorne et al., 1985).

Reverse genetics allowed for cloning of the eel electroplax sodium channel, thus providing the first amino acid sequence of an ion channel protein (Noda et al., 1984). Analysis of this sequence revealed the existence of four internally homologous domains, and hydropathy analysis predicted that each domain contained four transmembrane segments. Molecular modeling suggested the existence of six transmembrane segments (S1–S6) per domain, including a highly cationic S4 region containing positive charges at every third position (Guy and Seetharamulu, 1986). This model further proposed a reentrant loop between S5 and S6 with the S5–loop–S6 region constituting the channel pore. Fig. 1 depicts the currently accepted model of Na^+ channel architecture.

Cloned Na^+ channels have been expressed heterologously in *Xenopus* oocytes and mammalian cells. Injection of cRNA encoding rat brain Na^+ channel α subunits yields voltage-gated Na^+ currents detected by two-electrode voltage clamp.

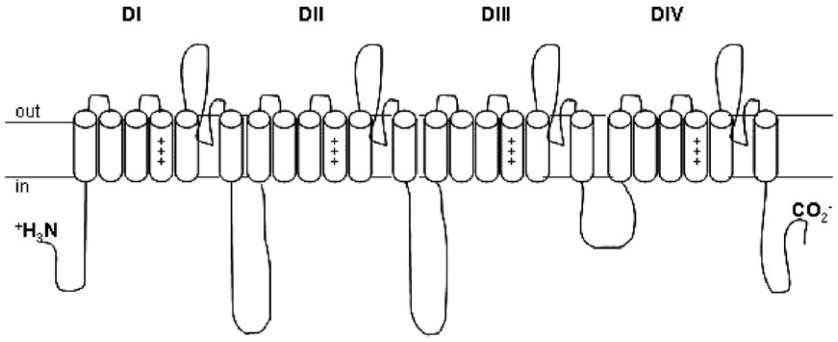

Fig. 1. Transmembrane architecture of the voltage-gated Na^+ channel. The deduced amino acid sequence indicates the presence of four homologous domains, each containing 6 putative transmembrane regions. These are indicated by cylinders. Re-entrant loops connecting the S5 and S6 regions of each domain are key elements of the channel pore. The positive charges shown on each S4 segment have been shown to be essential for voltage sensing.