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# The voltage-gated sodium channel: A major target of marine neurotoxins

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ABSTRACT

Voltage-gated sodium channels ($Na_v$) are key components for nerve excitability. They initiate and propagate the action potential in excitable cells, throughout the central and peripheral nervous system, thus enabling a variety of physiological functions to be achieved. The rising phase of the action potential is driven by the opening of $Na_v$ channels which activate rapidly and carry $Na^+$ ions in the intracellular medium, and ends with the $Na^+$ current inactivation. The biophysical properties of these channels have been elucidated, through the use of pharmacological agents that disrupt the molecular mechanism of the channel functioning. Among them, marine toxins produced by venomous animals or microorganisms have been crucial to map the different allosteric binding sites of the channels, understand their mode of action and represent an emerging source of therapeutic agents to alleviate or cure $Na^+$ channels-linked human diseases. In this article, we review recent discoveries on the molecular and biophysical properties of the $Na^+$ channel as a target for marine neurotoxins, and present the ongoing developments of pharmacological agents as therapeutic tools.



## 1. The sodium channel: molecular identity

Sodium channels, other than epithelial $Na^+$ channels (ENaC) or sodium leak channels (NALCN), belong to the superfamily of voltage-gated ion channels, which all share the property of carrying ions between cellular compartments in response to membrane voltage stimulation (Stock et al., 2013). Among them, the voltage-gated sodium channel is a large protein made of three subunits: the pore-forming α subunit is a 260 kDa protein, associated to one or two β1-4 auxiliary subunits of 30–40 kDa (Catterall et al., 2005). The α subunit encompasses 24 transmembrane segments. Precisely, it is arranged into four homologous but not identical domains (I to IV) made up of six helicoidal transmembrane segments S1–S6. Each domain plays the role of a "subunit-like" entity, so that the channel is arranged by the complex organization of these four domains around the pore. The four domains are linked by three different intracellular loops of unequal lengths. The pore inserts through the S5–S6 segments of each domain, and is surrounded by the S1–S4 segments responsible for the voltage sensing (Fig. 1A, B). Charged amino acids of the S4 segment lead the opening of the channel, as a response to a membrane depolarization. $Na^+$

*Abbreviations:* AP, action potential; ATX, antillatoxin; ATXII, anemone toxin II; CTX, ciguatoxin; GPT, Goniopora toxin; ICK, inhibitory cysteine knot; IFM, isoleucine-phenylalanine-methionine triad; KTX, kalkitoxin; $Na_v$, voltage-gated sodium channels; PbTX, brevetoxin; SaNaTx, sea anemone $Na^+$ channel toxins; STX, saxitoxin; TM, transmembrane; TTX, tetrodotoxin.

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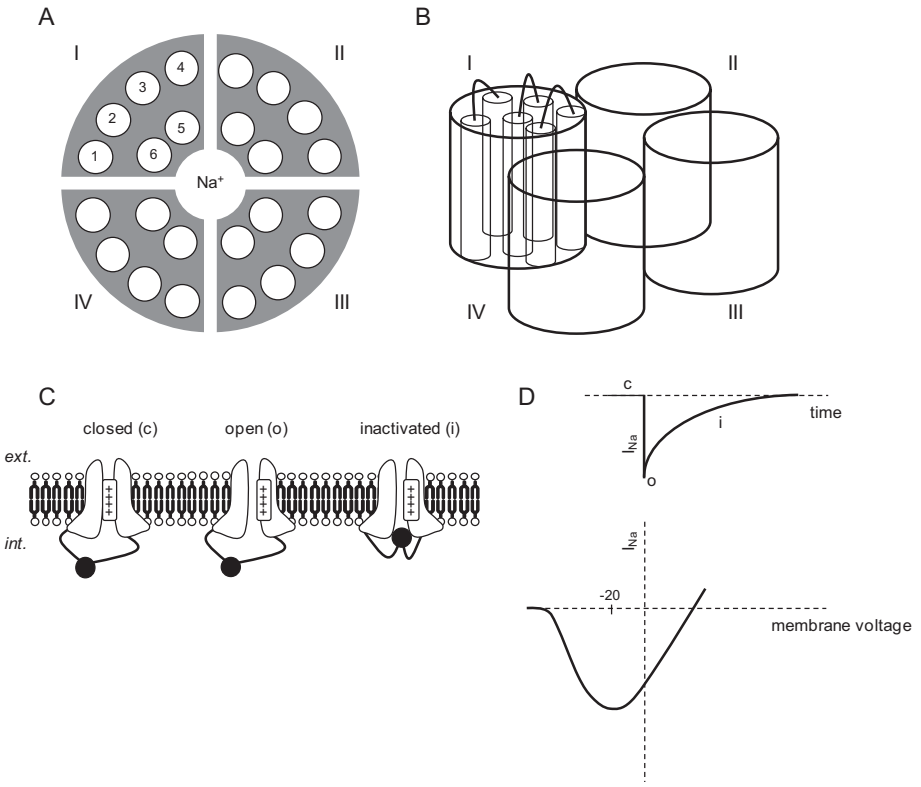


**Fig. 1.** Schematic representation of the α subunit of the $Na^+$ channel and gating mechanism. A and B: the α subunit is composed of 4 homologous domains, each comprising 6 transmembrane segments. S5 and S6 form the central and $Na^+$-selective pore of the channel. C and D: gating mechanism of the $Na_v$ channel. At resting potentials, $Na_v$ remain closed (c) and no current can be recorded. The channel is open (○) after a membrane depolarization which triggers the voltage sensor S4 to move. The central pore is then occupied by the inactivation gate, leading to the inactivation of the channel (i). Picture C represents a $Na_v$ channel in a closed state (left). A few mV depolarization of the membrane potential induces a movement of the positively charged S4 voltage sensor triggering a conformational change and opening of the channel (middle). The pore is then rapidly occluded by the inactivation gate (black circle) resulting in inactivation of the Nav channel (right). Traces in D depict a representation of a whole-cell voltage-clamp recording from a neuron (with a depolarizing test) and the corresponding current–voltage curve. The upper trace is the change in sodium current ($I_{Na}$) during depolarization. The downward deflection of the trace represents an inward movement of $Na^+$ ions into the cell. An inward $Na^+$ current rapidly develops (upper trace) and the activation is maximal for membrane potential of −20 to 0 mV (I/V curve).

ions then flow through this central channel inside the excitable cells during the rising phase of an action potential (AP). No structural determination of an animal version of the channel has been elucidated at high resolution so far, but the crystal structure of a bacterial $Na_v$ channel was recently published, that gave insights into the voltage sensors, the selectivity filter and the inactivation mechanism (Payandeh et al., 2011, 2012; Zhang et al., 2012). When expressed in heterologous systems, the α-subunit alone exhibits the properties of a fully functional $Na^+$ channel, but it is associated to β subunits in native cells. The accessory β subunits (1–4) are supposed to be one segment transmembrane proteins, with a large extracellular N-terminal and a short intracellular C-terminal, which modulate $Na_v$ channel biophysical properties and α subunit insertion in the membrane. For instance, the β4 subunit greatly modulates spider and scorpion toxin binding and pharmacology (Gilchrist et al., 2013). A $Na_v$ channel is organized in a dimer or a trimer with an α/β stoichiometry of 1:1 or 1:2 (Isom et al., 1992).

From the resting potential to the maximal amplitude of the AP, $Na_v$ channels shift from a close to an open state, and then rapidly inactivate (Catterall, 2000). In all types of excitable cells, APs ultimately cause an increase of intracellular calcium and either the subsequent release of a neurotransmitter/hormone or the contraction of a muscle or a cardiac cell. The opening of the channel, termed activation, is a fast and characteristic process at the molecular level (Fig. 1D). The channel can be considered a voltage-sensor through the transmembrane segment 4 of each domain. Indeed, several positively-charged and conserved amino-acids (arg or lys) of S4 are flexible and move from an internal membrane location, where they interact with negative charges at resting potential to the electrical field after depolarization, allowing the channel to open (Fig. 1C). Then, the channel inactivates. The inactivation process is a rapid mechanism, involving the intracellular linker segment between domains III and IV of the α subunit. Basically, a triad of residues (IFM) moves the linker from its cytoplasmic location towards the mouth of the pore, making a plug that stops the flow of $Na^+$ ions (Catterall, 2012). The fast inactivation is partly responsible for the depolarization phase of the AP. Most of the toxins targeting the $Na_v$ channel interfere with the mechanism of activation