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Molecular biology of insect sodium channels and pyrethroid resistance

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ABSTRACT

Voltage-gated sodium channels are essential for the initiation and propagation of the action potential in neurons and other excitable cells. Because of their critical roles in electrical signaling, sodium channels are targets of a variety of naturally occurring and synthetic neurotoxins, including several classes of insecticides. This review is intended to provide an update on the molecular biology of insect sodium channels and the molecular mechanism of pyrethroid resistance. Although mammalian and insect sodium channels share fundamental topological and functional properties, most insect species carry only one sodium channel gene, compared to multiple sodium channel genes found in each mammalian species. Recent studies showed that two posttranscriptional mechanisms, alternative splicing and RNA editing, are involved in generating functional diversity of sodium channels in insects. More than 50 sodium channel mutations have been identified to be responsible for or associated with knockdown resistance (kdr) to pyrethroids in various arthropod pests and disease vectors. Elucidation of molecular mechanism of kdr led to the identification of dual receptor sites of pyrethroids on insect sodium channels. Many of the kdr mutations appear to be located within or close to the two receptor sites. The accumulating knowledge of insect sodium channels and their interactions with insecticides provides a foundation for understanding the neurophysiology of sodium channels *in vivo* and the development of new and safer insecticides for effective control of arthropod pests and human disease vectors.

1. Introduction

1.1. Voltage-gated sodium channels

The nervous system enables animals to detect external and internal stimuli, integrate and process the information detected, and react with speed and coordination. It is hard to catch a cockroach and almost impossible to snatch a dragonfly out of the air because of the rapid electrical and chemical signaling within their nervous system. The electrical signals are comprised of action potentials (i.e., electrical impulses; Fig. 1A) propagating rapidly along axons and from one neuron to the next at synapses. The voltage-gated sodium channel is responsible for the initiation and propagation of action potentials along the axon (Fig. 1B).

The sodium channel forms a pore in the membrane that is highly selective to sodium ions (Fig. 1B). The opening and closing of the sodium channel is regulated by two gating processes, activation and inactivation (Fig. 1B). When a neuron is at rest (i.e., not firing), sodium channels are closed. When the membrane of a neuron is depolarized, sodium channels are activated (open). Influx of sodium ions through activated sodium channels, which further depolarizes the membrane, is responsible for the rising phase of an action potential (Fig. 1B). Within a few milliseconds after sodium channel opening, sodium channels are rapidly inactivated. The inactivation process is partially responsible for the falling phase of an action potential (Fig. 1B) and plays an important role in the termination of an action potential. Upon repolarization, the sodium channel recovers from inactivation and deactivates (i.e., the activation gate closes). Deactivation and recovery from inactivation complete the transition from the inactivated state to the resting

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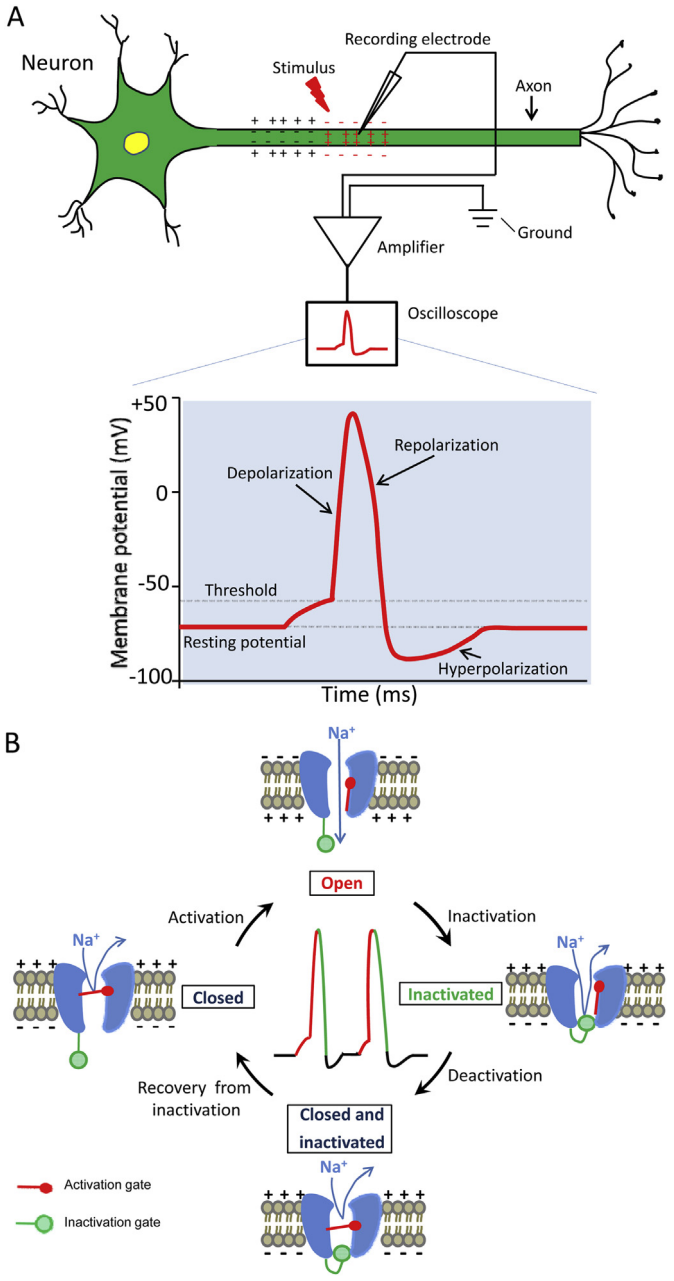

Fig. 1. Voltage-gated sodium channels and the action potential. (A) Recording of an action potential. A recording microelectrode, which inserts inside the axon (intracellular recording), is connected to an amplifier. The amplifier compares the potential difference between the tip of the recording electrode and another electrode (called ground) placed in the solution bathing the neuron. The potential difference can be displayed using an oscilloscope. In response to membrane depolarization, such as a depolarizing stimulus indicated in A, sodium channels open (i.e., activated), resulting in further depolarization of the membrane as indicated by the rising phase of the action potential. Sodium channel inactivation together with potassium channel activation helps terminate the action potential (repolarization and hyperpolarization). (B) Gating (i.e., opening and closing) of voltage-gated sodium channels. Please see the text for explanation.

state of the sodium channel (Fig. 1B), which allows the cell membrane to regain its resting excitable properties and prepare to fire another action potential (Fig. 1B). As such, sodium channels play a critical role in controlling electrical signaling in the nervous system and regulating membrane excitability. In addition, in response to prolonged depolarization (seconds to minutes), sodium channels progressively enter into more stable, slow-inactivated states. This process is known as slow inactivation, which is important for regulating membrane excitability, action potential patterns and spike frequency adaptation.

1.2. Structure and function of sodium channels

Mammalian sodium channels are composed of a pore-forming α-subunit and one or more β subunits. Multiple sodium channel α-subunits are found in mammals (Catterall, 2014; Goldin, 2002). Sodium channel α-subunits have four homologous domains (I–IV), each domain possessing six transmembrane segments (Fig. 2A). In each domain, segments 1-4 (S1–S4) constitute the voltage-sensing module, whereas S5, S6, and a membrane-reentrant loop connecting S5 and S6 segments (called the P-region) form the pore module (Fig. 2A–C). β subunits (β1–β4) are small transmembrane proteins that possess an extracellular immunoglobulin domain, a single transmembrane segment, and a short intracellular C-terminal domain (Brackenbury and Isom, 2011; Catterall, 2000). Coexpression of β subunits modulates sodium channel expression and gating properties (Brackenbury and Isom, 2011; Catterall, 2000).

Significant progress has been made in the past two decades in the understanding of the domain structures and amino acid motifs/sequences required for specific gating properties of sodium channels, including channel activation and inactivation. Most information has been derived from studies of α-subunits of mammalian sodium channels, with which insect sodium channels share high levels of sequence and functional similarities. Below is a brief summary of the current understanding of the structure–function relationship of sodium channels. Readers are referred to comprehensive reviews (Catterall, 2000, 2012; 2014) on this topic.

The ion selectivity of sodium channels is determined by the amino acids D, E, K, and A (the selectivity-filter motif "DEKA") in the analogous positions of domains I, II, III, and IV, respectively, of the α-subunit. Each S4 segment contains repeated motifs of a positively charged amino acid residue followed by two hydrophobic residues and serves as a voltage sensor of the sodium channel. In response to membrane depolarization, the S4 segments move outward, initiating conformational changes which lead to pore opening and inactivation of sodium channels. Short intracellular linkers between the S4 and S5 segments (L45) transmit the movements of the voltage sensing modules to the S6 segments during channel opening and closing. Fast-inactivation is achieved by the movement of an inactivation gate (formed mainly by the IFM motif in the short intracellular linker connecting domains III and IV), which physically occludes the open pore (Fig. 2A).

Since the publication of the first X-ray structure of a bacterial potassium channel KcsA (Doyle et al., 1998), homology models of sodium channels have been developed to predict binding sites of drugs, such as local anesthetics (Lipkind and Fozzard, 2005; Tikhonov and Zhorov, 2007) and toxins, such as tetrodotoxin (Lipkind and Fozzard, 2000; Tikhonov and Zhorov, 2005a) and batrachotoxin (Du et al., 2011; Tikhonov and Zhorov, 2005b). Currently, the mammalian voltage-gated potassium channel $K_v1.2$ crystallized in the open state (Long et al., 2005) and a bacterial sodium channel, Na_vAb, crystallized in the closed state (Payandeh et al., 2011) are used as reasonable templates to model eukaryotic four-domain sodium channels in the open and closed states, respectively. Four identical subunits of the bacterial sodium channel Na_vAb (i.e., a homotetramer) arrange around the pore axis in the way of four-fold rotational symmetry (Fig. 2D–F) (Payandeh et al., 2011). Accordingly, the four voltage-sensing modules are symmetrically arranged around the outer rim of the pore module. The voltage sensing module of one subunit is closely associated with the pore-forming module of the adjacent subunit (Payandeh et al., 2011), as in the $K_v1.2$ channel (Long et al., 2005). This arrangement