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Small molecule modulation of voltage gated sodium channels

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Voltage gated sodium channels are fundamental players in animals physiology. By triggering the depolarization of the lipid membrane they enable generation and propagation of the action potential. The involvement of these channels in numerous pathological conditions makes them relevant target for pharmaceutical intervention. Therefore, modulation of sodium conductance via small molecule binding constitutes a promising strategy to treat a large variety of diseases. However, this approach entails significant challenges: voltage gated sodium channels are complex nanomachines and the details of their workings have only recently started to become clear. Here we review – with emphasis on the computational studies – some of the major milestones in the long-standing search of a quantitative microscopic description of the molecular mechanism and modulation of voltage-gated sodium channels.

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Introduction: physiological role of voltage gated sodium channels (VGSCs)

Cells respond to stimuli from the environment by enabling the passage of ions across the plasma membrane, a process that results in the propagation of an electrical signal. Ion channels are the key players of this process, the membranes of excitable cells are studded with a myriad of these integral membrane proteins, which transduce chemical and electrical stimuli into currents of charged chemical species [1]. Owing to their pivotal role in cell physiology, a large number of genes encode for ion channels, especially in higher organisms [2]. Some of these channels are crucial for the physiology of animals: the emergence of a complex body plan in metazoa implied the necessity to coordinate the response of cells far away from each other and thus the development of a specialized electric network, that is, the nervous system [3–5].

Basic building blocks of the nervous system are the neurons, excitable cells with the ability of sustaining the propagation of electric signals with a defined waveform strictly preserved throughout the transmission process [6]. This requirement is satisfied thanks to the coordinated action of distinct types of ion channels, each allowing, at a precise time, the translocation of a specific ionic species [1]. Briefly, at resting conditions, the plasma membrane of the neuron is polarized; a perturbation of this resting potential (a depolarization) in a section of a neuron axon causes the activation of two types of channels: first those that enhance this perturbation and then those that counteract it and bring the neuron to its resting condition. Thus the electrical signal, so called action potential, is characterized by two phases: a rising and a falling phase [7].

The rising phase of the action potential results from the opening of voltage-gated sodium channels (VGSCs), which decreases the local charge imbalance responsible for the resting potential, thereby generating the rising phase of the action potential [8,9]. Thus VGSCs are *initiators* of the action potential and, as such, they are one of the most critical components of the nervous system. Perhaps not surprisingly, mutations in VGSCs can causative both neuronal hypoexcitability and hyperexcitability and, accordingly, have been found to be associated with inherited syndromes [10,11]. Owing to this crucial role played in diseases, VGSCs are among the most important targets of pharmacological agents.

Pharmacology of VGSCs

Many FDA approved drug molecules target voltage gated sodium channels (VGSCs), usually to reduce cell excitability via suppression of VGSCs activity. The most important examples are: local anesthetics (LA, such as lidocaine), general anesthetics (sevoflurane, isoflurane) anticonvulsants (carmazepine, lamotrigine), and antiarrhythmic drugs (mexiletine). The discovery of these drugs was in most cases serendipitous and thus occurred without a precise knowledge of the molecular target. As a result, these sodium channel modulators typically show weak affinity for the target and poor selectivity toward specific channel subfamilies. Given the ubiquity of

VGSCs, non-selective modulators often raise potential risks of severe side effects, thereby limiting the dose-range, the administration routes, or both. This lack of specificity is best discussed by recalling the major structural features of these channels (Figure 1).

Structure of VGSCs

VGSCs are membrane proteins that contain 24 transmembrane alpha-helices [8,12,13]. These alpha-helices constitute four homologous, though not identical repeats, conventionally denoted as DI through DIV, each constituted by transmembrane helices (S1 through S6). Sequence analysis indicates that these repeats share a common ancestor with the six-transmembrane-helix domain that characterizes voltage-gated potassium channels (VGPCs), usually referred to as the 6TM domain.

This homology suggests that VGSCs and VGPCs are characterized by the same structural architecture, although in one case (VGPCs) the structure results from the quaternary arrangement of four identical subunits, while in the other (VGSCs) it is the tertiary structure that shows an approximately fourfold symmetry and is referred to as pseudotetramer [14]. The recently determined structure of Cav1.1 [15], a pseudotetrameric voltage-gated calcium channel closely related to VGSCs, has provided solid experimental validation to this hypthesis.

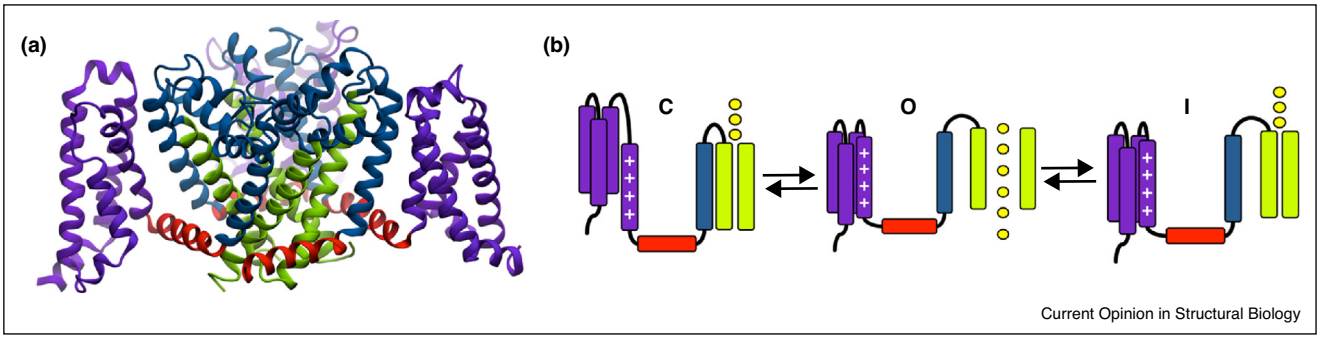
This evolutionary relationship between VGSCs and VGPCs raises the hypothesis that the ancestors of the former had the same primary structure as the latter (*i.e.*, a single 6TM domain), before the gene underwent two rounds of duplications [16]. This scenario is consistent with the large degree of observed sequence similarity between VGSCs and bacterial voltage-gated sodium selective channels, which show a primary structure constituted by a single 6TM domain assembling in homotetramers.

Similarly to all voltage-gated ion channels, VGSCs show an assembly of four S6 helices, one from each domain, lining the hydrophilic pathway through which conduction of ions occurs. There are two functionally crucial structural elements in this pathway: (i) the gate, that prevents diffusion of ions when the channels is closed thanks to the hindrance of bulky hydrophobic groups; (ii) the selectivity filter, which is selectively permeable to Na^+ ions [17••, 18–20]. Four amino acids, one from each domain, constitute the selectivity filter by forming a ring, the so-called DEKA motif, lining the conduction pathway [21].

Molecular mechanism of VGSCs

VGSCs open and close in response to a change in polarization of the lipid membrane. Their activation is governed by the same mechanisms used by other members of the voltage-gated ion channels superfamily like VGPCs. The structural element responsible for voltage sensitivity is called voltage sensor domain and is constituted by the assembly of the first four transmembrane helices (S1–S4), while the element directly controlling passage of the ions, the gate, is contained within the assembly of the last two transmembrane helices S5 and S6 [22,23••,24–27]. This occurs thanks to an allosteric coupling between the conformation of the pore and that of the voltage sensor domain. In particular, the helical segment S4 is characterized by a series of charged residues (almost invariably arginines) showing a periodicity of three amino acids along the sequence. This highly charged secondary structure element is poised to respond to a change in the electrostatic field across the membrane by moving along the membrane normal in a stepwise fashion. Specifically, S4 undergoes a helical screw motion that triggers the

Figure 1



Cartoon representation of the structure and mechanism of voltage gated sodium channels. The first four transmembrane (TM) helices (S1 through S4 from each subunit) constitute the voltage sensor domain (purple). The pore domain, instead, comprises two TM helices, S5 and S6 (blue and yellow, respectively. The pore and voltage sensor domains are connected by the linker domain (red). **(b)** The gating mechanism of VGSCs requires a coordinated motion of the voltage sensor and pore domains. Under depolarizing conditions, the positively charged S4 helix translates along the bilayer normal causing the splaying of the S6 helix bundle. The channel thus transitions from the closed (C) to the open state (O). The latter is unstable and decays to the inactivated state (I), a nonconductive state characterized by an activated voltage sensor domain and a closed pore domain.