Allosteric modulation of gating by agonists and antagonists studied in prokaryotic, voltage-gated sodium channels

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Clinical drugs and biological toxins, which modulate neuronal and muscle activity, take advantage of the multiple conformational states of voltage-gated sodium channel (Nav) proteins to achieve specific targeting and functional actions.

Prokaryotic channels (NavBacs), like many voltage-gated K channels, are homotrameric. In contrast, eukaryotic channels of the Nav1 family, have a single, major structural α-subunit, consisting of 4 homologous, but non-identical, domains (D1-D4), which show specific functional specializations. For example, D1-D3 are directly involved in sensing depolarization and driving fast voltage-dependent activation/opening. On the other hand, D4 appears to have a particular role in coupling between channel activation (opening) and channel inactivation to a non-conducting state, on prolonged depolarization. The 4-domain structure of eukaryotic Nav1 channels poses severe challenges to crystallization for the determination of high-resolution structures by X-ray diffraction. Thus, present attempts to glean a molecular understanding of Nav function largely depend on homology models built from available high-resolution structures of prokaryotic NavBac channels. A convoluted linkage between the voltage-sensing (VSD) domain, couples with the pore domain (PD), which opens rapidly on depolarization to allow ion conduction and the consequent generation of propagated electrical impulses.

Here, explore we explore ways in which different activating and inhibitory ligands may modulate gating to achieve contrasting functional effects. Prokaryotic NavBac structures form the basis of modeling, and give insight into the different molecular mechanisms by which the gating of voltage-sensitive sodium channels can be modulated, either allosterically, or directly, in response to ligand binding at different channel sites.

We illustrate cases of contrasting modulation by considering actions of 2 different types of inhibitors, μ-conotoxins and local anaesthetics (LAs), which block conduction by binding within the conducting pore, on opposite sides of the selectivity filter (external - μ-conotoxins, or internal – local anaesthetics). As a counterpoint, in particular to the LAs, we consider the action of batrachotoxin (BTX), which is perhaps the most dramatic of known Nav1 channel agonists. We can begin to understand these diverse actions in the context of homology models based on high-resolution molecular structures of prokaryotic Nav channels, determined within the last decade.

Both LAs and BTX exert apparently allosteric influences on channel gating, and both bind in the pore lumen on the cytoplasmic side of the selectivity filter. For LAs, the dominant effect is a “use-dependent” inhibition based on pore occlusion. In contrast, BTX is a powerful agonist because it produces a negative shift in the effective activation voltage, despite partial occlusion of the pore by the bound BTX molecule. For both LAs and BTX, the dominant allosteric actions are “use-dependent” (favoured by repetitive activation). Thus, LAs inhibit, while BTX predominantly activates, despite the fact that these agents bind to overlapping pore sites. In common with BTX activation of eukaryotic Nav1 channels, BTX modification of prokaryotic Nav channels, including NaChBac, is associated with a negative shift, of ˜−20mV, in the half-activation voltage. In addition, there is a progressive decrease in maximal conductance, during modification, consistent with a partial block of single-channel conductance by BTX, as reported previously for eukaryotic channels. Finally, mutation of NaChaBac residues, that are homologous to those important for BTX modification of eukaryotic channels, diminished the effects of BTX. Thus, the key functional changes induced by BTX in eukaryotic Nav channels are re-capitulated in prokaryotic Navs. These changes can be interpreted in the context a NaChBac homology model, in which the bound, rigid BTX molecule acts to stiffen the pore and inhibit closure of the channel’s activation gate.