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Voltage Gated Sodium Channel Associated Proteins and Alternative Mechanisms of Inactivation and Block

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Abstract

Voltage-gated sodium channels mediate inward current of action potentials upon membrane depolarization of excitable cells. The initial transient sodium current is restricted to milliseconds through three distinct channel inactivating and blocking mechanisms. All pore-forming alpha subunits of sodium channels possess structural elements mediating fast inactivation upon depolarization and recovery within milliseconds upon membrane repolarization. Accessory subunits modulate fast inactivation dynamics, but these proteins can also limit current by contributing distinct inactivation and blocking particles. A-type isoforms of fibroblast growth factor homologous factors (FHF) bear a particle that induces long-term channel inactivation, while sodium channel subunit Nav β 4 employs a blocking particle that rapidly dissociates upon membrane repolarization to generate resurgent current. Despite their different physiological functions, the FHF and Nav β 4 particles have similarity in amino acid composition and mechanisms for docking within sodium channels. The three competing channel inactivating and blocking processes functionally interact to regulate a neuron's intrinsic excitability.

Introduction

Action potentials in excitable cells are triggered by the transient activation of voltage-gated sodium channels upon membrane depolarization. Within milliseconds, inward sodium current is arrested by either inactivation or blocking mechanisms. Current arrest serves to preserve the transmembrane sodium gradient and enables a cell to digitize its electrical output as a temporal profile of action potentials. Three physically distinct channel processes serve to rapidly terminate the sodium current: 1) Fast inactivation is a transition to a dormant channel state from which recovery is rapid upon negative repolarization of the membrane, thereby rendering channels quickly available for reactivation. 2) Long-term inactivation generates a more stable dormant channel state from which recovery is far slower upon membrane repolarization, thereby opposing high frequency spike generation. 3) Open channel block is a dormant state from which channels reopen before deactivating or inactivating upon membrane repolarization. The resurgent sodium current generated upon repolarization contributes to the subsequent membrane depolarization cycle and thereby assists in high frequency spike generation. Each of these processes employs protein moieties on the cytoplasmic membrane face and may have related structural mechanisms despite divergent physiological consequences.

Fast Inactivation

The kinetics of fast sodium channel inactivation, its interdependence with channel activation, its essential structural moieties and the underlying channel conformational changes have been intensively studied for more than three decades, and there are many

excellent reviews on these subjects [1, 2]. This section updates and summarizes the principles of fast inactivation for subsequent comparisons to the more recently described phenomena of long-term inactivation and open channel block.

The alpha subunit of nine mammalian voltage-gated sodium channels (termed Nav1.1 through Nav1.9) bears four membrane-embedded homologous domains (I-IV), each consisting of six alpha helical segments (S1-S6), three cytoplasmic loops connecting the domains, and a cytoplasmic carboxyl-terminal tail [3] (Figure 1). The S6 segments of the four domains form the inner surface of the pore, while the S4 segments bear clusters of basic residues that constitute the channel's voltage sensors. At sufficiently negative membrane potentials, the electric field's inward pull on the voltage sensors is mechanically coupled through molecular hinges in the cytoplasmic S4/S5 linkers to pinch shut the inner half of the channel pore [4, 5]. Rising voltage allows for activation through outward sensor movement and relief of the pore's impingement [6].

Fast inactivation of sodium channels is coupled to voltage-driven conformational changes that lead to channel opening (Figure 2A). Three major findings have supported this conclusion: 1) Channel gating current mostly follows the time course of channel activation, while the relatively slower rate inactivation process is not paralleled by a necessary gating current [7]. 2) Voltage clamp protocols have shown a link between the voltage dependence of inactivation and the rate of channel activation [8]. 3) At weaker depolarization voltages where macroscopic sodium current onset and inactivation are slower, all single channel openings, regardless of their onset, are rapidly followed by inactivation [9, 10]. Under steady state conditions, the voltage dependence of channel inactivation has a $V_{1/2}$ that is 25-45 mV more negative than the $V_{1/2}$ for activation. Inactivation at more negative potentials, referred to as closed state inactivation, is evidence that rising voltage effects a sequence of sensor-driven conformational changes, all or most of which are needed for a channel to reach the open state, but only a subset of which are needed to render the channel competent for fast inactivation [11]. Mild proteolytic treatment of a channel's cytoplasmic face, application of an antibody directed against a channel's III/IV cytoplasmic loop, or amino acid substitutions within cytoplasmic regions of the channel abolish inactivation without affecting activation gating [12-14]. These findings argue that structural elements exposed to the cytoplasm become accessible or compatible for intramolecular interactions to effect inactivation following voltage-driven channel conformational changes.

Upon membrane repolarization to negative potentials, fast inactivated channels recover without passing through the open conducting state. Such repolarization drives voltage sensors inward, deactivating the channel and concomitantly destabilizing the cytoplasmic inactivation state structure. The rate of channel recovery is a function of the negative membrane potential, but again this reversal of inactivation is not directly voltage driven, but dependent upon the rate of sensor-driven deactivation transitions (Figure 2A) [11]. These findings are most consistent with a Markov state model for channel activation, inactivation, deactivation and recovery (Figure 2B) [11, 15]. The exact number of voltage sensor-driven closed state transitions from fully deactivated to open state is unknown, but computational models typically employ several closed state transitions to the open state (C1-C5, O) and accompanying fast inactivation states (I1-I6) to achieve kinetic behavior comparable to empirical data (Figure 2B)[15, 16]. Although not shown in the figure, there is evidence supporting at least two open channel states that differ by a late movement of the domain IV voltage sensor with concomitant effect on the channel cytoplasmic face, thereby increasing the rate of fast inactivation [17, 18].

The highly conserved hydrophobic isoleucine-phenylalanine-methionine triad in the short III/IV loop of all sodium channel alpha subunits is an essential component of the fast

inactivation mechanism [14]. Mutation of the central phenylalanine (F→Q) severely impairs inactivation, and full ablation of the triad (IFM→QQQ) abolishes inactivation altogether. The solution structure of the III/IV loop reveals that the IFM triad forms a hydrophobic cap atop a 10-12 residue alpha helical segment [19-21]. This III/IV epitope has sometimes been referred to as a tethered particle, plug, or lid that docks at the pore's cytoplasmic face to achieve fast inactivation. The central argument for this model came from experiments in which inactivation-defective IFM triad-mutated channel was treated with short synthetic peptides containing the IFM sequence and flanked by basic residues (e.g., the pentapeptide KIFMK) [22]. Upon depolarization, these peptides restricted the duration of sodium currents, similar to fast inactivation. However, evidence is scant that these peptides were structurally recapitulating fast inactivation. The IFM peptides were not reported to induce closed state inactivation at more negative voltages, and recovery of some channels from the peptide-mediated nonconducting state was slow. Rather than recapitulating fast inactivation, these peptides induced the distinct phenomena of open channel block and use-dependent long-term inactivation [22-25] and may have been mimicking the actions of FHF and channel beta subunit moieties (discussed in sections below).

Several clinical disorders result from sodium channel mutations that impair fast inactivation. Among disease-associated mutations affecting inactivation, childhood epilepsy-associated Nav1.1 mutations [26, 27], a skeletal myotonia-associated Nav1.4 mutation [28], and cardiac long QT syndrome-associated Nav1.5 mutations [29, 30] generate amino acid substitutions in the channel alpha subunit C-terminal domains. Some of these mutations render the inactivation state unstable resulting in elevated persistent sodium current upon depolarization, while other C-terminal domain mutations alter the voltage dependence of fast inactivation. The role of the C-terminal domain in inactivation is also supported by studies on chimeric sodium channels; C-terminal domain exchanges between pairs of alpha subunit isoforms alter voltage dependence of inactivation without affecting activation [31]. Documented physical interaction between the C-terminal domain and the III/IV loop of cardiac Nav1.5 may help explain the necessary roles of these regions in generating the fast inactivation state [32].

There are also compelling findings to support a physical interaction between the III/IV loop's IFM motif and the channel's III-S4/S5 cytoplasmic hinge region. Whereas an F→D mutation in the III/IV IFM triad of Nav1.2 almost fully ablates inactivation, the additional mutation of a III-S4/S5 hinge hydrophobic linker residue to lysine appreciably restores both open state and closed state fast inactivation [33]. These data argue that fast inactivation is driven by III/IV loop and III-S4/S5 linker hydrophobic interaction that can be functionally replaced by electrostatic interactions enabled by complementary charged polar residue substitutions. The role of an S4/S5 hinge [4] further suggests that fast inactivation may entail molecular interactions that suppress the electromechanical coupling between voltage sensor movement and inner pore widening.

The voltage dependence of sodium channel fast inactivation can be altered by accessory proteins that bind to the C-terminal domain of the alpha subunit [26, 34-36]. Navβ1 interactions with cardiac, skeletal muscle, or neuronal channel alpha subunits cause voltage dependence of fast inactivation to undergo hyperpolarizing shifts [26, 29, 37-39], while many fibroblast growth factor homologous factors (FHF) induce depolarizing shifts in inactivation [40-45]. The physical mechanisms through which accessory proteins alter the voltage dependence of channel inactivation without concomitantly affecting activation have not been reported.

FHF-Mediated Long-Term Inactivation

FHFs (also referred to as intracellular fibroblast growth factors) are small cytoplasmic proteins bearing β -trefoil structural cores and N-terminal effector domains [46, 47]. There are four FHF genes in mammals, and further family diversity is achieved through use of alternative transcriptional promoters and first coding exons to generate FHF variants with different N-terminal sequences. The β -trefoil core (Figure 1) provides a surface for 1:1 stoichiometric binding to the membrane-proximal portion of the sodium channel α subunit C-terminal domain, and the strong sequence conservation of the recognition surfaces allows all FHFs to interact with most, if not all, sodium channels [48]. One function of FHF binding is to raise the voltage dependence of channel fast inactivation and, consequently, to slow the rate of inactivation at specific voltages, the degree to which varies among FHF and channel isoforms [40-45].

A-type variants of FHFs (FHF1A, FHF2A, FHF4A) also induce sodium channel long-term inactivation [42, 44, 45]. Transition to the long-term inactivated state occurs within milliseconds following membrane depolarization, but recovery upon repolarization may be 100-fold slower than is required for recovery from fast inactivation. In contrast to fast inactivation, long-term inactivation has a higher voltage threshold closer to the $V_{1/2}$ of channel activation (Figure 2A) [45]. FHF-mediated long-term inactivation has been best described for Nav1.6, but other sodium channels including cardiac Nav1.5 are susceptible to this phenomenon as well [45]. Long-term inactivation is a process distinct from slow inactivation, which by contrast requires depolarization lasting on the order of seconds to affect a substantial percentage of channels [2].

A-type FHF-mediated Nav1.6 long-term inactivation is inhibited by prior fast inactivation [45]. Following brief depolarization sufficient to activate channels, the stochastic competing inactivation mechanisms drive a fraction of channels into long-term inactivation. Restoring strong negative membrane potential allows only fast-inactivated channels to recover over a 10-50 millisecond time range, after which another brief depolarization cycle again drives a fraction of available channels into long-term inactivation. Hence, channel availability decreases for each depolarization cycle if the inter-cycle negative potential intervals are not long enough to allow for recovery from long-term inactivation [42, 45]. Disabling or destabilizing Nav1.6 fast inactivation by III/IV loop IFM triad mutation [14] or by III-S4/S5 hinge mutation [33] dramatically increases the fraction of channels that can be driven into long-term inactivation by a single depolarization cycle [45]. We can approximate the behavior of a sodium channel in the presence of A-type FHF by employing a Markov model that adds long-term inactivated states (L3-L6) reached from closed or open states as alternatives to fast inactivation transitions (Figure 2B).

N-terminal residues of A-type FHFs are highly conserved among FHF family members and are required for induction of channel long-term inactivation. In FHF2A, hydrophobic residues at Ile-5, Leu-9, and Ile-10 along with basic residues clustered within positions 13-21 are required for long-term inactivation [45]. The positioning of these essential residues had suggested that an A-type FHF's N-terminus serves as an inactivation particle tethered to the complex of FHF core and sodium channel C-terminal domain. In support of this premise, Nav1.6 could undergo long-term inactivation in the absence of FHF if high concentrations of a 20-mer peptide corresponding to FHF2A's N-terminus were injected into cells through a patch pipette. Long-term channel inactivation mediated by FHF peptide showed the same voltage dependence, induction kinetics, recovery rate, and competition with fast inactivation as was observed for native A-type FHF protein [45].

Where does the long-term inactivation particle dock on the sodium channel, and how does this interaction block sodium current? Similarities between FHF-mediated long-term inactivation and the actions of small pharmaceutical compounds offer possible clues to the FHF mechanism. Aminoamide anesthetic/antiarrhythmic compounds (such as lidocaine, bupivacaine, and procainamide) bind to sodium channels on the cytoplasmic side of the ion selectivity filter [49]. Essential to their function are an aromatic moiety and a secondary or tertiary amine that binds to the channel pore, along with protonation of the amine that creates a cation permeation barrier [50, 51]. High affinity targeting of these compounds to channels requires depolarization to generate open and open/inactivated states that expose the compound's docking site within the pore [52]. A phenylalanine residue in the middle of the pore's IV-S6 helix situated between the selectivity filter externally and the fast inactivation machinery cytoplasmically is an essential residue for the binding of these anesthetic/antiarrhythmic compounds [53-55]. FHF long-term inactivation also requires strong depolarization to drive the sodium channel's conformation near to or into the open state, and the long-term inactivation particle bears essential hydrophobic and positively charged residues [45]. Furthermore, the channel's IV-S6 phenylalanine (Nav1.6 F1752) is among several pore-lining hydrophobic residues required for efficient FHF-mediated long-term inactivation (S. Zhao and M. Goldfarb, unpublished data). These findings suggest that sodium channel conformational changes driven by steep depolarization expose the site deep within the channel's cytoplasmic face for docking of the FHF particle, with one or more of the particle's cationic residues then imparting a sodium permeation barrier. Unlike the small aminoamide compounds, the FHF inactivation particle's action is prevented by fast inactivation, which may close off access of the bulky FHF particle to its docking site deeper in the channel [56], while small aminoamide compound binding to channels is stabilized by fast inactivation [57-59]. Additional channel and FHF mutagenesis studies will be required to validate mechanistic similarity between anesthetic block and long-term inactivation.

Small pentapeptides containing the channel III-IV loop's IFM triad that were designed to mimic an intrinsic fast inactivation particle may have actually been affecting sodium channels through a mechanism more akin to FHF-mediated long-term inactivation. First, inactivation mediated by KIFMK peptide showed slow recovery upon repolarization similar to A-type FHF peptide, so that a 10Hz train of 5 msec strong depolarizations yielded progressively decreasing peak currents (use dependence) [22, 25]. Second, sodium channel inactivation by KIFMK peptide had only been documented to occur from the open state, whereas native fast inactivation can also occur from closed states at more negative potentials. Third, inactivation driven by IFM-containing peptides required adjacent basic residues that are not native to the IFM region of the channel III/IV cytoplasmic loop [22, 60], but that are required native residues in the FHF inactivation particle [45].

Sodium Channel β 4-Mediated Open Channel Block and Resurgent Current

Resurgent sodium current is a secondary transient current generated by some classes of neurons when steep membrane depolarization and channel inactivation is followed by repolarization, with the most current generated in the -20 to -50 mV range [61]. Resurgent current is produced by the same channels that generate the transient sodium current during initial depolarization [61]. Although the peak macroscopic resurgent conductance is typically only 5-10% of the initial peak sodium conductance, the duration of resurgent current is protracted over tens of milliseconds, such that the overall charge transfer during resurgence can be up to 30% of that during the depolarization-induced transient current [61-63]. Single channel analysis has shown that resurgent channel openings upon voltage shifts from $+30$ mV to -30 mV last on average for a similar interval as initial channel openings following depolarization from -80 mV to -30 mV, but the resurgent openings have

slow onset with a time constant of several milliseconds, thereby explaining the smaller peak and longer duration of the macroscopic resurgent current [61].

Sodium channels are primed to generate resurgent current when steep membrane depolarization allows for a physical alternative to the fast inactivation mechanism for terminating the initial transient current. The alternative process has been termed open channel block, because it only occurs when channels achieve the open state, as opposed to fast inactivation that also occurs at more negative potentials from closed states. Channels in the open state transit more rapidly to the blocked state than they do to the fast inactivated state [15, 64], and directly unblock to the open state upon membrane repolarization to generate resurgent current before undergoing deactivation or fast inactivation [15, 61]. For sodium channels to undergo block, they must not only open, but the membrane voltage must be high enough to limit inward sodium current flow (Figure 2A) [68]. Sodium channel block has been described in a Markov model that includes a reversible transition between open (O) and blocked (OB) states [15]. To reflect more recent findings [68], I have added an intermediate permissive state (Op) in which open channels are transmitting little inward current (Figure 2B). $O \longleftrightarrow Op$ is not a structural transition, but is voltage- and $[Na^+]_{out}/[Na^+]_{in}$ -dependent, while transition to block ($Op \rightarrow OpB$) is voltage-independent, similar to fast and long-term inactivation transitions.

Resurgent current requires neuronal expression of the sodium channel $\beta 4$ subunit (Nav2.4) and is dependent upon Nav $\beta 4$'s short cytoplasmic tail. Disabling Nav $\beta 4$ function by RNA interference in cultured cerebellar granule cells [65] or by treatment of Purkinje cell sodium channel patches on their inside face with proteases [66] suppresses channel block and resurgent current, demonstrating that block is mediated on the channel's cytoplasmic face. Block and resurgent current can be restored in these treated cells or achieved de novo in hippocampal neurons lacking Nav $\beta 4$ by cytoplasmic exposure to a 14-mer soluble peptide corresponding to a membrane-proximal region of the Nav $\beta 4$ cytoplasmic tail [65, 67]. This Nav $\beta 4$ blocking peptide contains hydrophobic and basic residues, and a "mutant" peptide in which two adjacent lysines were replaced with alanines had no ability to induce channel block or resurgent current [67]. The basic residues on the Nav $\beta 4$ blocking particle may serve two purposes, first to create a cation permeation barrier upon particle docking to the open channel when the membrane is steeply depolarized, and second to render the particle sensitive to expulsion upon return to more negative potentials by repulsive interaction with external sodium ions [68]. The blocking particle is a unique sequence feature of Nav $\beta 4$, explaining why other channel beta subunits do not induce resurgent current. Block and resurgent current for neuronal-, cardiac-, and skeletal muscle-specific sodium channels can be mimicked by incubation with the KIFMK pentapeptide [22, 25, 69], presumably because this fast inactivation III/IV loop-derived peptide and the Nav $\beta 4$ particle share hydrophobic and cationic character.

Sodium channel block and resurgent current in neurons have been characterized most extensively for Nav1.6, which is the principal sodium conductance for neurons in the central nervous system [64, 70, 71]. Other sodium channels, including Nav1.1, also contribute to the resurgent current in neurons [72, 73]. Induction of block and resurgence by Nav $\beta 4$ peptide administration has also been demonstrated for Nav1.5 and Nav1.7 [69, 74]. It appears that a sodium channel's susceptibility to the Nav $\beta 4$ blocking particle is inversely correlated with the rate of onset of competing fast inactivation at strongly depolarized voltage [74, 75], and a sodium channel defective for fast inactivation could undergo complete block by the Nav $\beta 4$ peptide [69, 74]. Mutation of Nav1.5 at F1760 in the middle of the pore's IV-S6 helix renders the channel insensitive to Nav $\beta 4$ peptide, suggesting that the blocking particle docks deep within the cytoplasmic cavern of open sodium channels [69].

Nav β 4-mediated channel block and A-type FHF-mediated long-term inactivation have remarkably similar physical mechanisms despite their disparate physiological consequences. Both processes are mediated by small cytoplasmic peptide epitopes (particles) of hydrophobic and cationic character that engage with the channel when substantial depolarization has driven the channel near to or into the open state. In both cases, the mediating particles appear to dock at similar sites deep within the cytoplasmic cavern of the channel pore, as supported by their shared dependence upon a domain IV-S6 phenylalanine side chain in the cavern and their inhibition by fast inactivation. The two processes fundamentally differ in the rates of particle dissociation. Whereas the Nav β 4 particle dissociates rapidly upon membrane depolarization to generate a transient channel open state, the A-type FHF particle dissociates far more slowly. FHF particle dissociation has been modeled to proceed from closed channel states, analogous to fast inactivation (Figure 2), but even if particle dissociation were to yield a transient open state, a macroscopic resurgent current would not be detected, as the slow particle dissociation rate would prevent summation of unitary currents from simultaneous channel openings.

Regulation of Action Potentials by Sodium Channel Inactivation and Block

A neuron's intrinsic excitability is defined as the relationship between excitatory current input and action potential frequency output. Excitability is governed by the repertoire, abundance, subcellular distribution, and biophysical properties of voltage- and ligand-gated ion channels along with the electrotonic properties of a neuron. This section specifically focuses on the control of sodium channel inactivation and block as important determinants of excitability.

Many neurons are tuned to fire at frequencies over a broad dynamic range. For low frequency firing, minimum threshold input current induces a slow depolarizing voltage ramp that fast inactivates most sodium channels from the closed state, such that firing is largely dependent upon the persistent "window" sodium current triggered at the voltage intersection of steady state inactivation and activation curves [62, 73]. FHF proteins associated with sodium channels at axon initial segments of cerebellar granule neurons promote excitability at low current input by inducing a depolarizing 12-15 mV shift in the voltage dependence of channel fast inactivation without a concomitant effect on channel activation, thereby ensuring a small percentage of channel availability at slow voltage ramps [43]. If sodium channel density in a neuron is sufficiently high, this persistent current may alone drive the action potential upstroke, as put forth in neuronal computational models [15, 76]. Alternatively, the persistent current may provide a small but accelerated depolarizing voltage ramp capable of triggering more efficient opening of other sodium channels with different physiological dynamics or subcellular localization. This proposed two-step spike initiation model is supported by data on the differential distribution of sodium channel isoforms within axon initial segments of many neurons, with Nav1.1 restricted proximally and Nav1.6 residing more distally [77-79].

High frequency firing in response to large current inputs is dependent on both the modulation of sodium channel fast inactivation's voltage dependence by FHFs [43, 80] as well as by Nav β 4-mediated resurgent current [63-65]. During high frequency firing, the large input current (from excitatory synapses or through current clamp) counteracts a spike downstroke's voltage-gated potassium current to elevate the voltage of the afterhyperpolarization. FHF-mediated elevation of sodium channel fast inactivation's voltage dependence is required to allow sufficient channel recovery after each action potential to maintain channel availability throughout the spike train [43]. High frequency firing is also promoted by Nav β 4-induced block of some sodium channels following activation. Unblocking of channels during the interspike interval provides a resurgent

current to reduce depolarization time to spike threshold and also provides additional channel availability when threshold is reached. The degree of channel block during an action potential is determined by the fraction of sodium channels in association with Nav β 4 as well as the spike's voltage peak, since the rate of channel block as opposed to fast inactivation is enhanced by steep membrane depolarization. This voltage dependent dynamic may help explain why high frequency firing in cerebellar Purkinje cells with +40 mV spike amplitudes is more dependent upon Nav β 4 channel block and resurgency than is firing of cerebellar granule cells, where spike peaks are in the +10 to +20 mV range [64, 65]. In addition to its role for high frequency spike initiation at the axon initial segment, Nav β 4-mediated resurgent current is also required for faithful high frequency spike following at presynaptic axon terminals of auditory fibers [81].

Biological functions of native A-type FHF-mediated sodium channel long-term inactivation have not yet been documented. Injection into neurons of a 20-mer peptide corresponding to the FHF2A inactivation particle causes rundown of channel availability through multiple depolarization cycles and thereby prevents sustained high frequency spike generation [45]. A-type FHFs are detectable on axon initial segments following neuronal transfection with expression vectors [41, 43] and both hippocampal pyramidal neurons and cerebellar granule neurons display endogenous A-type FHFs at their axon initial segments [45, 82], yet these neurons generate sustained spike trains at high current inputs. A-type FHF function may be limited in these neurons by their association with only a fraction of the resident sodium channels. The sensitivity of a subset of sodium channels to long-term inactivation could in turn account for firing attenuation (reduced spike voltage peaks) or accommodation (reduced spike frequency) during continuous current inputs.

Sodium channels on the dendrites of hippocampal CA1 pyramidal neurons undergo a fast-onset use-dependent long-term inactivation that prevents backpropagation of high frequency spike trains from the soma through the dendritic arbor [83-86]. Many physiological parameters of dendritic channel long-term inactivation are indistinguishable from FHF-mediated long-term inactivation, including induction within milliseconds of depolarization, accumulated inactivation through depolarization cycles, recoveries requiring hundreds of milliseconds at resting potential, and accelerated recoveries upon membrane hyperpolarization [45, 85, 86]. A-type FHF immunofluorescence in hippocampal neurons is very strong [45], and weak fluorescence is evident along the dendrite [82], suggesting that A-type FHFs may be effecting the unusual inactivation dynamics of dendritic channels.

Summary

While fast inactivation is an intrinsic property of all vertebrate voltage-gated sodium channels, cytoplasmic association of channels with FHF and beta subunit accessory proteins can modulate fast inactivation dynamics and also establish competing inactivation and blocking mechanisms. FHF modulation of sodium channel fast inactivation's voltage dependence is essential for excitability of cerebellar granule and Purkinje cells over a broad dynamic range, while channel long-term inactivation induced by endogenous A-type FHFs is suspected, though not yet proven, to limit duration of high frequency excitation and conduction. Endogenous Nav β 4 induction of open channel block and resurgent sodium current is also required for firing at high frequencies in several classes of neurons. The effects of FHFs and Nav β 4 on excitability are likely to extend well beyond the cerebellum, as these accessory proteins are widely, though not uniformly, expressed in neurons throughout the central nervous system.

A-type FHFs and Nav β 4 contain membrane-tethered cytoplasmic inactivation and blocking particles that dock at potentially overlapping channel receptor sites exposed by strong

membrane depolarization. Upon binding, these particles may act as positively charged barriers to sodium permeation in a manner analogous to aminoamide anesthetic/antiarrhythmic compounds. By contrast, intrinsic fast inactivation results from interactions among several cytoplasmically exposed elements of the channel α subunit, including the III-S4/S5 hinge, the III-IV loop, and the carboxyl tail. While the IFM triad in the III-IV loop is essential for fast inactivation, loop-derived peptide elements may not be capable of physically recapitulating intrinsic fast inactivation, but rather may mimic the actions of FHF and Nav β 4 particles.

Pharmaceutical modulation of voltage-gated sodium channels has been used therapeutically for the management of pain disorders, cardiac conduction anomalies, myotonia, and epilepsy syndromes. Currently employed aminoamide blockers target sodium channels in the open and inactivated states, inhibiting both transient and resurgent sodium currents. A more comprehensive mapping of channel docking surfaces for A-type FHF and Nav β 4 particles may enable design of related small compounds with greater specificity for different channel states and improved therapeutic efficacies.

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Figure 1.

Topology and interactions of voltage gated sodium channel alpha subunit, beta subunit and FHF. **Nav α:** The alpha subunit of each sodium channel (Nav1.1 – Nav1.9) consists of four pseudo-homologous domains (D), each bearing six transmembrane helical segments (S) and partial reentrant loops between S5 and S6 that form the sodium ion selectivity filter. The S4 segments (blue) have periodic cationic residues that constitute the voltage sensors, while the S6 segments (red) define the inner walls of the channel beneath the ion selectivity filter. The channel has cytoplasmic amino (N) and carboxyl (C) tails, and the four domains are connected by cytoplasmic loops (not to scale). The small DIII/IV loop includes a short α -helical region and the IFM triad required for fast inactivation. The C-tail critically modulates fast inactivation and is the site for physical interactions with channel β subunits and FHF (arrows). **Nav β:** Beta subunits (Nav2.1 – Nav2.4) are single-pass transmembrane proteins with an extracellular immunoglobulin-like domain and a short cytoplasmic tail that mediates binding to Nav α . Nav β 4 bears a unique motif in its C-tail that serves as a channel blocking particle responsible for resurgent current. **FHF:** FHF (FHF1 – FHF4, each with multiple isoforms bearing different N-termini) are small cytoplasmic proteins that assume a β -trefoil fold bearing the surface for interaction with the Nav α C-tail. The A-type isoforms of FHF have N-terminal motifs that serve as long-term inactivation particles. Nav β 4 and A-type FHF particles terminate transient sodium current by competing with the Nav α fast inactivation machinery.

Figure 2. Sodium channel gating. **A)** Schematic flow diagram of fast sodium channel dynamics. Voltage driven conformation transitions (thick arrow) lead to channel activation upon depolarization (rightward, unfilled) and deactivation at more negative membrane potential (leftward, solid). Activating transitions enable intrinsic fast inactivation (downward arrows) from closed or open states, from which channels recover upon sufficient deactivating transitions. A-type FHFs enable alternative long-term inactivation (upward arrows) from near-open or open states. Navβ4 mediates channel block (diagonal arrow) at positive membrane potential, and expulsion of the blocking particle as membrane potential trends negative channels generates transient resurgent current. **B)** Markov state modeling of activation, inactivation, and block. Voltage-driven channel transitions are along the horizontal axis, while voltage-independent transitions are shown on orthogonal axes. For voltage-independent transitions, relative sizes of arrowheads indicate favored direction of transition. **Black:** Depolarizing membrane potential drives channels through closed states (C) towards an open conducting state (O), with concomitant increasing rates for transition to inactivated states (I) [11]. Sufficiently strong depolarization thereby generates transient sodium current before inactivation. Upon return to more negative potentials, inactivated channels deactivate (I6→I5→etc.) before recovering from inactivation (I→C), so that channels do not conduct as they reset. **Red:** A-type FHFs mediate an alternative competing mechanism for transitions to long-term inactivated states (L) [45]. Upon return to more negative potentials, deactivation rates (L6→L5→etc.) are far slower than following fast inactivation, increasing the time until channels are again available. **Green:** Positive

membrane potentials approaching the Na^+ reversal potential limits sodium flux through open channels, generating a permissive state (Op) for block by Nav β 4 (OpB). Subsequent return to negative potentials allows inward sodium permeation (OB), causing expulsion of the blocking particle (OB $\rightarrow$ O) and consequent resurgent current [15,68]. **Blue:** Additional states added to reflect the ability of open channels to transit to any of the three non-conducting states (Ip6, Lp6, OpB).