Abstract

The coincident cloning of the voltage-gated Na channel from the electric eel electroplax and development of patch-clamp methodology has allowed an explosive phase of investigation into the structural basis of cardiac Na channel function. Recognizing the importance of structural motifs that underlie gating (charged S4 segments, III–IV linker) and permeation (P-loops) have complemented new molecular information surrounding inherited cardiac arrhythmias, such as the chromosome 3-linked form of the long QT syndrome. Although the proarrhythmic potential recognized in the CAST trial [1] slowed the development of class I antiarrhythmic agents, our emerging understanding of the molecular pharmacology of Na channels may motivate strategies for Na-channel drug discovery that involve targeting particular structural domains.

Keywords: Sodium channels; Voltage-gated ion channels

1. Introduction

Voltage-gated sodium (Na) channels are transmembrane proteins responsible for the rapid upstroke of the cardiac action potential, and for rapid impulse conduction through cardiac tissue. As such, Na channel function is central to the genesis of cardiac arrhythmias and their complex pharmacology. Although a static crystal structure is now available for a voltage-gated potassium (K) channel from *Streptomyces lividans* [2], definitive structural data are not yet available for Na channels. Moreover, like K channels, Na channels are dynamic molecules that change their structural conformation in response to transmembrane electrical fields. Hence, our understanding of Na channel structure–function relationships is enhanced by studies in polarizable membranes or bilayers.

The first view of Na channel function was formulated in the elegant work of Hodgkin and Huxley [3]. Their studies provided a rationale for excitability based on the elementary properties of cation permeation (flux through the pore) and gating (dynamic conformational changes in response to membrane potential fluctuations). In the 1980s, Na channels were the first voltage-gated ion channels to be cloned and sequenced [4], ushering in a decade of molecular dissection exploiting the complementary strategies of genetic manipulation (e.g., site-directed mutagenesis) and patch-clamp electrophysiologic methods [5]. While these studies support the general features of ion translocation proposed by Hodgkin and Huxley [3], it is clear that the gating conformational changes are far more complex than first envisioned, and that the elementary processes of gating and permeation are linked both structurally and functionally.

This paper presents a contemporary view of cardiac Na channel structure and function. Mutagenesis studies elaborating the processes of permeation and gating will be described and then used as a scaffold to discuss related topics, including post-translational modification, α–β subunit interactions and antiarrhythmic pharmacology. Because the scope of the review is broad, the citations and discussion will be selective, and the reader is referred to more thorough treatments of particular subtopics [6–9].

2. Structural features of Na channel permeation

Our understanding of ion channel pore structure has
evolved with cloning and expression of the channel proteins (Fig. 1) [10,11]. The Na channel consists of a principal α-subunit composed of four homologous domains (I–IV), each containing six transmembrane segments (S1–S6). The four domains are attached to one another by cytoplasmic linker sequences. Conversely, the voltage-gated potassium (K) channels related to the Drosophila Shaker channel are encoded as single six-transmembrane segment subunits that later assemble as tetrad structures to produce functional channels. Although the amino acid sequences of the Na and K channels are not highly homologous, hydrophobicity analysis suggests a topological analogy. Hence, our first clues to the location and character of the Na channel pore came from structure–function studies on voltage-gated K channels. Studies have clarified that residues between the fifth and sixth transmembrane segments turn back into the membrane and line the pore, hence the term ‘P-loop’. Naturally occurring K-channel variants with differential sensitivities to tetraethylammonium (TEA), a well-known blocker of the K-channel pore [12,13], vary predictably in the P-loop [14].

The critical role of the P-loop in permeation and block was demonstrated by mutational analysis. Single-residue modifications in this region alter charybdotoxin binding (another pore-blocking peptide) [15], both internal and external TEA sensitivity [16,17], and cation selectivity and conductance [18]. Chimeric analysis definitively confirmed that the permeation properties associated with particular P-loop sequences are transferable between K channels [19].

Although the amino acid sequence differs markedly from that of the K channel, the Na-channel P-loop is highly conserved among species- and tissue-specific isoforms. Inspection of the primary structures of the P-loops in each domain (Fig. 2) reveals that each is unique. The structural basis of permeation thus differs fundamentally from that of K channels, in which four identical P segments can come together to form a K–selective pore. The four P-loop domains of the L-type calcium channel α1-subunit are also asymmetric, and are homologous to the Na channel (Fig. 2). In both Na and Ca channels, the P-loop asymmetries are critically linked to the unique permeation characteristics of channels. In domain III of the Na channel, a lysine in the selectivity filter region (K1418 in the human heart Na channel, Fig. 2) critically selects for Na+ over Ca2+ [20,21]. Conversely, Ca channels have a glutamate as the sequence-aligned residue in all four domains (Fig. 2). Although this ring of negative charge cooperatively provides a high-affinity Ca2+-binding site, the binding contributions from each of the domains exhibit considerable asymmetry [22]. Residues in the Na channel domain I P-loop also mediate divalent cation binding, as well as isoform-specific toxin affinity [23,24]. A cysteine that is one position C-terminal to the selectivity filter residue in domain I (Fig. 2) renders the cardiac channel insensitive to blockade by guanidinium toxins (TTX or STX), but sensitive to cadmium or zinc. Conversely, an aromatic residue at the equivalent position (the skeletal muscle or neuronal isoforms) renders the channel TTX sensitive, but cadmium insensitive. Furthermore, acces-
sibility mapping studies have revealed marked asymmetries in the positioning of each domain relative to the permeation pathway [25,26]. As such, the P-loop regions in each domain are uniquely involved in defining permeation and binding characteristics of the pore.

The positively charged guanidinium toxins compete with Na and other permeant cations for the same (or overlapping) sites in the external pore of the channel [27], and have been useful as probes to evaluate the structure of the outer pore. Detailed mutagenesis studies have mapped the residues in the P-loops of all four domains that mediate toxin sensitivity [28]. Given the small size and determined structure of the toxins (as derived from X-ray structural analysis), it has been possible to analyze these data using computational energy minimization algorithms for the intermolecular and intramolecular forces to predict structural characteristics for the individual P-loops [6,29]. The Lipkind–Fozzard model satisfactorily reproduced the effects of P-loop mutations on toxin affinity, and proposed a pore lined by four $\beta$-hairpins in a funnel-shaped structure, terminating in a narrowed region at the level of the putative selectivity filter (Fig. 2). Two carboxyl residues from domains I and II (D, E) formed an energetically favorable coordination site for binding Na$^+$ (in preference to K$^+$ or Ca$^{2+}$) and removing bound water molecules. Recall that mutation of the domain III residue (K$\rightarrow$E) renders the Na channel Ca$^{2+}$-permeant [20], supporting the evidence that at least three carboxylic acids are involved in coordinating Ca$^{2+}$. Although the structural details of the selectivity filter require additional investigation, the evidence suggests that Na and Ca channels possess structural analogies (and no doubt, common ancestry) in the pore regions [30].

While studies of toxin binding have been invaluable for defining general features of pore structure, ongoing studies involving cysteine-scanning mutagenesis of the P-loops (sequential replacement of native residues with cysteines) suggest that the details of secondary structure proposed in the Lipkind–Fozzard model require some revision. For example, a $\beta$-hairpin structure for the P-loops implies a periodic accessibility of residues to the aqueous pore. To examine the disposition of the P-loop side chains, hydrophilic methanethiosulfonate (MTS) reagents may be used to modify the thiol side-chains of each substituted cysteine in the pore. The MTS reagents react with mixed disulfides, adding bulky, charged moieties to free cysteins in an aqueous environment. Attachment of these moieties in the narrow pore region alters ion flux, as readily measured with single-channel patch–clamp methods. Cysteines placed at nearly all positions in the P-segments of all four domains can be modified by MTS reagents [26], a result inconsistent with either an $\alpha$-helical [31] or $\beta$-hairpin [29] periodic structure for the pore-lining segments. Furthermore, the ability of paired cysteines to form spontaneous disulfides bridging domains I and II implies that the P segments of these two domains are closely opposed (within 7 Å), a finding inconsistent with the P-loop packing constraints of $\alpha$-helices or $\beta$-hairpins [32]. The picture that emerges poses fewer constraints upon the molecular models: an asymmetric pore lined mainly by extended loops or random coils [32].

As the structural information expands, it still remains unclear how residues forming the putative selectivity filter permit selective flux of Na$^+$ over other cations by a factor of 100:1 or more, while at the same time allow high throughput rates approaching $10^8$ ions/s. Again, an analogy to the potassium channel may provide critical insights. Recent crystal structural data from the potassium channel from Streptomyces lividans suggest a structural motif for permeation and selectivity that may apply to all voltage-gated ion channels [2]. The major features are (1) a large water-filled cavity and hydrophobic channel-lining that minimizes the distance over which the cation interacts with the channel, while lowering the energy barrier for occupancy of the low-dielectric membrane center. (2) A relatively short selectivity filter that is lined by carbonyl oxygen atoms optimally positioned to coordinate and dehydrate a cation of the correct size. (3) A filter region that supports multi-ion occupancy, with ion–ion repulsion facilitating rapid conduction in a setting of high selectivity. Another structural motif recently described in Na channels, supporting an analogy between channels and enzymes, derives from evidence that the Na channel pore, like many enzymes, exhibits a high degree of conformational flexibility [33]. Pairs of cysteine residues engineered into the P-loops of domains I and II can form spontaneous internal disulfide bridges in specific patterns that could not arise without substantial mobility within the molecule, on the order of several angstroms [34,35]. Although the mechanism whereby pore motion supports permeation and selectivity is undefined, the importance of flexibility in ion translocation is underscored by evidence that disulfide crosslinking renders the channel less Na$^+$-selective [34].

3. Structural features of Na-channel gating

Hodgkin and Huxley [3] proposed that Na channels transit among various conformational states in the process of opening (‘activation’). They further proposed that a distinct conformation is entered when the channels shut during maintained depolarization (‘inactivation’), thereby providing a biphasic inward Na current. The activation and inactivation ‘gates’ were postulated to have intrinsic voltage dependence and to function independently [3]. While the essence of their gating prediction has withstood the test of time, the more recent molecular studies suggest that Na-channel gating processes are better understood as complex allosteric interactions among multiple structural domains.
3.1. Structural loci for inactivation gating

Site-directed mutations have implicated specific regions of the Na channel in gating. Inactivation can be disabled by antibodies directed towards [36], or by incision through [37], the cytoplasmic linker connecting domains III and IV (Fig. 3). Site-directed mutation of one or more residues that form a hydrophobic triplet (IFM) in the III–IV linker disrupts inactivation gating [38]. With inactivation disabled, Na currents exhibit a plateau of persistent inward current during maintained depolarizations (Fig. 4, left). Naturally occurring mutations in the III–IV linker also disrupt inactivation, and have been linked to skeletal muscle myotonias [39] as well as to inherited forms of the...
long-QT syndrome (see below) [40,41]. It is tempting to view the III–IV linker as a ‘lid’ that closes over the inner vestibule and, in this context, the IFM motif as a ‘latch’ that holds the inactivation gate shut. Nonetheless, peptide-binding studies suggest that inactivation may not involve simple occlusive block of the inner mouth of the pore [42]; rather, the III–IV linker may bind to a site on the channel that allosterically regulates or mediates pore closure.

Mutagenesis studies have identified a number of additional residues that reside in cytoplasmic loci consistent with ‘docking sites’ for the inactivation gate. Residues on the cytoplasmic end of the domain IV, S6 segment (Fig. 3) are thought to form part of the hydrophobic receptor site for the inactivation gate [43] or may stabilize the inactivated state through an indirect mechanism [44]. Residues in cytoplasmic loops proximal to the III–IV linker, including the domain III [45] and domain IV [42,46–49] S4–S5 loops also may form part of a ‘docking site’ if the III–IV linker is a fast inactivation particle. Indeed, Na-channel mutations linked to congenital neuromuscular syndromes spanning nearly the entire mutations linked to congenital neuromuscular syndromes the IFM mutations in the III–IV linker [38,63], although the III–IV linker is a fast inactivation particle. Indeed, Na-channel mutations linked to congenital neuromuscular syndromes spanning nearly the entire the α-subunit modify fast inactivation, suggesting that Na-channel inactivation depends upon complex ensemble interactions among many structural domains [50].

3.2. Activation and inactivation are coupled

The S4 segments in each domain contain large numbers of positively charged residues that underlie activation (Fig. 3), the voltage-dependent gating process responsible for channel opening [37]. While Hodgkin and Huxley [3] initially cast activation and inactivation as independent processes, gating current [51] and single-channel measurements [52] provided the first evidence that activation and inactivation are functionally coupled. This hypothesis has been elegantly confirmed by recent mutagenesis experiments involving mutations in the outer S4 segment of domain IV. Replacing the charged arginine residues with cysteines markedly slows the rate of inactivation during depolarization [53,54]. Indeed, naturally occurring mutations in the domain IV, S4 segment are implicated in both skeletal muscle myopathies [54] and the long QT syndrome (see below) [55]. Covalent modification of cysteines substituted into the S4 segment with bulky MTS reagents irreversibly reconstitutes rapid inactivation, as if the MTS appendage ‘locks’ the S4 segment in an outward position that eases binding of the inactivation gate to its receptor [56]. It appears that the domain IV–S4 charged segment serves as the voltage-sensor for both activation and inactivation, and that outward motion of this segment is a prerequisite to normal inactivation gating. This promiscuity does not apply selectively to the domain IV–S4 segment; recent studies suggest that the charged S4 segments in all four domains contribute to both activation and inactivation, but to varying degrees [57–59].

3.3. Na-channel mutations linked to arrhythmias

Patients with congenital long-QT syndrome are predisposed to life-threatening ventricular arrhythmias due to delayed ventricular repolarization, which has been linked to mutations in genes that encode the voltage-gated ion channels. One inherited form (LQT3) is associated with mutations in the cardiac Na-channel gene (SCN5A) [40,60,61]. In general, the Na-channel mutations linked to cardiac arrhythmias modify inactivation and reside at loci consistent with this gating effect (Fig. 3). The LQT3 mutations all reduce the stability of inactivation, generating a small noninactivating plateau of inward current during depolarization (Fig. 4, middle) [41,62]. This defect presumably causes persistent inward current during action potential repolarization, prolonging the QT interval and setting the stage for fatal ventricular arrhythmias. As predicted from their loci, these mutations induce inactivation gating defects that share qualitative similarities with the IFM mutations in the III–IV linker [38,63], although they are less severe in magnitude (Fig. 4).

However, detailed examination of the gating phenotypes of the mutations linked to cardiac arrhythmias reveal subtle distinctions. For example, the LQT3–ΔKPQ deletion mutant exhibits transient periods when the channels gate in an inactivation-impaired bursting mode [41]. Recently, a sporadic SCN5A mutation was identified in domain IV–S4 (R1623Q, Fig. 3) [64], a locus linked to activation–inactivation coupling. In contrast to the LQT3 mutations, R1623Q produced a three-fold prolongation of Na current decay (Fig. 4, right) [55,65]. Mutations in the Na channel have also been identified in families with idiopathic ventricular fibrillation and characteristic electrocardiographic features (Brugada’s syndrome, Fig. 3) [66,67]. In addition to a frameshift mutation that produces nonfunctional channels, a missense mutation was identified in the domain IV S3–S4 extracellular loop that destabilized Na-channel inactivation (increased recovery from inactivation and shifted steady-state inactivation to more positive membrane potentials). Like the domain IV–S4 segment, this contiguous extracellular loop has also been mechanistically linked to activation–inactivation coupling [68].

4. Local anesthetic/class I drug action

4.1. Local anesthetic receptor

Local anesthetic (LA)/class I antiarrhythmic agents are a structurally diverse group of compounds that suppress excitability in both neuronal and cardiac tissues by blocking Na current. The requirements for LA action are satisfied by a number of compounds that contain hydrophilic and hydrophobic domains separated by an amide or ester linkage. These compounds are commonly used to treat disorders of electrical excitability, including seizures.
and cardiac arrhythmias. Unfortunately, the success of the LA compounds in treating cardiac arrhythmias has been limited, and such therapy may have dangerous proarrhythmic potential [1].

The prevailing scheme for LA action, termed the modulated receptor model [69,70], attributes the complex time- and voltage-dependent effects of LAs to distinct binding affinities for the three putative gated conformational states of the Na channel (closed→open→inactivated) [3]. Mild suppression of Na current during isolated, brief depolarizations is attributed to low affinity binding for the closed channel conformation, while far greater current suppression during repetitive depolarization, also known as use dependence [71], is attributed to high-affinity binding to states occupied during depolarization (either open or inactivated). Importantly, this model implies not only distinct high and low affinity drug–receptor sites, but also a complex interrelationship between channel gating and drug action. A competing hypothesis, termed the guarded receptor model [72], suggests that voltage- and time-dependent drug effects are explained by a single drug receptor with access modulated by channel gating. Armed with molecular methods, pharmacologists and biophysicists have examined the drug-channel interaction in greater detail by altering residues predicted to form the LA receptor(s), as well as by modifying particular gating processes that critically impact LA action.

Some of the most intriguing insights have been offered by the molecular studies of Ragsdale et al. [73,74]. Specific residues in the S6 segment of domain IV figure critically in use-dependent block by local anesthetics (Fig. 5). Two particular mutations (F1764A and Y1771A in rat brain IIA Na channels) increase the IC50 for use-dependent action of lidocaine, etidocaine and phenytoin by up to two orders of magnitude. Voltage-clamp studies proposed that these effects resulted primarily from decreased drug affinity for the inactivated channel conformation. Indeed, residues at comparable S6 loci have been implicated in the quinidine block of K channels [75]. Similarly, sites in domain IV, S6 have been linked to both dihydropyridine and phenyalkylamine block of Ca channels [76,77], although residues in the S5 segments of both domains III and IV also appear to be involved in dihydropyridine block [78,79]. It is noteworthy that, in Na channels, at least one of the residues implicated in local anesthetic block (F1764) importantly affects inactivation gating [43,44]. While it is likely that domain IV, S6 forms part of the local anesthetic receptor, the fact that mutations in this location also influence inactivation gating makes it difficult to exclude the possibility that some of the mutational effects on drug action are indirect. In support of this notion, recent studies have shown that mutations in the outer pore, C-terminal to the selectivity filter and the putative LA binding site, alter inactivation gating and LA block [80].

A similar conundrum exists with mutations in the III–IV linker. Early voltage-clamp studies of LA action in nerve revealed that Na channels rendered inactivation-deficient by internal exposure to proteases exhibited marked resistance to use-dependent block [81]. More recent use of site-directed mutagenesis to selectively disable inactivation strengthens the hypothesis that gating structures poised on the cytoplasmic face of the pore are important for LA action. Replacing a hydrophobic triplet of residues in the cytoplasmic III–IV linker with glutamines (Fig. 4: IFM→QQQ) both disables fast inactivation [38] and eliminates use-dependent block of hH1 Na channels by 50 μM lidocaine [82]. Experiments that disable the inactivation gate to varying degrees offer additional insight [83]. With the addition of lidocaine, the channels with inactivation partly disrupted (F→Q) seem to recover their more rapid inactivating character, while channels with a more severe disruption (IFM→QQQ) are more resistant (Fig. 6).

Fig. 5. Sites of interaction between local anesthetics and the Na channel. Highly schematic view of the Na channel (represented as a cylinder, from Fig. 1) and possible interaction sites. The front and back walls of the cylinder are transparent, and the four P loops from domains I–IV are shown as they taper into the selectivity filter. Filter residues in domains I, III and IV prevent extracellular access and egress of the charged LA agent from below, as well as in the IV domain, S6 residue I1756. The domain III selectivity filter residue (K) repels the LA. Substitution of residues in domain IV, S6 segment and in the III–IV linker enhance use-dependent LA block.

Fig. 6. Comparison of lidocaine effects with graded III–IV linker mutations. Whole-cell currents were recorded from Xenopus oocytes expressing wild-type and mutant Na channels (rat skeletal muscle μ1). The currents were recorded at bath lidocaine concentrations of 0, 100, 300 and 1000 μM, as shown beside the QQQ mutant. Lidocaine speeds the inactivation of current through the FQQ channels, but has less kinetic effect on the QQQ currents. Modified from Balser et al., 1996 [83].
Hence, greater disruption of the inactivation gating process incrementally disables the inactivation-enhancing action of lidocaine. Rather than mimic inactivation through open-pore block with comparable binding kinetics, this finding supports the conclusion that lidocaine actually enhances inactivation gating, serving as 'molecular glue' [84], in support of the early predictions by Hille [69], Cahalan [81] and others [85,86]. When exposed to low concentrations of the class IB antiarrhythmic agents (lidocaine, mexiletine), channels with either the inherited [62,87,88] or spontaneous [55] long-QT mutations also recover inactivation gating behavior, approaching the wild-type phenotype, suggesting an analogy between the long-QT mutants and the engineered 'IFM' mutants in regard to class I drug action. Lidocaine also normalizes the recovery from inactivation in both F→S or mutant channels [83] and the LQT3 mutants [87].

It is tempting to explain many of the effects of class I agents on the long QT mutants in terms of inactivation-enhancing drug action. However, suppression of LQT3 Na current has not been examined with single-channel methods, and a kinetic scheme involving open-pore block with selective suppression of late openings has been proposed [88]. Moreover, a pore-plugging motif for LA molecules is well supported by evidence that the charged LA derivatives (e.g. QX-314) move deeply (50–70%) into the pore from the cytoplasmic side [83,89,90]. Consistent with this prediction for a hydrophilic local anesthetic binding site, P-loop residues in the putative selectivity filter (Fig. 2) markedly influence local anesthetic block [91]. Selectivity filter residues in domains I, II and IV limit external access (and escape) of hydrophilic LAs, and the domain III lysine residue repels the LA through an electrostatic interaction with the ionizable amino group (Fig. 5). It is of some interest that cardiac Na channels, unlike neuronal or skeletal muscle isoforms, are sensitive to external block by charged lidocaine derivatives [92]. A valine residue in domain IV, S6, just above the putative LA receptor site, inhibits extracellular block by QX-314 in neuronal channels, and substitution with the cardiac residue (threonine) restores external block [93]. These findings implicate the domain IV–S6 segment as a critical pore-lining locus that controls binding, access and egress of the hydrophilic LA moiety, and locate IV–S6 adjacent to the P-loop segments that comprise the selectivity filter [91].

How can we rationalize the effects of class I agents on inactivation gating, while at the same time acknowledge the pore-binding phenomena of the hydrophilic moiety? Studies of QX-314 block of papain-modified single cardiac Na channels have revealed two modalities of block, including a rapid-block process that reduces the unitary current, and a slower, more stable block that causes discrete interruptions in current. It was proposed that the rapid-block configuration may serve as a transition intermediate that catalyzes formation of the more stable block complex [89]. Hence, it is worth considering the hypothesis that binding of the charged LA moiety to a receptor deep in the pore, near the selectivity filter, provides a transition intermediate (rapid-block, low affinity) that leads to a more stable interaction between the hydrophobic tail of the LA and its (distinct) receptor (e.g., Fig. 5). It is plausible that the latter receptor involves residues that also have a role in inactivation gating (e.g., IV–S6 and/or the III–IV linker, Fig. 3). Studies of batrachotoxin-modified cardiac Na channels in planar lipid bilayers also suggest separate sites for fast lidocaine block of the open channel and kinetically slower block of the ‘inactivated’ channel [94,95]. Moreover, recent studies suggest that the residues in domain IV–S6 that reduce use-dependent LA block [73] may be more intimately associated with this stable block complex [96].

5. Isoform-specific features of cardiac Na channels

5.1. Modulation by auxiliary subunits

Auxiliary (β) subunits are important modulators of Na-channel gating and expression. Biochemical studies revealed two distinct β subunits (β1 and β2) associated with the brain Na channel [97]. Both β subunits have been cloned, and the deduced primary structures indicate that they are unrelated proteins of molecular weights 23 and 21 kDa, respectively [98,99]. Both contain a small carboxy-terminal cytoplasmic domain, a single membrane-spanning segment, and a large amino-terminal extracellular domain with several consensus sites for N-linked glycosylation and immunoglobulin-like folds. The human β1 subunit is widely expressed in skeletal muscle, heart and neuronal tissue, and is encoded by a single gene (designated SCN1B) [100]. Brain Na channel α-subunits are linked to the β3-subunit by a disulfide bond, and are attached to the β3-subunit noncovalently, while skeletal muscle Na channels are composed of only α- and β1-subunits [101,102].

The subunit composition of cardiac Na channels is less clear. Coexpression of β1-subunits with either neuronal or skeletal muscle α-subunits produces clear-cut effects on channel function. The current density increases, and activation and inactivation gating are both enhanced [103–106]. However, the functional role of the β1-subunit in the heart is less certain. While studies consistently show that β1-subunit coexpression in Xenopus oocytes enhances the current magnitude, the studies differ in regard to whether gating effects are present [107] or absent [100,108]. Antibodies directed to the cardiac α-subunit do not immunoprecipitate any accessory proteins [109]. Nonetheless, Northern blot analysis reveals β1 messenger RNA in heart [103], and it was recently shown in HEK-293 cells that coexpression of either the human or rat β1 isoform with the human heart (hH1) α-subunit shifts the voltage-dependence of steady-state inactivation to more depolarized membrane potentials [110] (it is of interest that β1 has
consistently shifted the voltage-dependence of inactivation to more hyperpolarized potentials in oocytes [103–105]). Furthermore, suppression of \( \beta \)-subunit expression in mouse atrial tumor (AT-1) cells using antisense oligonucleotides prevents the development of the mature (more rapid) gating phenotype [111].

The structural features of the noncovalent \( \alpha-\beta \)-subunit interaction are complex, and chimeric studies suggest that no single domain of the \( \alpha \)-subunit is involved [112]. Gating studies suggest that the domain I and IV P-loops (S5–S6) of the \( \alpha \)-subunit and the extracellular amino-terminal end of the \( \beta \)-subunit [113,114] are of considerable importance. While the intracellular domain of the \( \beta \)-subunit seems to have no effect on the rate of Na-channel inactivation, it was recently shown that a novel LQT3 mutation (D1790G) in the intracellular C-terminus of the hH1 \( \alpha \)-subunit (Fig. 3) eliminates the \( \beta \)-induced shift in the voltage-dependence of steady-state inactivation in HEK-293 cells [110]. Importantly, \( \beta \)-subunit interactions may influence LA/class I drug action. In oocytes, coexpression of \( \beta \) with cardiac [115] or skeletal muscle [116] Na-channel \( \alpha \)-subunits attenuates use-dependent block by lidocaine.

### 5.2. Isoform-specific gating and local anesthetic block

When lidocaine is administered systemically, cardiac Na channels are blocked preferentially to those in skeletal muscle or nerve. Although studies have suggested that cardiac Na channels may be intrinsically more sensitive to \( \alpha \)-blockers than are skeletal-muscle channels [107,119], and studies of the cardiac-specific functional effects [138], the structural features of the noncovalent \( \alpha-\beta \)-subunit interaction may be involved in this regulatory pathway [111].

#### 5.3. Second messenger regulation

Regulation of the Na channel by protein kinases is isoform-specific. The neuronal isoforms have a large intracellular linker between domains I and II that contains five consensus phosphorylation sites for cAMP-dependent protein kinase (PKA). Phosphorylation of these sites reduces current amplitude without significantly altering gating [124,125]. Conversely, the cardiac channel has just two serine residues in the I–II linker that are phosphorylated by PKA, and these sites are distinct from the consensus sites in the neuronal Na-channel [126]. When stimulated by PKA, cardiac Na-channel gating remains unchanged, but the whole-cell conductance increases (in contrast to the neuronal isoforms), suggesting that the distinct pattern of phosphorylation may be responsible for the isoform-specific functional effect [126–128]. The effects of upstream receptor-activated stimulation are less clear, with both increases and decreases in current being reported after treatment with cAMP-elevating agents or membrane-permeant cAMP analogs [129–133], and a rapid G-protein pathway of direct Na-channel modulation has been proposed [130,133]. In addition, in vitro studies suggest that cAMP may have a maturational role in cardiac Na-channel development. Na currents measured in cultured neonatal cardiac cells exhibit ‘immature’ slow gating kinetics, but coculture with membrane-permeant cAMP analogues or sympathetic neurons restores a ‘mature’ rapid-gating phenotype [134–136]. As discussed above, studies with \( \beta \), antisense in maturing AT1 cells suggest that an \( \alpha-\beta \) subunit interaction may be involved in this regulatory pathway [111].

Protein kinase C (PKC) also modulates cardiac Na channels, both reducing the maximal conductance of the channels and altering gating. Both effects are largely attributable to phosphorylation of a serine residue in the III–IV linker that is conserved in the neuronal and cardiac isoforms [137]. Interestingly, while PKC phosphorylation of this conserved serine reduces Na current in both isoforms, the gating effects diverge. Phosphorylation of this site in the cardiac isoform induces a negative shift in the voltage-dependence of steady-state inactivation (implying stabilized inactivation), while in brain Na channels, phosphorylation of the same residue slows and destabilizes inactivation [137]. Because the III–IV linker sequences are conserved, interaction of the phosphorylated serine with other domains may differ for the two isoforms. Studies have also implicated isoform-specific PKC sites in some of the cardiac-specific functional effects [138].

### Acknowledgements

Salary support provided by NIH R01GM56307 and the Clinician Scientist Award of the American Heart Association.
References


20 Nuss HB, Chiamvimonvat N, Perez-Garcia MT et al. Functional association of the beta 1 subunit with human cardiac (hH1) and rat skeletal muscle (mu1) sodium channel alpha subunits expressed in Xenopus oocytes. J Gen Physiol 1995;106:1171±1191.


30 Nuss HB, Tomaselli GF, Marban E. Cardiac sodium channels (hH1) are intrinsically more sensitive to tonic block by lidocaine than are skeletal muscle (mu1) channels. J Gen Physiol 1995;106:1193±1210.


33 Nuss B, Kambouris NG, Tomaselli GF et al. Isoform-specific differences in gating underlie the enhanced susceptibility of cardiac sodium channels to lidocaine. Circulation 1997;96:1±120.


