



## Basic Cardiac Electrophysiology

# The Cardiac Sodium Channel: Gating Function and Molecular Pharmacology

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J. R. BALSER. The Cardiac Sodium Channel: Gating Function and Molecular Pharmacology. *Journal of Molecular and Cellular Cardiology* (2001) 33, 599–613. Cardiac sodium (Na) channels are dynamic molecules that undergo rapid structural changes in response to the changing electrical field in the myocardium. Inherited mutations in *SCN5A*, the gene encoding the cardiac Na channel, provoke life-threatening cardiac arrhythmias, often by modifying these voltage-dependent conformational changes. These disorders (i.e., the long QT syndrome and Brugada syndrome) may serve as valuable models for understanding the mechanistic linkages between Na channel dysfunction and cardiac arrhythmias in more common, acquired conditions such as cardiac ischemia. In addition, the balance between therapeutic and adverse effects from Na channel blockade by antiarrhythmic compounds may be shifted by subtle alterations in Na channel function. This review examines recent studies that tie key loci in the Na channel primary sequence to its dynamic function, while examining the emerging themes linking Na channel structure, function, and pharmacology to inherited and acquired disorders of cardiac excitability. © 2001 Academic Press

KEY WORDS: Sodium channel, Antiarrhythmic drugs, Lidocaine, Flecainide, Long QT syndrome, Brugada syndrome.

## Introduction

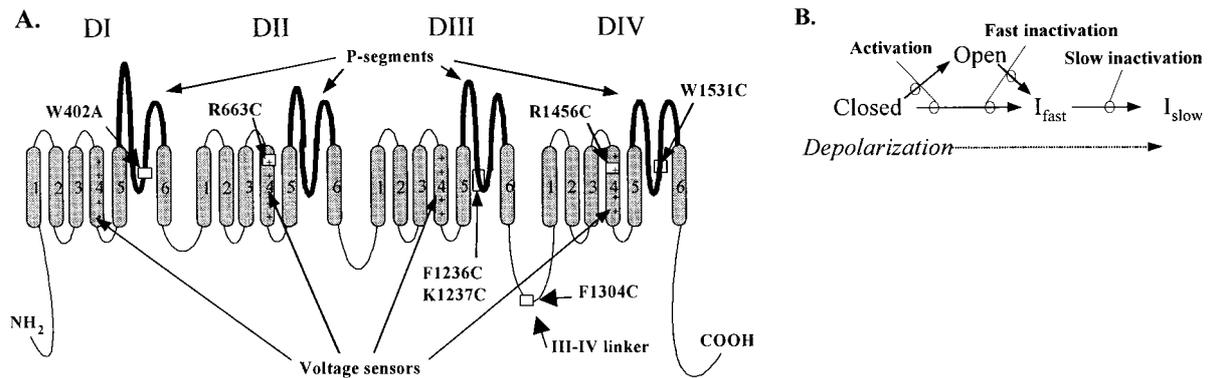
Voltage-gated sodium (Na) channels are transmembrane proteins responsible for the rapid upstroke of the cardiac action potential, and are therefore responsible for rapid impulse conduction through much of the myocardium. As such, cardiac Na channels are key molecular substrates in both inherited and acquired disorders of cardiac excitability. Na channel blockade as a general antiarrhythmic strategy has proven to be a dual-edged sword, with significant proarrhythmic risks.<sup>1</sup> However, current progress in resolving ion channel structure, function, and molecular pharmacology foreshadows precise strategies to modify favorably Na channel function and cardiac excitability.

A crystal structure is now available for a potassium (K) channel from *Streptomyces lividans*,<sup>2</sup> and high-resolution structural data are even becoming available for small segments of the Na channel.<sup>3</sup> At

the same time, Na channels are dynamic molecules that drastically change their structural conformation on a sub-millisecond time scale in response to the transmembrane electrical field, a process termed “gating”.<sup>4</sup> As genomic strategies increasingly link disorders in electrical excitability to cardiac Na channel mutations, functional studies of the “diseased” channels reveal pathologic gating defects. Whereas the salutary and toxic effects of antiarrhythmic drugs hinge critically upon gating transitions,<sup>5,6</sup> progress in drug design will require integration of the developing picture of Na channel structure with a refined image of gating function.

This review presents an emerging view of cardiac Na channel gating function and pharmacology. Structure–function relationships will be used as a scaffold to discuss mechanisms of arrhythmogenesis due to inherited Na channel mutations, as well as proarrhythmic effects of Na channel-blocking antiarrhythmic agents. As the discussion and citations

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**Figure 1** A: Topology of the voltage-gated Na channel. The S4 segments in each of the four homologous domains contain positively charged lysine and arginine residues that detect changes in the transmembrane field. The bold S5–6 linker sequences are “P-segments” that line the outer pore. The III–IV linker plays a major role in fast inactivation. Individual amino acid residues discussed in the review are numbered according to the original rat skeletal muscle clone.<sup>13</sup> B: Simplified scheme of the Na channel gating conformational changes in response to membrane depolarization. Activation and fast inactivation occur nearly simultaneously, such that channels sometimes reach the fast inactivated state ( $I_{fast}$ ) without opening (closed-state inactivation). Sustained depolarization induces channels to occupy stable, slow inactivated states ( $I_{slow}$ ).

will be selective, the reader is referred to more thorough treatments of related topics including Na channel regulation by second messengers and enzymes, details of cation permeation, and modulation by subsidiary ( $\beta$ ) subunits.<sup>7–10</sup>

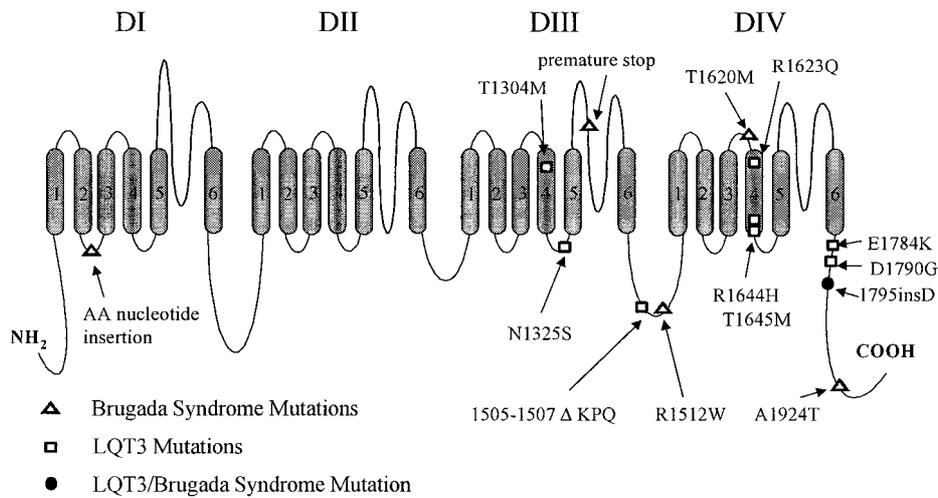
### Sodium channel gated states: linking structure to function

In contrast to the number and diversity of voltage-gated K channels, the voltage-gated Na channels are relatively small in number and are highly homologous in amino acid sequence. Of the 11 isoforms identified to-date, eight have been functionally characterized (see recent review by Catterall, 2000)<sup>9</sup> and reside in neuronal tissue, skeletal muscle, and cardiac muscle. Our understanding of Na channel structure and gating function has escalated with cloning and expression of the channel protein.<sup>11</sup> While many of the original studies correlating structure and function used skeletal muscle or neuronal isoforms, the themes that emerged proved to be generalizable to the cardiac isoform.

The tetrodotoxin “insensitive” human cardiac Na channel (hH1), encoded by the gene *SCN5A*,<sup>12</sup> is composed of a principal ( $\alpha$ ) subunit that includes four homologous subunits or domains [numbered I–IV, Fig. 1(A)], attached to one another by cytoplasmic linker sequences. Over the last decade, site-directed mutagenesis has been utilized in concert with patch-clamp electrophysiologic measurements

to define specific amino acid residues involved in voltage-dependent gating function.<sup>9</sup> Figure 1(B) shows the principle gated states occupied by Na channels as the cell membrane is depolarized. Upon depolarization, channels undergo a process termed “activation”, involving the concerted, outward movement of all four charged S4 segments that leads to opening of the channel pore.<sup>14,15</sup> At the same time, depolarization triggers the initiation of “fast inactivation” [Fig. 1(B)], a distinct form of channel closure from which opening does not occur. Like channel opening, inactivation is tied to the outward movement of the S4 sensors, but primarily those in domains III and IV (D3S4, D4S4: notation used throughout the text).<sup>16–18</sup> Consistent with this dual role for the S4 voltage sensor is the observation that activation and fast inactivation gating are tightly coupled,<sup>16–20</sup> and proceed more or less simultaneously.<sup>21</sup> Hence, any given Na channel may open prior to inactivating, or alternatively, may inactivate without ever opening [so-called “closed-state inactivation”, Fig. 1(B)].<sup>22</sup> As discussed below, closed-state inactivation may profoundly influence the action of anti-arrhythmic compounds, especially when modified by inherited mutations.<sup>23</sup>

Fast inactivation also critically involves the III–IV linker [Fig. 1(A)],<sup>24–26</sup> which may function as a “lid” that occludes the pore,<sup>27,28</sup> binding to sites situated on or near the inner vestibule.<sup>29–36</sup> Studies of “gating current”, produced by the movement of charged residues through the membrane electric field, are providing new insights into the relationship between S4 motion and the inactivation “lid”. Gating currents are slowed or “immobilized” by fast in-



**Figure 2** Inherited mutations in the cardiac Na channel. The topology of the Na channel is shown in the same style as Figure 1, and indicates mutations (numbering follows the human cardiac Na channel, hH1) linked to the Long QT syndrome and Brugada syndrome. Mutations tend to cluster in and around the S4 segments, the III–IV linker, and the C-terminus, consistent with the key roles of these loci in channel gating function.

activation,<sup>19</sup> suggesting the possibility that binding of the III–IV linker may lock the S4 sensors in an outward position. However, recent studies indicate that the D4S4 gating charge is immobilized by inactivation independent of whether the III–IV linker “lid” is bound,<sup>37</sup> suggesting additional gating mechanisms must underlie charge immobilization.

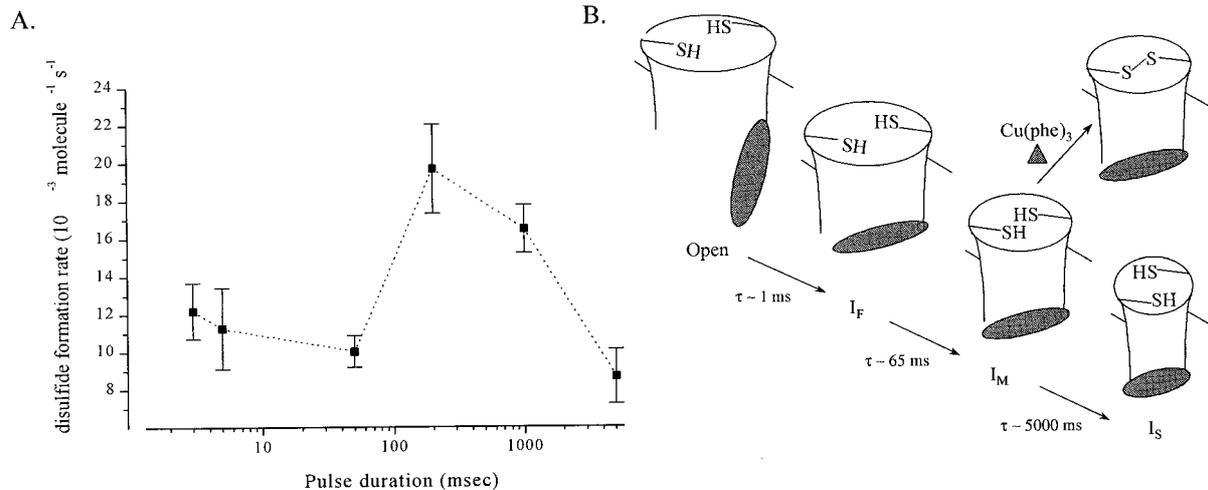
### Slow inactivation: dynamic rearrangement in the outer pore

Whereas fast-inactivated Na channels recover rapidly (within 10 ms) during the hyperpolarized interval between stimuli, upon prolonged depolarization Na channels progressively enter “slow” inactivated states [ $I_{\text{slow}}$ , Fig. 1(B)].<sup>38</sup> These more stable, non-conducting states have diverse lifetimes ranging from hundreds of milliseconds to many seconds.<sup>39,40</sup> An “intermediate” kinetic component of slow inactivation ( $I_{\text{M}}$ ) develops in cardiac Na channels with a time course relevant to the duration of the cardiac action potential (100 s of milliseconds).<sup>41,42</sup> Although the structural determinants of slow inactivation are less well defined than fast inactivation, both site-directed mutations and chimeric analyses suggest a key role for the P-segments,<sup>43–46</sup> linker sequences between the S5 and S6 segments in each domain that bend back into the membrane and line the outer pore [Figs 1(A), 2].

Slow “C-type” inactivation in K channels involves a dynamic rearrangement of the outer pore structure<sup>47</sup> that even alters the characteristics of the

permeation pathway.<sup>48</sup> Studies of induced disulfide bridges in the outer pore of the Na channel also reveal a flexible structure,<sup>49,50</sup> suggesting an analogous relationship may exist between the pore and slow inactivation.<sup>46</sup> A channel engineered to include dual P-segment cysteine substitutions (Fig. 1: K1237C–W1531C) forms an internal disulfide bond that occludes the pore when exposed to an oxidizing catalyst.<sup>49</sup> Disulfide bond formation was dependent upon channel depolarization, and the reaction was most rapid with depolarizations of intermediate length [200 ms; Fig. 3(A)].<sup>46</sup> It was therefore postulated that the two P-segment cysteines are approximated more closely in the intermediate ( $I_{\text{M}}$ ) inactivated state than in either the fast-inactivated ( $I_{\text{F}}$ ) or slower-inactivated ( $I_{\text{S}}$ ) states [Fig. 3(B)].<sup>46</sup> The emerging picture of the Na channel pore incorporates a high degree of conformational flexibility during both gating and permeation, and may even suggest analogies between Na channels and enzymes.<sup>52</sup>

Just as the S4 segments “transduce” the changing membrane potential to the fast-inactivation gating structures, the D4S4 sensor may have an analogous role in slow inactivation. Here again, structure–function motifs in the K channel parallel those in the Na channel. Recent studies utilizing patch-clamp recording and voltage-clamp fluorometry reveal that the K channel outer pore interacts directly with the S4 voltage sensor during slow (C-type) inactivation.<sup>53</sup> In Na channels, slow inactivation gating is amplified by sulfhydryl modification of a cysteine engineered at the third outermost arginine residue in D4S4 [R1456C; Fig. 1(A)].<sup>54</sup> This effect



**Figure 3** Disulfide bond formation in the outer pore catalyzed by  $100 \mu\text{M}$   $\text{Cu}(\text{phe})_3$ . A:  $I_{\text{Na}}$  was generated in *Xenopus* oocytes expressing K1237C–W1531C channels at a pulse rate of 0.05 Hz. The pulse duration (abscissa) ranged from 3 ms to 5 s. The rate at which peak  $I_{\text{Na}}$  decreased reflects the rate of interdomian disulfide bond formation (ordinate). Disulfide formation was greatest at intermediate pulse widths. B: The diagram illustrates sequential occupancy of three inactivated states (fast, intermediate, and slow:  $I_{\text{F}}$ ,  $I_{\text{M}}$ , and  $I_{\text{S}}$ ) as the length of the depolarization is extended. Time constants for entry into the three inactivated states were derived from experimental protocols<sup>46</sup> and from the literature.<sup>51</sup> The scheme suggests that when the “intermediate”  $I_{\text{M}}$  state is occupied, the 1237 and 1531 cysteines are spatially optimized for disulfide bond formation. Reprinted with permission from BENITAH JP, CHEN Z, BALSER J, TOMASELLI GF, MARBAN E. Molecular dynamics of the sodium channel pore vary with gating interactions between P-segment motions and inactivation. *Journal of Neuroscience* 1999; 19: 1577–1585. Figures 4 and 8.

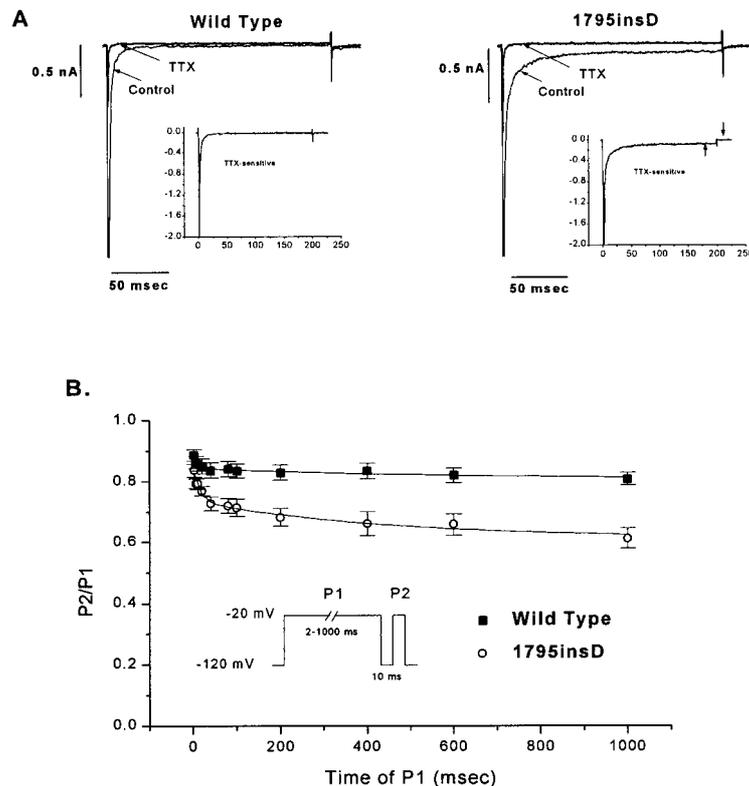
was antagonized by alanine substitution in the domain I P-segment [Fig. 1(A), W402A],<sup>54</sup> a substitution known to inhibit the intermediate component of slow inactivation.<sup>55</sup> These results motivate future studies to define functional linkages between voltage-sensing, slow inactivation, and the dynamic structure of the Na channel pore.

### Inherited cardiac Na “channelopathies” modify gating function

The Na channel normally opens only briefly ( $\sim 1$  ms), and then “fast inactivates” as the action potential commences, producing a large inward current that rapidly extinguishes. Once inactivated, channels normally cannot reopen until the membrane potential hyperpolarizes (during diastole), allowing a period of recovery. Mutations in the cardiac Na channel linked to an autosomal dominant form of the long QT syndrome (“LQT3”, Fig. 2),<sup>56</sup> produce electrocardiographic QT interval prolongation and a peculiar polymorphic ventricular tachycardia (Torsades de Pointes). These mutations invariably disrupt fast inactivation<sup>57–59</sup> and allow repeated reopening during sustained depolarization, evoking a small, persistent Na current during the action

potential plateau [Fig. 4(A)]. This excess inward current, essentially a gain of Na channel function, delays cellular repolarization and predisposes patients to polymorphic ventricular tachycardia. Surprisingly, the size of this sustained current is miniscule ( $\sim 0.5$ –2%) compared to the rapid inward Na current that develops immediately upon depolarization (“peak  $I_{\text{Na}}$ ”).<sup>58–60</sup> Nonetheless, linkage between this sustained current, action potential prolongation, and pro-arrhythmic activity has been validated in quantitative modeling<sup>61</sup> that illustrates the highly non-linear relationship between Na channel function and cardiac excitability.

Consistent with the role of the III–IV linker in fast inactivation (Fig. 1), the first reported LQT3 mutation was a deletion of three residues in this region (1505–1507  $\Delta\text{KPKQ}$ , Fig. 2).<sup>57</sup> Three other mutations reside near the cytoplasmic face of the channel (N1325S, R1644H, T1645M)<sup>57,58,62</sup> and may influence the motion or binding of the III–IV linker during fast inactivation. Mutations in other regions of the Na channel that are critical to fast inactivation elicit LQT3. Two *SCN5A* mutations reside in D3S4 and D4S4 (T1304M, R1623Q, Fig. 2),<sup>62,63</sup> the voltage sensors linked closely to inactivation.<sup>18</sup> In addition to “destabilizing” inactivation (i.e. allowing reopening of inactivated channels), open R1623Q channels exhibit a slowed



**Figure 4** A mutation in the C-terminus alters two gating processes, and provokes LQT3 and Brugada syndrome.<sup>42</sup> A: The 1795insD mutation induces a sustained component of inward current, similar to other LQT3 mutations (i.e.  $\Delta$ KPQ). The inset in each figure shows the TTX-sensitive current ( $30 \mu\text{M}$ ), obtained by subtraction, and shows a significantly larger TTX-sensitive persistent current (arrows) for the mutant. The persistent current was only  $0.41 \pm 0.1\%$  of peak  $I_{\text{Na}}$  for wild-type, but was  $1.41 \pm 0.2\%$  for 1795insD ( $P < 0.01$ ). B: An enhanced slow kinetic component of inactivation was also seen in 1795insD. Development of slow inactivation was evaluated using the inset voltage clamp protocol. The solid lines show least squares fits of a single exponential function ( $y = Ae^{-t/\tau}$ ) to the data. The mutation increased the magnitude of the slow inactivation component (A) from  $0.06 \pm 0.008$  (wild-type) to  $0.19 \pm 0.01$  (1795insD,  $P < 0.001$ ). The time constant of slow inactivation ( $\tau \sim 100$  ms) did not change significantly.<sup>42</sup> Reprinted with permission of Lippincott, Williams and Wilkins from VELDKAMP MW, VISWANATHAN PC, BEZZINA C, BAARTSCHEER A, WILDE AA, BALSER JR. Two distinct congenital arrhythmias evoked by a multidysfunctional  $\text{Na}^{+}$  channel. *Circulation Research* 2000; 86: E91–E97. Figures 1 and 3.

rate of entry into the fast-inactivated state, causing a “gain of function” by prolonging the Na current decay [see Fig. 5(A)].<sup>65,66</sup>

A highly conserved acidic domain in the C-terminus also appears to be a “hot-spot” for LQT3 mutations (Fig. 2). While two charge-altering LQT3 mutations in this region (E1784K, 1795insD)<sup>42,67</sup> evoke small, sustained currents similar to  $\Delta$ KPQ, a third charge deletion (D1790G) alters the voltage-dependence of inactivation but may not evoke sustained Na current.<sup>68</sup> Computational analysis suggests that D1790G alters the kinetics of Na channel inactivation at membrane potentials near the action potential plateau, thereby changing the balance of other currents to evoke action potential prolongation.<sup>69</sup> The structural mechanism whereby

the C-terminus influences fast inactivation remains uncertain; studies of multiple simultaneous charge neutralizations in the C-terminus do not elicit a more severe or additive effect,<sup>67</sup> suggesting that this acidic region does not participate in fast inactivation directly as a charged “binding particle”. Rather, mutations in the C-terminus may impose allosteric effects on more proximal gating domains.

A distinct class of idiopathic ventricular arrhythmias, collectively known as the “Brugada syndrome”,<sup>70</sup> trace their origin to recently-identified *SCN5A* mutations. Unlike patients with the long QT syndrome, these patients manifest distinctive electrocardiographic features that include right bundle branch block and elevation of the ST segment in leads V1 to V3. The mutations traced to

Brugada syndrome seem to arise at scattered loci, and initial efforts to characterize these disorders using heterologous expression yielded diverse and puzzling results.<sup>71</sup> Identified frameshift and splice-donor mutations (Fig. 2; a premature stop codon and “AA” nucleotide insertion) would render the channel entirely non-functional,<sup>72</sup> in contrast to the “gain-of-function” effects seen in LQT3. Similarly, a Brugada syndrome mutation in the domain III P-segment (R1432G) eliminated the Na current entirely,<sup>73</sup> possibly related to its location in the pore. Two additional Brugada syndrome mutations in the III–IV interdomain linker (R1512W) and the C terminus (A1924T)<sup>74</sup> did not eliminate channel function entirely, but shifted the voltage-dependence of steady-state inactivation to more negative membrane potentials, thereby reducing the availability of channels to open (a “loss-of-function”). Conversely, studies of Brugada mutation (T1620M) in *Xenopus* oocytes revealed opposite results: a positive shift in the voltage-dependence of inactivation and hastened recovery from inactivation,<sup>72</sup> consistent with an increase in Na channel availability. At the same time, studies of T1620M in cultured mammalian cells found hastened development of fast-inactivation<sup>75</sup> and slowed recovery from fast inactivation,<sup>75,76</sup> consistent with a loss-of-function. Complicating the picture further are recently identified *SCN5A* mutations that should produce entirely non-functional channels, yet appear to cause only isolated cardiac conduction defects.<sup>77</sup>

Further insight into the linkage between loss- and gain-of-function and these arrhythmia syndromes has emerged from analysis of a unique C-terminal *SCN5A* mutation that causes affected individuals to manifest heart-rate-dependent features of both LQT3 and Brugada syndrome: the QT interval is prolonged at slow heart rates, and distinctive ST-segment elevations occur with exercise.<sup>42,78</sup> Consistent with the notion that LQT3 is elicited by a gain of function, and Brugada syndrome by loss of function, the mutation (1795insD) has opposite effects on two distinct kinetic components of Na channel inactivation (*fast* and *slow*).<sup>42</sup> Like many other LQT3 mutations, 1795insD disrupts fast inactivation, causing sustained Na current throughout the action potential plateau [Fig. 4(A)], prolonging cardiac repolarization at slow heart rates. At the same time, 1795insD augments the intermediate kinetic component of slow inactivation [Fig. 4(B), denoted “ $I_M$ ” in Fig. 3]. This gating effect slows the recovery of Na channels between stimuli, causing loss-of-function mainly at rapid heart rates (consistent with the 1795insD

clinical phenotype)<sup>42</sup> due to the relatively brief diastolic interval. Moreover, recent studies of the Brugada T1620M mutant reveal analogous enhancement of the  $I_M$  slow inactivation component.<sup>79</sup> Taken together, these results may suggest that loss-of-function in the Na channel is, after all, a consistent mechanistic feature of the Brugada syndrome, and raise the possibility that enhanced slow inactivation is a general pro-arrhythmic mechanism in idiopathic VF.

Inspection of Figure 2 suggests that *SCN5A* loci linked to Brugada syndrome are scattered compared to those associated with LQT3. However, there is an intriguing non-random “clustering” of Brugada sites adjacent to several LQT3 loci. For example, T1620M (Brugada) resides on the external linker between S3 and S4 in domain IV, only three residues C-terminal to the outermost S4 arginine (R1623Q; LQT3). The observed gating effects of R1623Q on fast inactivation<sup>65,66</sup> and T1620M on both fast and slow inactivation<sup>75,79</sup> are consistent with the structure/function data (discussed above) supporting the role of D4S4 in both gating processes.<sup>16,17,54</sup> Analogous clustering of LQT3 and Brugada syndrome mutations appears in the III–IV linker (Fig. 2). While these mutations typically disrupt inactivation (LQT3), a recently identified Brugada syndrome mutation (R1512W) slowed recovery from inactivation,<sup>73,74</sup> and lies only five residues away from the  $\Delta$ KPQ LQT3 deletion. A number of LQT3 and Brugada mutations also lie side by side in the C terminus and influence both fast and slow inactivation (Fig. 2). The surprisingly diverse effects of these vicinal mutations emphasize the importance of future studies aimed at defining the 3-dimensional protein structure.

### Loss of Na channel function: a general pro-arrhythmic mechanism?

Inquiry into the mechanisms whereby loss of Na channel function may induce ventricular fibrillation predates the Brugada syndrome–Na channel linkage studies,<sup>80,81</sup> and was motivated partly by the pro-arrhythmic consequences of Na channel blockade in the CAST trial.<sup>1</sup> In short, the epicardial myocardial cell action potential duration is much more sensitive to factors reducing  $I_{Na}$  than the endocardial action potential, due to a prominent transient outward potassium current ( $I_{to}$ ) that counterbalances  $I_{Na}$  in the epicardium. Hence, conditions that significantly reduce  $I_{Na}$  (i.e. Brugada syndrome mutations<sup>82,83</sup> or cardiac ischemia<sup>84,85</sup>) may selectively shorten the



Y1586) increased the  $IC_{50}$  for depolarization-dependent block by lidocaine and phenytoin up to two orders of magnitude.<sup>95,96</sup> In addition, mutations in D1S6 (Fig. 6; N434, L437) also reduce drug affinity, suggesting the D1S6 and D4S6 segments together may form a domain interface site for antiarrhythmic drug binding.<sup>97,98</sup> At the same time, mutation of F1579 disrupts fast inactivation gating,<sup>29,30</sup> and substitution of the D1S6 residue N434 markedly alters both fast and slow inactivation gating.<sup>97</sup> The extent to which these S6 loci influence antiarrhythmic drug affinity by their effects on inactivation gating is difficult to resolve.

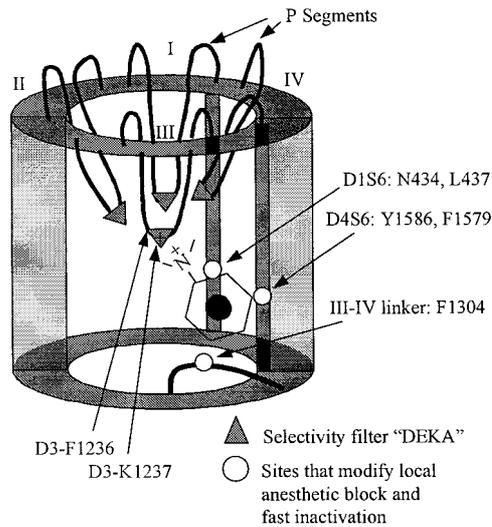
However, the dual role of S6 residues involved in drug binding and inactivation gating may be more than coincidental. Recent spin labeling studies of the putative S6 ("M2") domain in the KcsA channel suggest the alpha helical segment undergoes a 20° twist during gating.<sup>99</sup> If analogous movements occur in Na channel S6 segments, the particular residues mediating drug binding may undergo positional changes during membrane depolarization that alter the drug binding site and contribute to gating-dependent binding affinity. In fact, studies examining diverse amino acid substitutions in Na channel D4S6 and D1S6 loci suggest that the physicochemical properties (aromaticity, polarity) of the residues correlate with block affinity *only* when the channel is inactivated. Hence, the channel inactivation process may alter S6 side chain orientations in a manner that facilitates drug binding.<sup>97,100</sup>

Experiments with permanently charged lidocaine derivatives (i.e. QX-314) suggest that the hydrophilic moiety approaches its receptor in the pore from the intracellular (cytoplasmic) side.<sup>101,102</sup> This implies that the putative drug binding residues in S6 lie intracellular to the cation "selectivity filter", which is formed by the "DEKA" residues contributed by the P-segments of each domain (Fig. 6).<sup>103</sup> However, P-segment residues within the "DEKA" ring, and even those immediately adjacent to the ring, are accessible only from the outside.<sup>104</sup> Hence, the selectivity filter residues appear to "bridge" the inner and outer pore regions (Fig. 6), and limit external access (and escape) of hydrophilic compounds. Additionally, the P-segment filter residue in domain III (Fig. 6; K1237) is positively charged, and repels the ionizable amino group of lidocaine.<sup>105</sup> These findings suggest proximity between the putative drug receptor loci in S6 and the P-segment selectivity filter regions (Fig. 6),<sup>105</sup> and may provide a rationale for antiarrhythmic drug-induced conformational changes involving the outer P-segment (see below, "Use-dependent Na channel block").

## Inherited mutations: "probing" for pro-arrhythmic molecular mechanisms

Whereas the inherited *SCN5A* mutations provide valuable insights into Na channel gating mechanisms, these mutant channels prove useful as "probes" for investigating the molecular pharmacology of antiarrhythmic drugs, and also provide insight into how particular agents may be beneficial, or even harmful, in patients who carry mutations. Na channel blockers may be particularly useful in managing patients with LQT3 disorders.<sup>106,107</sup> The sustained inward currents through Na channels that harbor LQT3 mutations are potently inhibited by lidocaine and its analogues,<sup>58,108</sup> a pharmacologic effect that may serve to hasten repolarization in patients with LQT3 disorders.<sup>109</sup> Initial studies of drug action posited that lidocaine may avidly bind to the open LQT3 channel pore, or may even "repair" the fast inactivation of open channels.<sup>65,110</sup> However, studies of the R1623 LQT3 mutant reveal an unanticipated mechanism for lidocaine action on sustained current.<sup>23</sup> Patch-clamp experiments using heterologously-expressed mutant channels revealed that not only the sustained current component, but also the peak  $I_{Na}$  was unusually sensitive to lidocaine [Fig. 5(A)]. Further studies in drug-free conditions revealed that "closed-state" inactivation of R1623Q channels was actually increased [see Fig. 1(B)],<sup>23</sup> in contrast to the more obvious mutation-induced disruption in open-state inactivation.<sup>65</sup> Using a quantitative Markov model, it was demonstrated that state-dependent lidocaine affinity could further augment closed-state inactivation through an allosteric effector mechanism [Fig. 5(B)],<sup>23</sup> providing a parsimonious explanation for lidocaine suppression of both peak  $I_{Na}$  and the sustained R1623Q inward current. Notably, the same model was sufficient to reproduce observed effects of lidocaine on sustained Na currents induced by other LQT3 mutations (i.e.,  $\Delta$ KPQ),<sup>23</sup> suggesting an allosteric interaction between lidocaine and the closed-state inactivation mechanism may be generally applicable.

While Na channel blockers may have beneficial effects on the sustained current induced by LQT3 mutants, the enhanced sensitivity of R1623Q peak  $I_{Na}$  to lidocaine [Fig. 5(A)] raises concerns regarding the pro-arrhythmic potential of Na channel blockade in this setting, by reducing the epicardial Na current (i.e. Brugada syndrome). In this context, it was recently observed that flecainide, a potent Na channel blocker, elicited ECG ST elevation in a number of patients carrying LQT3 mutations.<sup>111</sup> If the closed-state inactivation gating characteristics of R1623Q are recapitulated in other Na channels carrying



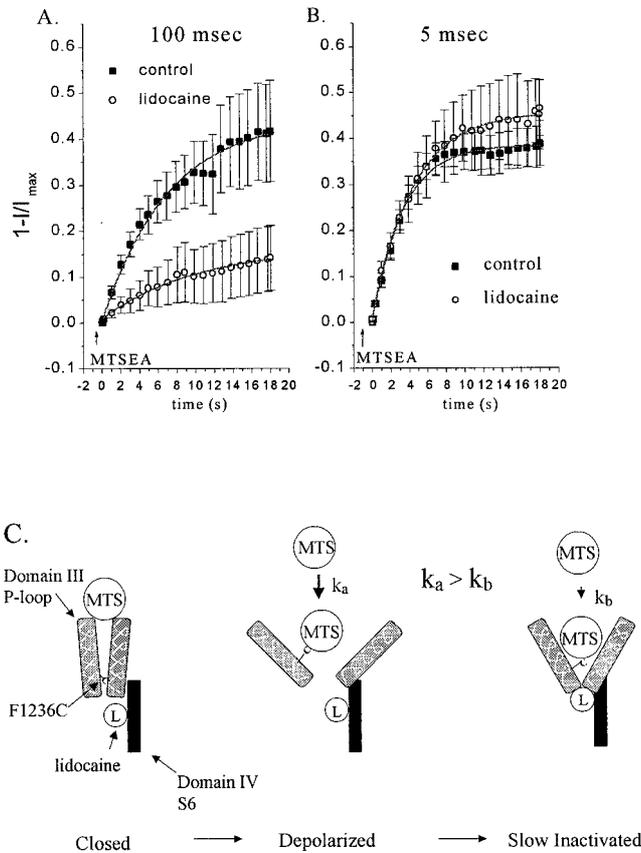
**Figure 6** Highly schematic view of the Na channel pore, lined by the four homologous domains (I–IV), with a lidocaine analogue residing in the pore. The outer pore is formed by the P-segments, while the S6 segments line the inner pore. P-segment selectivity filter residues bridge the outer and inner pore regions. Residues in the selectivity filter (i.e., K1237) and the domain I and IV S6 segments interact with antiarrhythmic drugs (see text). Residue numbers correspond to the rat skeletal clone.<sup>14</sup> to facilitate comparison with Figure 1. Adapted from *Cardiovascular Research*, 42, BALSER JR, Structure and function of the cardiac sodium channels, pp 327–388, Figure 5, copyright 1999, with permission from Elsevier Science.

LQT3 mutations, the associated pharmacologic sensitivity may at once engender both a therapeutic benefit (via sustained current suppression) and proarrhythmic risk (due to peak  $I_{Na}$  suppression). In this context, it was recently shown that the 1795insD mutation, linked to both the Brugada and LQT3 syndromes, exhibits pronounced closed-state inactivation.<sup>112</sup> Peak 1795insD  $I_{Na}$  was four-fold more sensitive to Na channel blockade by procainamide compared to wild-type channels,<sup>112</sup> and procainamide worsened the clinical phenotype in affected patients.<sup>78</sup>

A direct interaction between Na channel blockers and the fast-inactivated conformational state would readily explain how augmented closed-state inactivation potentiates drug action. In fact, an interaction between lidocaine and fast inactivation was originally postulated from data showing enzymatic disruption of fast inactivation reduces lidocaine block,<sup>113,114</sup> and was supported by later studies showing mutagenic disruption of the III–IV linker also reduces lidocaine block.<sup>115</sup> However, more recent

studies suggest that these fast-inactivation gating effects on lidocaine action may be indirect, or allosteric in nature. By utilizing MTS reagents and a rapid perfusion system to covalently modify an engineered cysteine in the III–IV linker “latch” [F1304C, Fig. 1(A)], it was possible to monitor the position of the latch at a time scale relevant to fast-inactivation gating.<sup>116</sup> Lidocaine accelerated depolarization-dependent “binding” of the latch cysteine to its receptor, suggesting that the drug promoted fast-inactivation gating upon initial depolarization,<sup>116</sup> consistent with the postulated drug-induced facilitation of closed-state inactivation in LQT3 channels.<sup>23</sup> However, upon subsequent repolarization, recovery of drug-bound channels was typically slow (100 s of milliseconds), but recovery of the latch residue was rapid. Hence, lidocaine block either directly or indirectly augmented fast inactivation, but did not “trap” the fast-inactivation gate, and therefore cannot explain the more sustained features of use-dependent block<sup>116</sup> (see below).

If lidocaine does not “trap” channels in the fast-inactivated state, how does the drug facilitate rapid, closed-state inactivation when the channel is suddenly depolarized? Studies examining the S4 voltage sensors during lidocaine block provide useful insights. Gating current measurements show that lidocaine and its quaternary derivatives substantially reduce the maximal gating charge ( $I_g$ ) associated with channel depolarization, and also decrease the slope of the gating-charge voltage dependence.<sup>117,118</sup> Moreover, site 4 toxins (that specifically inhibit movement D4S4 sensor) have an additive effect with lidocaine to suppress the maximal gating charge, further suggesting that lidocaine does not effectively “stabilize” the fast inactivation gate.<sup>118</sup> Taken together, the studies suggest that lidocaine analogues may alter closed-state inactivation by influencing the voltage sensors coupled to inactivation gating. Recent studies examining the linkage between S4 motion and closed-state inactivation<sup>119</sup> lend support to this postulate. MTSET modification of a cysteine substituted for the outermost arginine [Fig. 1(A); R663C] in D2S4 shifts the voltage-dependent behavior of the S4 segment to more hyperpolarized potentials, and also slows its outward movement during depolarization. These effects on the activation sensor paradoxically cause the channel to exhibit an unusual degree of closed-state inactivation. Whether antiarrhythmic drugs can inhibit movement of the S4 segments in an analogous direct manner, or alternatively through an allosteric (indirect) mechanism, remains uncertain.



**Figure 7** MTSEA modification of F1236C channels is modulated by slow inactivation and use-dependent lidocaine block. A and B: HEK-293 cells expressing F1236C channels are exposed to trains of either (5 ms) or intermediate length (100 ms) depolarizations. Peak  $I_{\text{Na}}$  (relative to pre-MTSEA) is plotted in a manner that reflects matching cumulative depolarization time. The recovery interval between pulses was sufficient to avoid accumulation of slow inactivation. In lidocaine-free conditions, MTSEA modification was more rapid when the pulse duration was brief, suggesting slow inactivation renders the 1236 cysteine less accessible (compare panels A and B). In addition, lidocaine ( $100 \mu\text{M}$ ) markedly reduced the depolarization-dependent MTSEA modification rate when the pulse duration was 100 ms, but not 5 ms. C: A conceptual model of P-segment motion and MTS accessibility during slow inactivation and lidocaine (L) block (see text for description). Reproduced from *The Journal of General Physiology*, 2000, 116, pp. 653–661 figures 5 and 6 by copyright permission of The Rockefeller University Press.

### Use-dependent Na channel block, slow-inactivation, and the P-segments

The studies discussed above provide insight into the sensitivity of Na channels to blockade that ensues immediately upon depolarization (“first-pulse” block), which relates primarily to block that develops during a stimulus delivered after a long diastolic pause. Additional mechanisms may be involved in modulating drug affinity that develops during repetitive “trains” of action potentials, and persists long after the channel is repolarized (“use-dependent” block).<sup>120</sup> For LQT3 patients who carry the D1790G mutation, flecainide hastens cardiac repolarization while lidocaine has no effect.<sup>107</sup> This disparity is manifest in studies of heterologously expressed D1790G channels, which reveal mark-

edly increased use-dependent block sensitivity to flecainide (compared to wild-type channels), but not lidocaine.<sup>121</sup> The mutation resides in the C-terminus, a locus not previously associated with Na channel blockade, so the means whereby D1790G selectively alters the channel sensitivity to use-dependent flecainide block is unknown.

Studies of lidocaine blockade during a number of interventions (mutations, cation substitutions,  $\beta$  subunits) that modify slow inactivation<sup>54,122–124</sup> support an early proposal;<sup>125,126</sup> that slow recovery of drug-bound channels between depolarizing stimuli involves an interaction between the drug and slow-inactivated conformational states. Recent studies<sup>127</sup> utilized an engineered cysteine in the domain III, P-segment [Fig. 1(A); F1236C] as a “sentinel” for slow-inactivated motion of P-

segments, and demonstrated state-dependent accessibility of this residue to covalent modification by MTS-ethylammonium (MTSEA) applied from outside (but not inside) the cell. Trains of brief, 5 ms depolarizations sufficient to open and fast inactivate channels (but not induce slow inactivation) produced more rapid MTSEA modification [Fig. 7(B)] than longer (100 ms) pulse widths [Fig. 7(A)], suggesting slow inactivation inhibits depolarization-induced MTEA accessibility of the cysteine side chain [Fig. 7(C)]. Importantly, lidocaine further inhibited the depolarization-dependent sulfhydryl modification induced by these sustained (100 ms) depolarizations, but not by brief (5 ms) depolarizations [Fig. 7(A,B)]. Electrostatic interactions between the adjacent P-segment residue (K1237) and the lidocaine amino terminus suggest that S6 residues forming a putative lidocaine receptor lie in proximity to the P-segments (Fig. 6).<sup>105</sup> Hence, it was postulated that the structural rearrangements associated with slow inactivation move the Na<sup>+</sup> channel P-segments into positions that stabilize the interaction between lidocaine and the pore [Fig. 7(C)]. The association of Brugada syndrome (T1620M and 1795insD) with enhanced slow inactivation raises the possibility that this gating defect exacerbates the Brugada phenotype during exposure to Na channel blockade.

Considered together, the data suggest that fast and slow gating both play important roles in Na channel blockade, and inherited mutations that alter these gating processes may substantially influence the response to antiarrhythmic drugs. Fast inactivation, particularly from closed states, predominantly influences the component of Na channel blockade that develops rapidly upon depolarization, whereas pore conformational changes associated with slow inactivation influence more sustained components of block. Future studies are likely to provide a deeper understanding of how the pore-lining segments and the S4 voltage sensors rearrange during antiarrhythmic drug binding. When coupled with emerging structural data, these insights will better inform the dynamic structure of the modulated receptor hypothesis,<sup>5,6</sup> and will facilitate the design of more effective and selective antiarrhythmic agents.

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