Review

Biophysical defects in voltage-gated sodium channels associated with long QT and Brugada syndromes

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The activity of voltage-gated sodium channels contributes to onset and duration of the cardiac action potential through an intricate balance with the activity of other ion channels. Activation of sodium channels leads to membrane depolarization and Phase 0 of the cardiac action potential. Sodium channel fast inactivation contributes to Phase 1, the initial repolarization. Slow inactivation and closed state fast inactivation determine channel availability and, thus, overall membrane excitability. Defects in any of these biophysical states or transitions between them, imparted by (over 170 reported thus far, including both Long QT3 and Brugada syndromes) mutations in the (over 2000) amino acids that compose the sodium channel protein, can lead to channel dysfunction that manifests as an abnormal cardiac action potential and electrocardiogram. A causal relationship between several such abnormalities and the panoply of sodium channel mutations have led to a greater understanding of the molecular underpinnings of cardiac arrhythmias as well as a deeper appreciation for the intricacies of sodium channel function. Here, we review the literature regarding these causal relationships from a perspective of the biophysical properties of sodium channels.

Introduction

Recent advances in genetic screening have led to an explosion in the number of diseases identified as having congenital basis. A subset of congenital diseases is due to mutations in the genes that encode voltage-gated ion channels and are now termed 'channelopathies'. Concurrent advances in molecular cloning and electrophysiological analysis have led to a greater understanding of the biophysical underpinnings of channelopathies. Once thought to be relatively rare, it is becoming increasingly apparent that channelopathies have a much higher incidence than originally thought. A broad range of ion channel mutations are now known to underlie cardiac arrhythmogenesis, and these congenital defects are a major contributor to, if not the primary cause of, sudden death.

Voltage-gated ion channels are a class of membrane proteins that control signaling in electrically excitable cells, and are characterized by being sensitive to transmembrane voltage and by their ionic selectivity. Voltage-gated sodium channels (NaV,s) are a closely related subset of ion channels that open in response to membrane depolarization and are preferentially permeated by sodium ions. Although NaV,s show far greater homogeneity in their gating properties than their potassium selective counterparts, subtle differences in their biophysical characteristics, distributions, expression levels, and pharmacological sensitivities lead to a wide range of electrical properties in cells and an even wider range of drug interventions that can modify those electrical properties.

Long QT syndrome (LQT) and Brugada syndrome (BrS) are two cardiac arrhythmogenic disorders that arise from ion channel mutations. Both these disorders have been related to mutations in voltage gated sodium channels, and (thus far) exclusively from the ion channel that arises from the SCN5A gene: NaV1.5. An ever-increasing number of NaV1.5 mutations are associated with LQT and BrS. Recent reviews have focused on clinical aspects of these disorders and their molecular underpinnings. Rather than list the mutations and their effects on sodium channel function (examples of which may be found elsewhere), [www.fsm.it/cardmoc/] we here present the various biophysical properties of voltage-gated sodium channels and discuss how these properties are adversely affected by emblematic mutations that underlie LQT and BrS.

Sodium Channel Function

The principal role of NaV,s is to initiate the action potential. The rising phase of the action potential in most electrically excitable cells, including cardiac myocytes, is due to the rapid influx of sodium ions through NaV,s. Membrane depolarization leads to a rapid sequence of gating events that precede, and result in, channel opening. Cumulatively called activation, these state transitions involve a series of molecular movements that occur in the microsecond time domain. Activation is quickly followed by another series of molecular movements that block the depolarizing flow of sodium ions into the cell. This gating step, fast inactivation, occurs in the millisecond time domain (although there is a far greater range of time constants for inactivation than for activation) and, together with potassium channel activation, help to terminate the action potential. Once the membrane potential is restored to its resting, hyperpolarized voltage,
Na\textsubscript{v}s recover into their closed state, a process known as deactivation. Na\textsubscript{v} deactivation participates in action potential repolarization in some cells in which the action potential time course is very fast.\textsuperscript{5} Yet another form of inactivation, called slow inactivation, plays an important role—along with closed-state fast inactivation—in regulating the number of sodium channels available for activation. Channels enter the slow inactivated state over a time course of seconds to, in some cases, minutes of sustained (experimentally) or repetitive (in vitro) depolarization. Slow inactivation is pharmacologically and structurally distinct from fast inactivation, although there may be an inverse relationship such that fast inactivated channels are resistant to slow inactivation.\textsuperscript{6-9}

**Structural Correlates of Sodium Channel-Gating**

The structural basis for some of these kinetic states has become reasonably well elucidated through a combination of analyzing the biophysical sequela of mutations, through mutant cycle analysis using channel blocking drugs, and by comparison to crystal structures and models of other, principally potassium, ion channels. The canonical Na\textsubscript{v} alpha subunit structure consists of four homologous domains (D1-DIV), each with six membrane spanning alpha helices (S1-S6).\textsuperscript{10} As in most other voltage-gated channels, the fourth membrane-spanning segment (S4) in each domain carries a disproportionately number of positively charged, hydrophobic residues, a lysine or an arginine in every third position. Mutational analysis has revealed that the S4 membrane spanning segments move in response to changes in the electrical field; they are therefore known as “voltage sensors”.\textsuperscript{2} Further structural analysis revealed that the cytoplasmic chain of residues between DIII and DIV contains a motif of amino acids that are both necessary and sufficient to impart fast inactivation.\textsuperscript{12-15} The IFMT sequence of amino acids in the DIII-DIV cytoplasmic linker is surrounded by a number of charged residues and, although fast inactivation is not independently voltage-sensitive, these charge residues may electrostatically interact with counterparts in the membrane spanning segments to stabilize the inactivated state.\textsuperscript{16,17} The molecular model that has emerged from a variety of experimental approaches is as follows: in response to membrane depolarization, the S4 voltage sensors translocate from their hyperpolarized-favored positions into their depolarized-favored positions.\textsuperscript{18} (A significant controversy in the field exists over the nature of S4 translocation, but that discussion is beyond the scope of this review.) When the S4s move in response to depolarization, charge is moved through the electrical field. This change can be measured, in the absence of ionic current (which masks charge movement), as a gating current associated with activation. It is likely that S4 movement occurs following a specific sequence in Na\textsubscript{v}s because, unlike in most homotetrameric potassium channels, the heteromeric structure of the Na\textsubscript{v} alpha subunit has an unequal number of charged residues in each of the four voltage sensors. Each S4 movement can be represented as a single step in a sequence of activation steps leading to channel opening. Thus, four voltage dependent steps lead to a final, concerted conformational change in the ion permeation pathway that is made up of amino acids linking the S5 and S6 segments, as well as cytoplasmic portions of those segments themselves. The final step in activation is associated with pore hydration and is apparently a voltage-independent step.\textsuperscript{19}

Shortly following activation (with or without subsequent channel opening), the fast inactivation particle located within the DIII-DIV linker\textsuperscript{12,13,15,20} undergoes a conformational change and binds to the S4-S5 cytoplasmic linkers in DIII and DIV.\textsuperscript{21-24} This binding results in a blockade of the inner pore mouth and the flow of sodium ions is terminated. The voltage sensor in DIV appears to be a critical link between activation and fast inactivation (and may, in fact, play only a limited role in activation).\textsuperscript{25} Upon repolarization and subsequent inward movements of one or more S4 voltage sensors, the ion permeation pathway collapses, the binding site for the inactivation particle becomes less accessible and fast inactivation becomes destabilized. It is noteworthy that channel opening is not requisite for fast inactivation; closed-state inactivation occurs at voltages more hyperpolarized than necessary for channel opening, indicating that voltage sensor movement, but not channel opening, is necessary for movement of the DIII-DIV cytoplasmic linker and binding of the IFMT motif.

Sustained or repetitive depolarizations that result in slow inactivation may lead to voltage sensor movements secondary to activation, since slow inactivation (unlike fast inactivation) appears to have some independent voltage sensitivity.\textsuperscript{26,27} The secondary, slow S4 movement may lead to a collapse of the permeation pathway;\textsuperscript{28} mutations in the S5-S6 linkers in sodium channels disrupt slow inactivation.\textsuperscript{29} Little else is known about the structural underpinnings of slow inactivation, although mutations in S6 membrane spanning segments and in the C-terminus have been shown to affect slow inactivation.\textsuperscript{30,31}

**Sodium Channel Isoforms: Differences in Localization and Gating**

Nine isoforms of sodium channel \(\alpha\)-subunits have been identified.\textsuperscript{32} Early classification identified tissue-specific isoforms (e.g., Na\textsubscript{v}1.5 was considered to be the ‘cardiac’ isoform) based on the tissues in which they were originally identified. It has become increasingly evident, however, that multiple Na\textsubscript{v} isoforms are expressed in single tissue types and single cells. Thus, Na\textsubscript{v}1.1, Na\textsubscript{v}1.3, Na\textsubscript{v}1.5 and Na\textsubscript{v}1.6 all appear to be expressed in the ventricular myocardium, with Na\textsubscript{v}1.5 apparently restricted to intercalated disks, whereas Na\textsubscript{v}1.1, Na\textsubscript{v}1.3 and Na\textsubscript{v}1.6 are localized within the T-tubules.\textsuperscript{33,34} In another example of differential distribution, Na\textsubscript{v}1.1 and Na\textsubscript{v}1.3 are expressed in central cells of the SA node, whereas Na\textsubscript{v}1.5 is expressed in peripheral nodal cells.\textsuperscript{35} Table 1 lists the isoforms, distributions and some of the drug sensitivities for sodium channels that have been identified within the myocardium.

In addition to the \(\alpha\) subunit, which by itself is capable of forming functional ion channels in heterologous expression systems, most excitable tissues also express \(\beta\) subunits. Four known \(\beta\)-subunits co-localize with \(\alpha\)-subunits: \(\beta1–\beta4\).\textsuperscript{36-40} These subunits variously modulate sodium channel gating and expression levels through direct interactions with the \(\alpha\)-subunit and through interactions with anchoring proteins.\textsuperscript{41,42}

**Cardiac Disorders Associated with Sodium Channels: Long QT and Brugada Syndrome**

The two primary cardiac diseases that have been associated with mutations in sodium channels include Long QT syndrome (LQT) and Brugada syndrome (BrS). LQT is typically identified by an electrocardiogram signature that includes a longer-than-normal interval between the Q wave and the T wave.\textsuperscript{43} Brugada syndrome, first introduced in 1992,\textsuperscript{44} is characterized by an elevated ST interval in
The rate-corrected QT interval (QTc) is used as a standardized measure because the QT interval normally varies with heart rate. A QRS complex to the completion of the T wave, the QT interval. The ST segment and T wave represent ventricular repolarization and therefore extend the duration from the onset of the QRS complex to the completion of the T wave, the QT interval. Because the QT interval normally varies with heart rate, a heart rate-corrected QT interval (QTc) is used as a standardized measure of the QT. QTc is calculated using the equation: QTc = QT/√RR, where RR is the time in seconds between peaks of the QRS complex. An individual is considered to have an abnormal QT when their QTc > 440 ms for males and QTc > 460 ms for females; however, this is considered an approximation. Individuals with LQT-associated mutations are often found with QTc below the suggested interval time and healthy individuals carrying no mutations sometimes have abnormally long QTc intervals.

In LQT3, the form of LQT associated with sodium channel mutations, a typical ECG pattern is characterized by a large extension of the ST segment (the time between the completion of the S-wave and the onset of the T-wave) and a relatively narrow, peaked T-wave. This differs from the majority of LQT patients in that LQT1 sufferers display a very large and prolonged T-wave with a relatively short ST, and LQT2 sufferers show a low-amplitude, bifid T-wave. LQT1 and LQT2 represent 80–90% of those with LQT, whereas LQT3 represents only 5–8%. Figure 1 shows a pharmacological induction of the LQT3 waveform in the ventricular endocardial action potential (top row), m-cell cell action potential, (second row), epicardial action potential (third row), and the ECG (bottom row), using anthopleurotoxin to destabilize sodium channel fast inactivation.

The timing of QT-prolonging current is an important aspect of the emergent ECG pattern. Mutations that produce persistent sodium current elongate Phase 2 of the cardiac action potential, which manifests as increased ST duration. The T wave from an ECG coincides largely with Phase 3 of the cardiac action potential and appears indicative of the surge in repolarizing potassium currents. LQT 1 and 2 are the result of decreased potassium currents from Kv7.1 and HERG channels respectively. These channels have delayed currents following initial depolarization of the action potential; their effects do not manifest until Phases 2 and 3. This explains their different effects on T-wave shape. Stemming from their increased late sodium current, LQT9 patients exhibit ECG patterns similar to those of LQT3. Another ECG abnormality associated with LQT3 is a bifid T-wave. This differs from the majority of LQT patients in that LQT1 suffers display a very large and prolonged T-wave with a relatively short ST, and LQT2 sufferers show a low-amplitude, bifid T-wave. LQT1 sufferers display a very large and prolonged T-wave with a relatively short ST, and LQT2 sufferers show a low-amplitude, bifid T-wave.
Sodium Channel Activation Defects

Sodium channel activation is conferred by the voltage-dependent movements of the S4 voltage sensors in each domain, which leads to channel opening. The voltage dependence of activation is assessed by comparing the amplitude of current to the voltage used to elicit activation. This comparison results in the current-voltage, or I(V), curve. Application of Ohm’s law converts the I(V) curve to a conductance-voltage, or g(V), curve. The slope of the g(V) curve indicates the relative amount of charge that moves during S4 translocation. The midpoint of the g(V) curve indicates the relative voltage dependence of activation. Shifts of the g(V) midpoint along the voltage axis in the positive direction indicates that more voltage is required for activation and excitability is therefore decreased. Conversely, shifts of the g(V) midpoint in the negative means that the channels require less provocation to activate and excitability is increased.

Shifts in the voltage dependence of activation often occur in mutant cardiac sodium channels but are generally small, have limited effects, and are not believed to be a direct cause of LQT. By increasing the magnitude of sodium channel window current (see below), hyperpolarized shifts activation can indirectly produce LQT3. Depolarized shifts of activation, however, stabilize the channels’ deactivated state, thus acting to decrease channel open probability. Positive shifts of activation associated with LQT3 occur in conjunction with other channel defects, such as faster recovery from fast inactivation and persistent current, which mask the effects of decreased activation probability.

Charge-neutralizing LQT3 mutations of DIV/S4 have been characterized that appear to have no noticeable affect on channel activation. R1626P produced no measurable changes on activation. Instead, persistent current, as well as slower onset of inactivation, changes in the apparent valence, and a hyperpolarized shift in the midpoint of steady-state fast inactivation curve were all observed. Mutations such as R1626P create new questions as to the function of DIVS4 in channel activation and point to its apparent role linking activation and fast inactivation.

Because of its apparently limited contribution to the LQT3 phenotype, comprehensive characterization of activation is often overlooked in studies of LQT3-associated mutations. Detailed kinetic analysis, and an inspection of gating currents and molecular movements assessed using fluorescence-tagged residues, may elucidate a better understanding of the mechanisms that underlie both LQT3 and sodium channel activation.

Although most mutations that cause Brugada syndrome primarily affect inactivation properties, some mutations, including G752R, G1319V and S1710L have been demonstrated to induce a depolarizing shift in the g(V) curve. Such a shift is predicted to induce a loss of function consistent with BrS, because a greater depolarization is required to induce channel activation. The mutations, however, are located in channel regions not typically associated with the voltage dependence of activation (i.e., the S4 voltage sensors and the pore region). Interestingly, however, residue 1710 is located proximal to the DIV portion of the selectivity filter, and may contribute to the putative link between voltage sensor movement and the channel opening.

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Defects in Onset and Recovery from Fast Inactivation

Sodium channels enter the fast-inactivated state in a time-dependent and indirectly voltage-dependent transition. The onset of fast inactivation is usually described by one, and sometimes two, time constants obtained by fitting exponential functions to the decay of current during a test pulse. The time constants that describe the kinetics of fast inactivation are voltage-dependent; the onset of inactivation is faster at more depolarized test potentials. Recovery from fast inactivation is also described in terms of one or two time constants, derived from a double pulse protocol in which the amplitude of two test pulses are compared when the second test pulse follows a hyperpolarizing recovery step of varying duration and voltage. Recovery is also, thus, indirectly voltage-dependent.

Mutations in Na\textsubscript{v}1.5 that slow the onset and hasten the recovery of fast inactivation contribute to LQT3. Pathogenic mutations have been isolated throughout cytoplasmic regions that are commonly associated with channel inactivation. Several charge-altering mutations in DIVS4 voltage sensor have also been linked with altered inactivation kinetics and LQT3. R1644H increases rate of recovery, R1626P decreases the rate of onset, and R1623Q does both, although none of these LQT3 mutations have significant effects on channel activation.

One of the most common effects of mutations that cause BrS is a defect in the onset of fast inactivation. Paradoxically, some mutations slow inactivation onset, including G752R, R1232W and T1620M. These results contrast with the faster Phase 1 repolarization that underlies ST-segment elevation. Decreased channel expression in these mutants may help explain why overall excitability is lower in these particular BrS mutants.

Persistent Current

Persistent current in sodium channels is a small, inward current present during prolonged depolarizations such as the cardiac action potential. Persistent current is caused by defects in fast inactivation that result in the channel reentering the open state from the fast-inactivated state. Persistent current in Na\textsubscript{v}1.5 is the primary cause of the autosomal dominant, congenital LQT3 disorder. In LQT3, late reopenings occur during Phases 2 and 3 of the cardiac action potential. These late reopenings prolong Phase 2 and delay repolarization of the membrane. Figure 3 shows persistent currents associated with LQT.

Gain of function mutations in Na\textsubscript{v}1.5 manifest throughout the channel’s ca. 260 kDa amino acid chain. Nearly all of the Na\textsubscript{v}1.5 mutations that cause persistent current in Na\textsubscript{v}1.5, however, are found in either cytoplasmic regions of the channel (i.e., the inter-domain linkers and the C-terminus), or in the DIVS4 voltage sensor. Mutations that occur intracellularly could either manifest directly on the channel itself or stem from alterations in channel modulation via intracellular proteins. For example calmodulin has been shown to modulate C-terminus stabilization of inactivation associated with the DIII–DIV linker.

The first mutation causally associated with LQT3 was discovered in 1995. A 9 base pair deletion in cDNA was identified in two unrelated families with LQT. The deleted base pairs resulted in the loss of amino acids 1505-lys, 1506-pro and 1507-gln (ΔKPQ). ΔKPQ is found in a conserved region of the DIII–DIV linker. Not surprisingly, ΔKPQ had major effects on the properties of Na\textsubscript{v}1.5 fast inactivation. Biophysical analysis of ΔKPQ, heterologously expressed in tsA201 cells, showed increased window current, faster recovery from and slower onset of fast inactivation and, most notably, persistent current. Further analysis showed that the loss of Q1507 via mutations del1507Q-1508K-1509P, del1507Q and Q1507A produced persistent current in Na\textsubscript{v}1.5. This identified Q1507 as an important amino acid in channel fast inactivation. During fast inactivation, the hydrophobic residues IFMT of the DIII–DIV linker bond with A1330 of the DIII S4–S5 linker. Alterations of the charged and polar amino acids surrounding the IFMT particle disrupts the stability of the IFMT-A1330 link and allows late re-openings of the channel during inactivation.
Charge altering mutations in the S4 domains of sodium channels have broad effects on channel kinetics. The mutation R1626P, located in DIVS4, is associated with LQT3 and has drastic effects on channel gating. The exchange of positively-charged arginine with neutral proline decreases the net positive charge of the S4 segment and causes defects of inactivation without any significant effects to activation. Prolines also introduce major changes in protein structure, such that R1626P is predicted to exert allosteric as well as electrostatic effects on channel function. When expressed in HEK 293 cells, R1626P mutants show persistent current, slowed onset of fast inactivation, changes in the apparent valence of fast inactivation, and a hyperpolarized shift of the midpoint of the steady-state fast inactivation curve. Although the S4 segments are traditionally believed to mediate channel activation, R1626P clearly demonstrates the role of DIVS4 in the control of fast inactivation. The exact method of modulation is debatable, although electrostatic interactions with the surrounding negative residues are thought to be critical, and other studies on channel deactivation have provoked the hypothesis that charged DIVS4 residues electrostatically interact with charges surrounding the IFMT motif in the DIII–IV linker.

The C-terminus in NaV1.5 is 242 amino acids long, located on the intracellular side of the membrane. The C-terminus has been divided into a proximal and a distal portion. The distal portion lacks any discernable tertiary structure and has yet to be associated with channel kinetics or voltage dependence. Truncation of the distal portion of the C-terminus of NaV1.5 at L1921 has no significant effects on channel function. The proximal portion, however, is comprised of six α-helices that appear to have a modulatory role on sodium channel inactivation. Specifically, amino acids E1773 through D1816 make-up α-helix1, most of α-helix2, and comprise the highly conserved, acidic region of the C-terminus. The majority of C-terminus mutations for LQT3 are found with in this area.

Work by Wei et al. demonstrated the importance of the acidic domain of the C-terminus in channel inactivation. The biophysical effects of LQT3 mutation E1784K include persistent current (2–4% of peak current), a slowed onset, and a hyperpolarized shift in the steady-state fast inactivation curve. Wei et al. further studied the acidic domain's role in channel inactivation using two channel constructs. The constructs were made with multiple, charge-neutralizing glutamine substitutions (construct 1: E1773, E1780, E1781 and E1784; construct 2: E1788, D1789, D1790 and D1792) and both showed persistent current and depolarizing shifts in the voltage dependence of fast inactivation, defects consistent with the LQT3 phenotype. Although mutations in α-helix6 linked to LQT3 have yet to be isolated, α-helix6 is believed to have a role in channel inactivation as well. Truncation at amino acid S1885, which clips off α-helix6, creates bursting channel behavior and persistent current. A bipolar structure in the C-terminus arises from the net positive charge in α-helix6 in opposition to the net negative charges in α-helices1 and 2. Mutations that alter the polarity of the C-terminus are pathogenic. This suggests that the C-terminus electrostatically interacts with other channel regions, and that the interaction is dependent on the balance of charge within the proximal C-terminus.

Unlike in LQT, persistent current is not a hallmark of BrS. To date, relatively few mutations underlying BrS have been shown to evoke a persistent current. T353I is in the pore region of DI and induces a hyperpolarizing shift in the voltage dependence of steady state fast inactivation, a decrease in current amplitude, and a persistent current. M1766L, a mutation caused by a single nucleotide in NaV1.5 (5298 A > C) that resulted in a case of fatal IVF, induces a gain of function phenotype by increasing late sodium current. The persistent current and expected gain of function in both mutations is over-ridden by a decrease in peak current. Curiously, the apparent decrease in expression relative to WT-NaV1.5, but not the persistent current in M1766L, is rescued by mexilitine. Thus, both T353I and M1766L could be classified as both a LQT and a Brugada ‘crossover’ mutations, since they both show loss- and gain-of-function (when the loss of function properties are rescued). Residue 1766 is located in the proximal C-terminus, and the increase in persistent current caused by this mutation is consistent with reports of C-terminus contributions to fast inactivation.

**Defects in the Voltage Dependence of Fast Inactivation**

Although fast inactivation is not directly voltage dependent, its apparent voltage dependence—and channel availability—can be measured from the steady-state inactivation curve. Prepulses to voltages more negative than required to activate the channels, if sufficiently long, will cause the channels to enter the fast-inactivated state from the closed state. Closed-state inactivation is greater at more depolarized voltages. The midpoint of the sigmoidal relationship between channel availability and voltage can shift along the voltage axis similar to shifts in the g(V) curve. A shift of the steady-state fast inactivation curve in the positive direction means that fewer channels are inactivated—and more are available—at any given potential. Conversely, a negative shift in the midpoint of the steady state fast inactivation curve indicates a decrease in channel availability.

Increased channel availability from positive shifts in the steady-state inactivation curve does further the development of LQT3 in the individual, but it is by no means necessary. In fact, it appears negative shifts of inactivation, which decrease channel availability, appear to be more common. Despite many varying characteristics between them, these mutations all have one defect in common, persistent current. V1777M displayed a 12 mV shift of inactivation in the hyperpolarized direction, the effects of which were completely negated by persistent current. These mutants underscore the overwhelming effect of persistent sodium current on cardiac dysfunction in LQT3.

Decreased channel availability due to negative shifts in the steady-state inactivation curve is common among BrS mutations. In view of the fact that ST-segment elevation in BrS arises as the result of faster onset of inactivation, it is somewhat surprising that some BrS mutations produce shifts in the midpoint of fast inactivation that are associated with increased channel availability, including T1620M. This effect is likely swamped by a decrease in channel expression. Other BrS mutations, e.g., L567Q and S1710L, produce the more predictable result associated with a loss in excitability. Both these mutations cause a hyperpolarizing shift in steady-state fast inactivation as well as a more rapid onset of inactivation.

**Window Current**

Window current (WC) arises from an overlap of the steady-state activation and fast inactivation curves. In WC, channels have been activated; however, membrane voltage is not sufficiently depolarized to allow complete inactivation. Channels flip between the open and
inactivated states producing current similar to late persistent current. Ordinarily, WC is extremely small, -0.5% of normalized peak current amplitude, and observed in all forms of sodium channels. Similar to persistent current, increases and/or changes in the voltage range of WC can have adverse results on cardiac function. Figure 4 shows that a variety of changes in the voltage dependence of activation and/or fast inactivation can induce changes in WC.

Increased WC is the product of depolarizing shifts of the g(V) curve midpoint, depolarizing shifts in the fast inactivation midpoint, or decreases in the apparent valence. N1325S, a mutation in the DIVS4–S5 linker, demonstrates the roles of these defects in LQT3.67 Expressed in tsA201 cells, N1325S channels show mild but not significant convergent shifts in the midpoints of FI and activation, in addition to a decrease in the apparent valence of both curves.67 The augmented WC increases late sodium current in Phase 3 of the cardiac action potential. This, in combination with a mild persistent current, produces LQT3.

Due to its relatively negative voltage range, WC manifests in Phase 3 of the cardiac action potential. As the cell rapidly repolarizes in Phase 3, the voltage potential passes through the active range of WC. Channels momentarily return to the open state and delay repolarization of the cell membrane. I1768V, a mutation in the channel’s DIVS6, shows how WC can cause LQT3 without persistent current. I1768V channels in HEK 293 cells showed a two-fold increase in their rate of recovery from fast inactivation, positive shifts of fast inactivation and WC, a mild increase in WC, and less slow inactivation.85,86 Clancy et al., using heterologous expression and computer simulations, showed that the increased recovery from FI and positive shift of window current increased Phase 3 sodium current.87 The increased current resulted in arrhythmogenic early afterdepolarizations during cardiac action potential clamp and would produce the delayed ventricular repolarization of LQT.87 A mutation in the DIII3–S4 linker, an area associated with the binding of the fast inactivation particle, also shifts the WC voltage range with no persistent current. The mutation, A1330T, depolarizes the WC into a voltage range in which the repolarizing effects of K+ current would be reduced, leading to action potential prolongation.88

The alterations of WC appear small compared with overall channel current. R1644H and N1325S increase WC to 2.5% and 1% of peak current, respectively. I1768V has almost no increase in WC. However, these subtle changes have profound effects on cardiac function and the health of the individual. These mutations, like those that produce persistent current, demonstrate the delicate balance of ionic currents in the cardiac action potential.

An increase in the amplitude of window current was reported when the BrS mutation, T1620M was co-expressed with the β1-subunit.69 The increased overlap of steady state activation and fast inactivation voltage dependencies is entirely due to a depolarizing shift in the midpoint of the steady state fast inactivation curve. This effect is paradoxical in view of the fact that a depolarizing shift in the midpoint of inactivation is associated with increased channel availability due to a destabilization for fast inactivation, yet BrS is associated with more rapid entry of sodium channels into the fast inactivated state. The size of window currents also were decreased in a BrS mutation with symptoms that were unmasked by fever.89 In H681P (located in the DII–DIII linker), the midpoints of both activation and fast inactivation were found to be hyperpolarized relative to WT NaV1.5, but the fast inactivation curve is shifted to a greater extent resulting in less overlap and a smaller window current. These shifts, and the decreased window current, are consistent with a decrease in sodium channel availability and a stabilization of the fast-inactivated state.

Slow Inactivation Defects

Arrhythmias due to LQT3 are non-exertional; they occur during rest or sleep.90-92 As such, slow inactivation might be predicted to have a limited role, if any, in LQT3 arrhythmogenesis. The insertion of aspartate at location 1795 of the C-terminus (1795ΔasD) is a slow inactivation-modifying, LQT3-associated mutation. 1795ΔasD produces persistent current, a destabilization of FI, which produces a prolonged QT.93,94 The mutation also decreases sodium peak current and augments slow inactivation.93,94 Together these defects produce a phenotype that fits both LQT3 and BrS. At rest, the individual displays a prolonged QT from the channel’s persistent current; however, at elevated heart rates, augmented SI decreases sodium current.94 This, in conjunction with an already decreased peak current, produces ST elevation.94

Several BrS mutations induce defects in NaV1.5 slow inactivation. The BrS mutation G1319V, located in the DIII5–S5 cytoplasmic linker, renders the channel less available for activation by depolarizing the g(V) curve, hyperpolarizing the steady state fast inactivation curve, slowing recovery from fast inactivation, and causing a two-fold increase in the onset of slow inactivation.95 Enhanced slow inactivation was also observed in a double mutation, K1527R/A1569R, that caused asymptomatic BrS.88 These mutations were also associated with a hyperpolarization of the fast inactivation midpoint. The kinetics of slow inactivation has been widely interpreted to range from tens of milliseconds to minutes. The shorter end of this
time domain has also been referred to as intermediate inactivation. Intermediate inactivation was stabilized in T1620M (expressed as a single mutation).\textsuperscript{97} Thus, BrS generally results in a stabilization of the slow inactivated state, which would be expected to reduce sodium channel availability and thus decrease cell excitability.

**Defects in Channel Expression**

Alterations in membrane expression of sodium channels can have drastic effects on the electrophysiological properties of the heart. Caveolae are 50 to 100 \( \mu \)m invaginations of the cellular membrane that act as reservoirs of Na\( _\alpha_{1.5} \) channels.\textsuperscript{98} The Na\( _\alpha_{1.5} \) channels are only made available following stimulation of the \( \beta \)-receptor pathway.\textsuperscript{99,100} \( \beta \)-receptor activation triggers the binding of the primary scaffolding protein of aLQT in the heart, caveolin-3 (CAV3), with the G-protein subunit G\( _{\text{wt}} \). The caveolar neck then opens, and the reservoir of Na\( _\alpha_{1.5} \) channels become available for activation. It was shown that \( \beta \)-receptor mediated caveolar current increases sodium current by as much as 35\% in rat myocytes.\textsuperscript{98}

To date, eight mutations associated with LQT type 9 (LQT9) have been isolated in the gene that encodes CAV3.\textsuperscript{53,101,102} Those thus far characterized, F97C, S141R, V14L, T78M and T79R, uniformly increase late sodium current and displayed 3- to 5-fold increases in magnitude thereof.\textsuperscript{53,101} As discussed previously, persistent sodium current is a primary cause of LQT3. The individual carrying the F97C mutation displayed a prolonged QT only during acute administration of an anti-asthmatic \( \beta \)-agonist inhaler.\textsuperscript{53}

Drug-induced LQT is the most common form of acquired LQT (aLQT) and is the consequence of both altered channel expression and kinetics. Several subclinical mutations in Na\( _\alpha_{1.5} \) can increase an individual’s susceptibility to drug-induced aLQT with administration of classically anti-arrhythmic drugs, such as quinidine or sotalol, and gastric motility agents like cisapride.\textsuperscript{103} L1825P is a loss-of-function mutation in the C-terminus of Na\( _\alpha_{1.5} \) associated with aLQT following administration of cisapride.\textsuperscript{104,105} L1825P channels display numerous defects including, but not limited to, decreased current density, 8-fold increase of persistent current, a hyperpolarized shift of the \( V_{1/2} \) of steady state fast inactivation curve, a depolarized shift of the \( V_{1/2} \) of steady state activation curve, and increased WC.\textsuperscript{104,105} Carriers of the mutation, however, have shown normal ECGs with an apparent healthy cardiac function. Only after exposure of the rapid delayed rectifier potassium channel (\( I_{\text{Kd}} \)) blocker cisapride do individuals develop the characteristics of aLQT.\textsuperscript{105} Expression of L1825P in tsA201 cells with acute administration of cisapride at 1 \( \mu \)mol/L showed no alterations in L1825P channel kinetics or current.\textsuperscript{105} Following prolonged (\( \geq 48 \) hours) cisapride administration, however, L1825P channels expressed in CHO cells showed a dose dependent increase in sodium current.\textsuperscript{106} Persistent current density increased by 4-fold to 5.04 \pm 0.77 pA/\( \mu \)F and cell surface expression increased from 9–30\% of wild type channels.\textsuperscript{104} Acting in conjunction with cisapride’s effect on \( I_{\text{Kd}} \), function, the increased membrane expression allows the persistent current of L1825P to delay ventricular repolarization and produce LQT, although it remains to be shown that similar effects are seen in myocytes. This mutation also demonstrates a modulatory role of the C-terminus in proper Na\( _\alpha_{1.5} \) protein trafficking.

Some BrS mutations clearly affect the level of channel expression, at least in heterologous expression systems such as HEK cells. The double mutant R1232W/T1620M fails to express at levels similar to WT Na\( _\alpha_{1.5} \).\textsuperscript{70} When the charged arginine at position 1232 was replaced with a similarly charged lysine, however, functional channels were produced by the R1232K/T1620M double mutant. Immunostaining and confocal studies indicate that the R12332W/T1620M double mutant co-localizes with calnexin, an ER-specific chaperone protein. It thus appears that a charged residue in position 1232 may be necessary for proper folding and adequate membrane surface expression. Curiously, the expression defect is not seen in Xenopus oocytes.\textsuperscript{106,107}

\( \beta \)-Subunit Interactions

Modulating \( \beta \)-subunits of voltage-gated sodium channels are much smaller than the pore forming \( \alpha \)-subunit but have large roles in channel gating and expression. \( \beta \)-subunits are single transmembrane proteins with a short cytoplasmic C-terminus and a long, extracellular, Ig-like N-terminus.\textsuperscript{108} There are four isoforms of the \( \beta \)-subunit (\( \beta_1 \)-\( \beta_4 \)) encoded by genes SCN1B, SCN2B, SCN3B, SCN4B. All four \( \beta \)-subunits are found in cardiac muscle. Subunits \( \beta_3 \) and \( \beta_4 \) have similar homology and bind with the channel’s \( \alpha \)-subunit through a disulfide link at the DIV/S5–S6 extracellular linker.\textsuperscript{3,108,109} Subunits \( \beta_3 \) and \( \beta_4 \) have similar homology and associate with the \( \alpha \)-subunit through noncovalent interactions.\textsuperscript{108}

Different \( \beta \)-subunits localize to different locale in cardiac muscle. \( \beta_1 \) is found in the intercalated discs and t-tubules, \( \beta_3 \) to just the t-tubules, and \( \beta_2 \) and \( \beta_4 \) to specific regions of the intercalated discs.\textsuperscript{3,109} A mutation in the \( \beta_4 \), L179F, is believed to be the sole cause of long QT type 10 (LQT10) and marks the first sodium channel \( \beta \)-subunit mutation associated with an inheritable cardiac disease.\textsuperscript{55} Heterologous expression of L179F with the WT Na\( _\alpha_{1.5} \) showed mild increase in WC magnitude as well as a 2.5-fold increase in persistent current.\textsuperscript{55} The heart rate-corrected QT interval (QTc) of the subject was significantly larger than family members who also carried the mutation, suggesting a multifactorial cause within the subject.

The function of WT \( \beta_4 \) is not well understood. Coexpression of WT\( \beta_4 \) with Na\( _\alpha_{1.5} \) appears to have little effect on channel function. \( \beta_4 \) has been shown to negate the modulatory effects other \( \beta \)-subunits have on channel function. Despite its unclear role in cardiac function, this mutation demonstrates the importance of \( \beta_4 \) in normal cardiac function.

As previously noted, co-expression of the \( \beta_1 \) subunit with T1620M exacerbates biophysical defects associated with BrS.\textsuperscript{69} This observation is consistent with a variety of reports which show the \( \beta_1 \) subunit stabilizes fast inactivation.\textsuperscript{110,111} Stabilization of fast inactivation promotes action potential repolarization and is predicted to contribute to the differential abbreviation of action potentials in right ventricular epicardial cells.

**Conclusion**

Although the prevalence of LQT and BrS are relatively low, their often fatal consequences makes them highly important disorders from a clinical standpoint. An ever-increasing number of identified sodium channel mutations underline these diseases, coincidentally providing a rich insight into structure/function relationships. On one hand, LQT3 arises from a prolongation of the ventricular action potential primarily due to a destabilization of fast inactivation in...
sodium channels. On the other hand, Brø typically arises from more rapid entry into the fast-inactivated state. A host of other factors, such as the voltage dependencies of activation, fast inactivation and slow inactivation, and alterations in channel expression levels, also play into the intricate balancing act that determines the relative availability of sodium channels. This availability, combined with repolarizing influence of potassium channels, sets the level of membrane excitability that results in action potential probability and duration. One is left with the profound impression that seemingly minor changes in the biophysical properties of sodium channels can produce major, sometimes fatal, results in terms of cardiac function. Since some of these changes in biophysical properties occur in regions of the channel that have not been previously associated with either gating, selectivity, or permeation, one is also left with the impression that there is much more yet to be learned about the relationship between channel structure and function.

Two important avenues require exploration. First, rational drug design based on individual effects of individual mutations is particularly poignant in light of the vast heterogeneity of effects imparted by individual mutations. Second, the identification of endophenotypes—a concept that, until recently, has been primarily used to help identify psychiatric disorders—might lead to earlier detection of cardiac disorders. It is remarkable, given the ventricular distribution of NaV1.5 being apparently restricted to intercalated disks, that profound disorders arise from mutations in this isoform. Since other sodium channel isoforms are also broadly distributed in the ventricles,33 it seems reasonable to predict that mutations in these other isoforms also give rise to cardiac rhythm disorders. Given the prevalence of e.g., NaV1.1 in the brain, such mutations (if not fatal) should elicit endophenotypes that might serve as an early warning system for potentially fatal cardiac arrhythmias. A few reports have noted a relationship between syncope and BrS, for instance, and a recent report co-identified febrile seizures (commonly associated with NaV1.1 and β1-subunit mutations) with BrS.112 In this family, however, a mutation was also identified in SCN5A. This points to the alternative scenario that NaV1.5 is expressed in the brain.113,114 In any case, seizure activity could serve as one possible endophenotype for cardiac rhythm disorders, and prudent clinical practice might include a comprehensive ECG when seizures present in early childhood. Although it may not seem practical to screen using ECGs for low-incidence disorders like LQT or BrS, a prolonged P-wave has been identified as an endophenotype for the far more common disorder of atrial fibrillation.115

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