A hot topic
Temperature sensitive sodium channelopathies

Csilla Egri and Peter C. Ruben*
Department of Biomedical Physiology and Kinesiology; Simon Fraser University; Burnaby, BC Canada

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Abbreviations: BrS, brugada syndrome; ECG, electrocardiogram; FS, febrile seizure; GEFS⁺, generalized epilepsy with febrile seizures plus; HRA, hyperthermic-induced respiratory alkalosis; LQT, long QT syndrome; Na⁺, voltage-gated sodium channel; PMC, paramyotonia congenita; Q¹⁰, temperature coefficient; SMEI, severe myoclonic epilepsy of infancy; V¹/², half-maximal voltage

Perturbations to body temperature affect almost all cellular processes and, within certain limits, results in minimal effects on overall physiology. Genetic mutations to ion channels, or channelopathies, can shift the fine homeostatic balance resulting in a decreased threshold to temperature induced disturbances. This review summarizes the functional consequences of currently identified voltage-gated sodium (Na⁺) channelopathies that lead to disorders with a temperature sensitive phenotype. A comprehensive knowledge of the relationships between genotype and environment is not only important for understanding the etiology of disease, but also for developing safe and effective treatment paradigms.

Sodium Channels: A Brief Introduction

A brief introduction to the structure and function of the voltage-gated sodium channel (Na⁺) is required in order to understand how mutations to this family of proteins lead to disease.

α Subunit

The large (260 kDa) pore forming α subunit of Na⁺ consists of four homologous domains (D1–DIV) each with six transmembrane spanning helices (S1–S6). To date, 10 isoforms of Na⁺ α subunits have been found, each with slightly different biophysical properties. Typically, Na⁺,1.1, 1.2, 1.3 and 1.6 are preferentially expressed in the central nervous system, and Na⁺,1.7, 1.8, 1.9 and Na⁺,x are preferentially expressed in the peripheral nervous system. Na⁺,1.4 is predominantly expressed in skeletal muscle, and Na⁺,1.5 predominates in cardiac muscle. The differential expression of Na⁺,x isoforms often leads to their description as “neuronal,” “cardiac” or “skeletal.” Each isoform, however, can be expressed diffusely throughout the body, so this specificity of terminology is becoming a less accurate depiction of Na⁺ localization.

Regardless of their location, the general function of Na⁺ is the same. They are the main determinants of action potential initiation and propagation in electrically excitable cells. Segments S5–S6 and their extracellular linker constitute the Na⁺ permeable pore and selectivity filter, allowing passage of Na⁺ ions down their electrochemical gradient. Na⁺ are not constitutively open, but are gated by changes in membrane voltage. A high density of positively charged amino acids in the S4 segment of each domain allows the segment to shift in response to changes in the electric field. Thus, the S4 segments are referred to as the channels’ ‘voltage sensors’. This voltage dependent conformational change initiates a series of events leading to channel activation. Shortly after channel activation, four hydrophobic amino acid residues (IFMT) located within the intracellular linker of DIII and DIV, also undergo a conformational change and bind to the S4–S5 cytoplasmic linker of DIII and DIV. This “fast-inactivation particle” blocks the ion permeating pore and inactivates sodium current (INa) within a matter of 1–2 ms, causing fast inactivation. There is small area of overlap between the voltage dependence of inactivation and activation, where channels activate but fail to inactivate. This is referred to as ‘window current’ and results in a small, late component of INa at this range of membrane potentials. Late current can also result from failure of channels to fully fast inactivate, or a subpopulation of channels with slower inactivation kinetics, producing subthreshold persistent currents. Na⁺ mutations which increase persistent current also increase cellular excitability.¹

Channels can quickly recover from fast inactivation, within a range of 5–10 ms, upon return to a hyperpolarized potential. The time course of entry into and recovery from fast inactivation determines channel availability for subsequent action potentials. Sodium channel fast inactivation is responsible for the absolute refractory period of an action potential and allows propagation in a unidirectional manner because previously activated channels have not yet recovered from fast inactivation and cannot be re-excited.
Another form of channel inactivation, termed slow inactivation, develops more slowly, in the range of seconds to minutes in response to sustained or repetitive depolarization. Accumulation of channels into the slow inactivated state in response to repetitive depolarizations is called use-dependent inactivation, and is an important regulator of cellular excitability in high frequency firing neurons. The transition into the slow inactivated state is thought to involve further movement of the S4 segment during depolarization (sustained or repetitive) resulting in a change in configuration of the permeation pathway and thus inactivation of current.\(^2\,^3\)

Recovery from the slow inactivated state also takes much longer than from the fast inactivated state, and displays a so-called “memory” because recovery time is extended with increasing duration of membrane depolarization.\(^4\) The long time course of development and recovery from slow inactivation is a major determinant of cellular excitability, and dictates the number of channels available to open in response to subsequent depolarizations. Thus, any shifts in voltage dependence, time of onset, or recovery from slow inactivation would have profound consequences on neuronal excitability.

**Auxiliary β Subunits**

Although experimental evidence shows that the α subunit is functional on its own, native channels typically associate with one or two auxiliary β subunits 1–4.\(^2\) Each is significantly smaller (33–36 kDa) than the α subunits and is composed of an N-terminal extracellular immunoglobulin-like domain important for α-β interaction, one transmembrane segment, and a short intracellular C-terminal segment.

The β\(_1\) isoform appears to be the predominant subunit to associate with cardiac and neuronal channels, but its exact role as an Na\(_\text{v}\) modulator is unclear. A comprehensive view of β\(_1\) function is difficult as functionality differs depending on the Na\(_\text{v}\) isoform and the experimental expression system used. However, we know that α-β interactions are functionally important in the brain, heart, as well as skeletal muscle.\(^6\,^9\) This review highlights only the role of the non-covalently associated β\(_1\) subunit, whose general function is to modulate channel gating and kinetics as well as aid in trafficking to the membrane.

**Sodium Channels and Temperature Sensitivity**

Before delving into sodium channelopathies conferring temperature sensitive phenotypes, it is worthwhile to discuss what is currently known about how temperature affects native channels.

Due to the technical challenges associated with performing electrophysiological experiments at physiological temperatures, most of what we know about Na\(_\text{v}\) function has been collected electrophysiological experiments at physiological temperatures,\(^7\) while skin temperature is significantly lower and averages around 33°C.\(^8\) Temperature of the external environment, as well as internal heat produced by increased metabolism, can perturb the normothermic state. Within certain limits, the human body is able to handle such changes. Beyond a core temperature of 40–41°C, however, an adult would begin to experience the convulsive effects of hyperthermia, or outside the range of 3–45°C feel noxious cold or heat on the skin, or below a muscle temperature of 35°C begin to experience muscle stiffness.\(^29\,^30\) Certain disorders, however, can lower the bodies’ natural thermal threshold.

**Temperature-Sensitive Sodium Channelopathies**

Mean core body temperature is tightly regulated and ranges between 36.5–37.5°C,\(^7\) while skin temperature is significantly lower and averages around 33°C.\(^8\) Temperature of the external environment, as well as internal heat produced by increased metabolism, can perturb the normothermic state. Within certain limits, the human body is able to handle such changes. Beyond a core temperature of 40–41°C, however, an adult would begin to experience the convulsive effects of hyperthermia, or outside the range of 3–45°C feel noxious cold or heat on the skin, or below a muscle temperature of 35°C begin to experience muscle stiffness.\(^29\,^30\) Certain disorders, however, can lower the bodies’ natural thermal threshold.

**Paramyotonia Congenita**

A prominent temperature sensitive sodium channelopathy is the hereditary neuromuscular disorder paramyotonia congenita (PMC). Dr. Ezra Clark Rich first described this disorder in the late 1800s in his own family. His aunt, affected by the disorder, showed no muscular impairments at normal temperature but displayed dramatic facial myotonia (muscle stiffness) upon exposure to cold.\(^106\) Cold induced myotonia is still a hallmark of PMC...
today, and can be aggravated by increased activity (paradoxical myotonia). With intensive cooling, the myotonia can give way to periodic paralysis of the muscles.24,31

PMC is caused by mutations in the skeletal muscle sodium channel isoform NaV1.4. Most PMC mutations impair channel inactivation, leading to persistent I_Na, and subsequent reduction of membrane excitability responsible for the periodic paralysis phenotype, while others increase membrane excitability resulting in muscle stiffness.26-31,32 This may seem counterintuitive, but a gradient exists between severity of inactivation impairment and resulting phenotype. Minor impairments to fast inactivation (between 8–20% non-inactivating channel population) result in a late I_Na that depolarizes the membrane just enough to bring it closer to action potential threshold, thus increasing the probability of action potential firing and muscle stiffness.33 Severe impairments to fast inactivation (greater than 20% non-inactivating channel population) however, result in a late I_Na that can significantly depolarize the membrane to about -40 mV (from a normal resting value of about -90 mV).34 At this potential, many remaining channels are in the inactivated state, rendering the membrane refractory and resulting in weakness or paralysis. Why cold or, in paradoxical myotonia, warmth exacerbate PMC symptoms is yet to be determined.

It is noteworthy that temperature-dependent phenotypes, such as myotonic episodes, depend on the threshold properties of membrane excitability. Such episodes may be evoked by subtle, temperature-dependent changes in channel gating. Future studies could investigate these temperature-dependent triggers using simulations and incorporating stability analyses. A recent review of skeletal muscle channelopathies by Jurkat-Rott et al. (2010) indicates that the temperature sensitive phenotype of PMC is not due to mutations altering temperature sensitivity, but is more dependent on the slowing of channel kinetics at cooler temperatures.31 Cooling the bath solution slows channel inactivation to a greater extent than activation,34 resulting in increased channel open time. Thus, the overall effect of cooling on the action potential would be increased amplitude, duration and refractory period.30 These changes to the muscle action potential, however, cannot explain the lack of adverse musculoskeletal effects in normal individuals compared with those with PMC. In fact, many PMC mutations characterized in vivo suggest a special temperature sensitive mechanism.26,35,36,105 Even for those mutations in which no changes in temperature sensitivity were found,37,38 observing no change in Q10 of one kinetic parameter may not characterize temperature dependence due to different rate constants for each state transition.

This disparity is well illustrated by the study of two PMC mutations; a proline to serine mutation at position 1,158 (P1158S) and an isoleucine to threonine mutation at position 693 (I693T) in NaV1.4.26,37,38 Both these mutations are implicated in cold induced paralysis without subsequent myotonia, as well as in another spectrum of congenital muscle disorders, hyperkalemic periodic paralysis.26,38 The two groups that investigated P1158S reported similar results. Cooling the external recording solution resulted in a hyperpolarizing shift in activation, fast and slow inactivation, as well reduced propensity for slow inactivation.26,38 These changes to P1158S gating were not apparent at 37 or 32°C (choice of “warm” temperature varied between the two laboratories) and were significantly different from WT.

For the mutant I693T, however, cooling from 22 to 16°C produced no significant changes to V1/2 of activation or fast inactivation in either mutant or WT.37 This may suggest that I693T is not temperature sensitive; however, slow inactivation, shown to be more temperature sensitive than fast inactivation, was not investigated in the study. Nevertheless, the temperature induced left shift in activation and impairment of slow inactivation seen in P1158S offers an explanation for cold induced exacerbation of PMC symptoms. Steady depolarizing current due to incomplete slow inactivation at colder temperatures would result in a reduction of subsequent membrane excitability, thus play a role in the paralytic phenotype of PMC.

Further evidence of impaired slow inactivation upon exposure to cold is provided by analysis of a glutamine to lysine mutation at position 270 (Q270K) in NaV1.4.24 Similar to other PMC mutations, Q270K impairs fast inactivation, but slow inactivation is affected to a greater extent upon cooling.24 The voltage dependence of slow inactivation was similar between mutant and wild type at 30°C, but upon cooling to 20°C and 11 mV depolarizing shift was observed for Q270K.35 This leftward shift of slow inactivation is consistent with that observed for P1158S, and potentially contributes to PMC related weakness and paralysis.

Impairments to slow inactivation are typically associated with paralysis in PMC, whereas changes to other biophysical parameters such as fast inactivation are associated with myotonias.23 Several of the mutations implicated in cold induced myotonias occur at the amino acid site 1,448, and exchange the native arginine with proline, cysteine, serine or histidine (R1448P/C/S/H).26,37,38 This site, located on the S4 segment of DIV (Fig. 1), is implicated in the coupling of activation to inactivation, and these mutations share a common destabilization of fast inactivation.26,35,36,39,40 The severity of the mutation reflects the severity of the PMC phenotype, decreasing from proline (most severe) to histidine.39,41 In vitro analysis of temperature sensitivity, however, does not show such a clear gradient. R1448P, which apparently has no temperature sensitive alterations (see Table 1), has the most severe PMC phenotype, while the most temperature sensitive mutation, R1448H, has the mildest phenotype.32,35

It is not easy to pinpoint the exact cause of these disparities, as the choices of expression system, inclusion or exclusion of auxiliary subunits, electrophysiological technique, as well as recording solutions all complicate the comparison of experimental results. It is difficult to argue that one technique is better than another, and the non-uniformity of experimental methods across laboratories increases the difficulty of arriving at a consensus regarding the biophysical basis of cold sensitive PMC.

**Febrile Seizures**

Febrile seizures (FS) are common in the general population, occurring in approximately 2–5% of children aged 6 mo to 5 y.42 The infant brain is known to be more sensitive to perturbations in homeostasis due to extensive neuronal network.
development and remodelling, but why some children respond to fever by seizing and some don’t is still unknown. Genetic predisposition is a likely factor, as incidence of FS and recurring FS is higher in patients with a family history of epilepsy or prior FS.53 Mutations have been mapped to several chromosomal loci: FEB2 on chromosome 19p, FEB5 on chromosome 6q, FEB6 on chromosome 18p, FEB7 on chromosome 21q22, FEB9 on chromosome 3p24.2-p23 and FEB10 on 3q26.44-49 Specific mutations to genes SCN1A and SCN9A encoding isoforms Na1.1 and Na1.7, respectively, have also been identified and associated with familial febrile convulsions.50-52 Increased temperature sensitivity, either of the immature brain or due to mutations, is a likely mechanism of FS. Other factors may also be involved in seizure genesis. Inflammatory cytokines produced during infection and fever may increase neuronal excitability, or febrile electrolyte imbalances may perturb ionic homeostasis.53,54 Another possible mechanism is hyperthermia-induced respiratory alkalosis.

Hyperthermia-induced respiratory alkalosis (HRA) has been the subject of recent debate in the literature. As body temperature rises during febrile states, the compensatory increase in breathing rate expels CO2 rapidly enough to result in a mild increase in intracortical pH, a phenomenon which is apparently more pronounced in infants.55 The HRA theory suggests that it is the increased pH which precipitates seizure onset, not the effect of temperature itself. Animal studies support this hypothesis, showing increased breathing rate and resultant rise in intracortical pH preceding seizure onset in heated rat pups.56-58 Although HRA involvement in seizure genesis is documented in animals, the extent to which this affects humans is controversial.59 The temperature elevation necessary to induce respiratory alkalosis in rat pups is approximately 9°C (a seizure threshold of 41°C from baseline of 33°C).57,60 Even in infants, who tend to have a higher peak febrile temperature compared with adults (40–41°C compared with 38–38.5°C in adults),61 a 4°C change in body temperature may not be sufficient to induce respiratory alkalosis extensive enough to cause neuronal imbalance.

When hyperventilation and increased pH are excluded as possible causes for thermally induced convulsions, animal and electrophysiology studies provide extensive evidence that temperature has a direct role in seizure genesis. Hyperthermia induced seizures in rat pups is a well-documented phenomenon, and shows age dependence; only young rats are susceptible to reversible hyperthermic seizures.62-66 A reliable method for studying thermally induced epileptiform activity which removes some of the potential complications of in vivo analysis is studying brain slices in vitro. Young rat hippocampal tissue slices heated by 3–4°C produce ictal-like discharges, which disappear upon returning to baseline temperature.67 This febrile model also shows age-dependent seizure activity, reflecting observations in humans.67

Evidently, increased temperature can perturb neuronal networks enough to cause epileptogenesis. To our knowledge, no in vitro experiments investigating the effects of temperature on any specific Na+ mutation associated with FS have been performed to date. Clearly, temperature plays a major role in increasing neuronal excitability. Characterizing the temperature sensitivity of FS mutations would help clarify the mechanisms of this disease at the molecular level.

**GEFS+**

Generalized epilepsy with febrile seizures plus (GEFS+), a clinical subset of familial FS, is a multifaceted pediatric epilepsy syndrome. Numerous mutations to a variety of neuronal ion...
C121W was the first mutation to be associated with GEFS+, and the first mutation in the β subunit to be implicated in disease. The mutation occurs in the Ig-loop of the β1 subunit, disrupting interaction with pore forming α subunit. Electrophysiological studies in heterologous expression yield conflicting reports as to the functional effects of this mutation. Development of a knock in mouse model by Wimmer et al. has helped shed some light on the seizure-causing mechanism of this mutation in vitro.

Brain slices from heterozygous C121W (CW) mice display increased neuronal excitability, evident by increased tonic firing frequency, lowered threshold and higher action potential amplitude. Most interesting, however, is that CW mice have increased temperature sensitivity. Action potential recordings from brain slices at 22 and 34°C revealed statistically significant differences between wild type and CW at 34°C that weren’t apparent at 22°C. Live CW mice also displayed a decreased thermal seizure threshold compared with their wild-type littermates.

### Table 1. Summary of results for thermosensitive sodium channelopathies

| Disorder                  | Gene  | Amino acid mutation (ref.) | Na+ species and expression system | Main results                                                                 |
|---------------------------|-------|----------------------------|----------------------------------|-----------------------------------------------------------------------------|
| Brugada syndrome          | SCN5A | T1620M<sup>81</sup>       | Human variant expressed in a tsA201 cell line | Increasing the temperature from 22–34°C resulted in:                       |
|                           |       |                            | - β1 did not affect peak I<sub>N</sub> in oocytes (measured at 22°C only) |
|                           |       |                            | - HEK cells co-expressed with β1 showed reduced peak I<sub>N</sub> (22 and 32°C) |
|                           |       |                            | - incubation of HEK cells at lower temperature (26°C) attenuated current reduction (suggests αβ interaction results in temperature dependent trafficking defect in mammalian cell line) |
|                           |       |                            | V1340I<sup>96</sup>             | Human variant expressed in HEK cells                                       |
|                           |       |                            | -V1340I in SCN5A yields a non functional channel |
|                           |       |                            | -V1340I in SCN5A-ΔI077Q splice variant resulted in 50% current reduction and faster recovery of FI at higher temperatures (32, 37, 40°C) compared with WT-delQ at 22°C |
| L325R<sup>18</sup>       | SCN5A | -                 | Human variant expressed in HEK cells | Increasing the temperature from 23–33°C resulted in:                       |
|                           |       |                            | -80% reduction of I<sub>N</sub> at 22°C |
|                           |       |                            | -increasing the temperature from 22–42°C attenuated current reduction (inconsistent with ECG phenotype) |
|                           |       |                            | -suggest hyperthermic arrhythmia mediated through WT Na<sub>1.5</sub> |
| Long QT3 syndrome         | SCN5A | ΔKPQ<sup>22</sup>         | Human variant expressed in HEK cells | Increasing the temperature from 23–33°C resulted in:                       |
|                           |       |                            | -decreasing frequency dependent run down of I<sub>N</sub> (no temperature data) |
| Febrile seizures          | SCN1A | No specific amino acid mutation<sup>81</sup> | Heterozygous SCN1A null mice | -age dependent lowered thermal seizure threshold (39.5°C for mutant mice vs. 42.5°C for WT) |

AP, action potential; Fl, fast inactivation; Hyper/HypoPP, hyperkalemic and hypokalemic periodic paralysis, respectively; I<sub>N</sub>, sodium current; V<sub>a</sub>, voltage dependence of half maximal activation; V<sub>h</sub>, voltage dependence of half maximal fast inactivation; V<sub>s</sub>, voltage dependence of half maximal slow-inactivation; WT, wild type.

Channels have been identified and a classification system based on their chromosomal location has been implemented. Listing only those involving the Na<sub>v</sub> family and associated subunits; GEFS+ Type 1 is associated with mutations to SCN1B encoding the β<sub>1</sub> subunit of Na<sub>v</sub>; GEFS+ Type 2 with SCN1A encoding Na<sub>v</sub>1.1; and GEFS+ Type 7 with SCN9A encoding Na<sub>v</sub>1.7. The phenotypes associated with the various GEFS+ mutations vary between affected individuals, but the most common symptoms are febrile seizures (FS). As mentioned previously, FS are quite common in young children and have an age dependent decrease in prevalence. Patients with GEFS+, however, display FS persisting beyond the age of 6.

Presumably, Na<sub>v</sub> mutations associated with GEFS+ cause the FS phenotype by altering the temperature sensitivity of the channels, thereby lowering the seizure threshold temperature. Of the many Na<sub>v</sub> mutations of GEFS+, the only mutant to have its temperature sensitivity analyzed to date is a cysteine to tryptophan mutation in the auxiliary β<sub>1</sub> subunit (C121W).
Further experiments by Egri et al. complement the findings of Wimmer et al. and elucidate a putative temperature sensitive role of C121W at the molecular level. Temperature elevation from 22–34°C resulted in increased channel excitability of Na\textsubscript{V} 1.2+β\textsubscript{1} (C121W) compared with Na\textsubscript{V} 1.2+β\textsubscript{1} and the Na\textsubscript{V} 1.2 α subunit alone. Na\textsubscript{L}1.2+β\textsubscript{1}(C121W) at 34°C displayed decreased use-dependent inactivation, increased persistent current and window current, and delayed onset of, and accelerated recovery from, fast-inactivation.\textsuperscript{15} This suggests that expression of wild-type β\textsubscript{1} is protective against increased channel excitability induced by...

### Table 1. Summary of results for thermosensitive sodium channelopathies

| Disorder                              | Gene  | Amino acid mutation\textsuperscript{WT} | Na\textsubscript{V} species and expression system | Main results                                                                 |
|---------------------------------------|-------|------------------------------------------|--------------------------------------------------|-------------------------------------------------------------------------------|
| Paramyotonia congenita                | SCN4A | Q270K\textsuperscript{24}               | Human variant expressed in HEK cells             | -depolarizing shift in V\textsubscript{a} upon cooling from 30–20°C          |
| R1448C and T1313M\textsuperscript{36} |       |                                          | Human variant expressed in oocytes               | -slowing of open-state and FI-state deactivation in R1448C was more temperature sensitive than in WT (measured at 22, 15 and 10°C) -temperature sensitivity of T1313M similar to WT -neither mutant exhibited significant shift in V\textsubscript{a} upon cooling, whereas WT displayed 15mV depolarizing shift |
| R1488P\textsuperscript{32}           |       |                                          | Human quadriceps sarcolemma                      | -no difference in temperature sensitivity between mutant and wild type (measured at 15, 22 and 30°C) |
| R1448H and M1360V\textsuperscript{35} |       |                                          | Human variant expressed in HEK cells             | -compared with WT; both mutations have slower FI entry and faster FI recovery (measured at 15, 25 and 35°C) -in R1448H only, decreased temperature from 34°C resulted in: -hyperpolarizing shift in V\textsubscript{a} -increased window current |
| P1158S\textsuperscript{26,38}        |       |                                          | Human variant expressed in HEK cells and a tsA201 cell line\textsuperscript{28} | *associated with Hyper/HypoPP as well as PMC cooling of mutant channels from 32–22°C\textsuperscript{38} or from 37–25°C\textsuperscript{26} resulted in: -hyperpolarizing shift in V\textsubscript{a} and V\textsubscript{h} -depolarized shift in V\textsubscript{a} -persistent I\textsubscript{Na} due to incomplete SI |
| F1473S\textsuperscript{20}          |       |                                          | Human variant expressed in HEK cells             | -mutant displays increased persistent current and slower inactivation at all temperatures (15, 22 and 30°C) both which are exacerbated by cooling (PMC phenotype possibly due to threshold phenomenon) |
| I693T\textsuperscript{37}           |       |                                          | Human variant expressed in HEK cells             | -cooling from 22–16°C produced no significant changes to V\textsubscript{a} or V\textsubscript{h} between mutant and WT |
| C121W\textsuperscript{77}           | SCN1B |                                          | Heterozygous C121W knock in mice                 | -decreased thermal seizure threshold by 0.44°C compared with WT mice -increased AP frequency and amplitude at 34°C compared with 22°C |
| C121W\textsuperscript{15}           |       |                                          | Rat variant expressed in CHO cells              | C121W compared with WT at 34°C (22°C control): -increased I\textsubscript{Na} \textsubscript{rest} and window current -decreased use-dependent inactivation -decreased rate of FI onset -increased rate of FI recovery |
| Erythromelagia                        | SCN9A | L858F\textsuperscript{37}              | Human variant expressed in HEK cells             | -decreased temperature from 35–25°C or 16°C caused a depolarizing shift in V\textsubscript{a} of mutant but not WT (resulting in V\textsubscript{a} of mutant closer to that of WT at 16°C) |

AP, action potential; FI, fast inactivation; Hyper/HypoPP, hyperkalemic and hypokalemic periodic paralysis, respectively; I\textsubscript{Na}, sodium current; V\textsubscript{a}, voltage dependence of half maximal activation; V\textsubscript{h}, voltage dependence of half maximal fast inactivation; V\textsubscript{s}, voltage dependence of half maximal slow-inactivation; WT, wild type.
Severe Myoclonic Epilepsy

Severe myoclonic epilepsy of infancy (SMEI) was previously considered part of the extreme spectrum of phenotypes for GEFS+ Type 2. Although the mild phenotype of GEFS+ Type 2 is due to missense mutations to SCN1A, SMEI is due to nonsense mutations resulting in loss of function. Hence, they are now considered separate disorders. The reduction of functional Na\textsubscript{1.1} in SMEI results in severe infantile-onset epilepsy, often precipitated by fever, and result in developmental and psychomotor delay.

To date, no in vitro functional studies have been performed on individual Na\textsubscript{1.1} mutations to assess their sensitivity to temperature, but mouse models of SMEI do show age dependent thermally induced seizures. SMEI mice have a targeted gene deletion of one copy of SCN1A, resulting in reduced whole cell I_{Na}, specifically in pyramidal and hippocampal inhibitory interneurons. A reduction of I_{Na} in inhibitory neurons may result in increased neuronal network excitability by a disinhibitory mechanism. This increased neuronal excitability plays a role in decreased thermal threshold for seizure development. SMEI mice display epileptic activity upon raising core temperature to 39.5°C, while wild type mice have no seizure activity up to 42.5°C.

The biophysical mechanism underlying heat induced seizures associated with loss of Na1.1 channels is not known. Because Na1.1 are not the only neuronal sodium channels, it is possible a reduction in Na1.1 increases the proportion of I_{Na} driven by other Na\textsubscript{1.x} isoforms, such as Na1.2. As previously mentioned, Na1.2 has a relatively large Q\textsubscript{10} value compared with other Na\textsubscript{1.x}, and opens at more hyperpolarized potentials at higher temperatures.

Erythromelalgia

Erythromelalgia is a chronic pain syndrome where mild warmth (32–36°C) can induce attacks of burning pain of the skin which is alleviated upon cooling. While secondary erythromelalgia can be a symptom associated with thrombocytopenia, diabetes, peripheral neuropathy or use of certain drugs, primary erythromelalgia is an inherited disorder caused by mutations to SCN9A. SCN9A encodes peripheral nerve Na\textsubscript{1.7}, which is preferentially expressed in dorsal root ganglion (mainly of nociceptive neurons) and sympathetic ganglion.

To date, 9 SCN9A mutations have been identified which cause primary erythromelalgia, all of which share a common hyperpolarizing shift in V\textsubscript{1/2} of activation. A rightward shift in activation can increase neuronal excitability and, since Na\textsubscript{1.7} can be found in nociceptive neurons, may cause a lowered threshold for pain sensation. The link between pain and elevated skin temperature in erythromelalgia may lie with the co-expression of Na\textsubscript{1.7} in neurons with temperature sensitive transient receptor vanilloid (TRPV) channels, although this association has yet to be fully worked out.

The relief of pain upon cooling the skin has, however, been investigated in conjunction with a known Na\textsubscript{1.7} mutation, L858F. In agreement with previous studies on erythromelalgia mutations, L858F causes a hyperpolarizing shift in V\textsubscript{1/2} of activation in comparison to WT. This divergence between mutant and WT is lessened upon cooling due to a depolarizing shift in L858F which is not present in WT. This brings the V\textsubscript{1/2} of activation of the mutant closer to that of WT, decreasing the hyperexcitability of the neuron and contributing to the alleviation of pain upon cooling of the skin.

Brugada Syndrome

Brugada syndrome (BrS) is an autosomal dominant arrhythmogenic disorder with a high risk of sudden cardiac death in young adults. It is associated with mutations in the SCN5A gene encoding the cardiac sodium channel isoform, Na\textsubscript{1.5}, typically resulting in loss of function and reduced I_{Na}. Diagnosis of BrS relies on absence of any structural heart disease as well as manifestation of a characteristic ECG phenotype. ECG abnormalities include ST-segment elevation in precordial leads V1–V3 and apparent right bundle branch block, that can degenerate into ventricular fibrillation. The ECG phenotype can be explained by the decreased I_{Na} that decreases the initial depolarizing force in the cardiac action potential. This results in an all or none repolarization due to the heightened contribution from outward potassium currents. This effect is greatest in right ventricle which has a high level of transient outward potassium current. The loss of the epicardial action potential dome and premature action potential termination primarily in the right ventricle creates a transmural voltage gradient leading to the apparent ST segment elevation on the ECG.

The pathophysiology of BrS is generally transient, and influenced to a large extent by genetic and environmental factors. Fever is a common environmental trigger of BrS, and can unmask BrS ECG changes and ventricular arrhythmias, that cease upon return to a normothermic state. While fever has been known to trigger arrhythmic events in individuals with normal hearts, it is likely that underlying Na\textsubscript{1.5} mutations result in altered temperature sensitivity, contributing to abnormal transmural gradients during hyperthermic conditions.

Genotyping of febrile induced BrS patients reveals several Na\textsubscript{1.5} mutations and polymorphisms which have been analyzed for temperature sensitivity in vivo. A threonine to methionine substitution at position 1620 (T1620M) in Na\textsubscript{1.5} expressed in oocytes at room temperature showed accelerated recovery from and depolarized shift in steady-state fast inactivation. These findings, however, are inconsistent with the ECG characteristics of BrS, and it appears that arrhythmogenic effects of this mutation are only revealed at elevated temperatures. Electrophysiological recordings from mammalian cells at 34°C (compared with 22°C) show hastened inactivation kinetics and depolarization the V\textsubscript{1/2} of activation, reducing the I_{Na} contribution during the depolarizing
phase of the cardiac action potential. Decreased $I_{Na}$ at elevated temperature is the likely trigger for ventricular arrhythmias in patients carrying this mutation.

The functional effects of T1620M may be dependent on $\beta_1$ co-expression along with the polymorphism R1232W found in some febrile BrS patients. Wan et al. did not show temperature dependent shift in gating kinetics for this mutant, but reported a temperature dependent defect in trafficking to the membrane mediated by co-expression of $\beta_1$.

The channel splice variant that the mutation expressed may also influence function. Samani et al. expressed a valine to isoleucine (V1340I) mutation identified in patients with febrile induced arrhythmias in two splice variants; SCN5A and SCN5A with a deletion of a glutamine at position 1077 (Q1077Δ). V1340I in an SCN5A background produced a non functional channel, whereas V1340I in the SCN5A-Q1077Δ variant resulted in decreased current and increased recovery from fast inactivation with elevated temperature. Keller et al. (2005) analyzed the loss of function mutations: L325R, which replaces a leucine with arginine, and R535A which results in an arginine deletion at position 535. They showed an 80% current reduction with L325R, while R535Δ yielded a non-functional channel. Temperature analysis of L325R failed to produce effects consistent with ECG abnormalities observed in BrS. Keller et al. (2005) proposed that the autosomal dominant pattern of inheritance of BrS results in a dominant negative reduction in the number of functional sodium channels. Thus, it could be argued that febrile arrhythmias are not mutation specific but instead are mediated through the inherent temperature sensitivity of wild-type channels in conjunction with reduced $I_{Na}$.

A year later, Keller et al. (2006) characterized the newly identified phenylalanine to serine mutation at position 1344 (F1344S) in Na$_{1.5}$ and observed similar temperature dependent Na$_{1.5}$ current reduction. The reduction in current for F1344S was attributed to a significant right shift in the $V_{1/2}$ of activation, which was exacerbated at elevated temperatures. Action potential modeling of the right ventricle shows that a 50% reduction in $I_{Na}$ in conjunction with elevated temperature results in premature termination of the action potential, consistent with the BrS phenotype.

Elevating temperature of a canine model of heart tissue also supports a non-mutation-specific mechanism of hyperthermic arrhythmias. Morita et al. demonstrate a temperature induced pro-arrhythmic state due to shortening of the action potential duration and creation of a large intramural dispersion gradient of action potential duration.

Regardless of the varied ionic mechanisms, it is clear that fever is an important risk factor for arrhythmias and hyperthermia may be a stronger provocative test for BrS than sodium channel blockers in some patients. Close monitoring and antipyretic treatment of BrS patients can be recommended as a preventative therapy.

Long QT Syndrome

Long QT syndrome (LQTS) is a congenital heart disease that results in lengthened action potential duration and increased QT interval on the ECG. The lengthened QT interval can induce arrhythmic events such as torsades de pointes which can degenerate into ventricular fibrillation and sudden cardiac death. A majority of LQTS arises from mutations to different ion channels involved in the cardiac action potential, and is classified into different types depending on the mutation. LQTS Type 3 (LQT3) is estimated to underlie 10–15% of all LQTS and results from mutations to Na$_{1.5}$. It is typically increased late $I_{Na}$ which is the main contributor to LQT3 associated arrhythmias.

Genotype to phenotype correlations are not clear cut; however, as several mutations identified in LQT3 overlap with those for BrS. Brugada syndrome patients, as mentioned above, can be sensitive to febrile induced arrhythmias, thus hyperthermia may also be a variable implicated in LQT3 cardiac events. This correlation is highly plausible, as febrile illness has been shown to further lengthen the QT interval and precipitate arrhythmias in LQT Type 2, a LQT variant caused by potassium channel mutations. The link between hyperthermia and arrhythmias is complex, and there is evidence that elevated temperature increases the transmural dispersion gradient of repolarization, precipitating arrhythmias.

While there are no specific LQT3 Na$_{1.5}$ mutations identified which display enhanced temperature sensitivities, biophysical analysis close to physiological temperature is recommended for characterization of these mutations in vivo.

One such mutation investigated at close to physiological temperature is the three amino acid deletion in Na$_{1.5}$ ΔKPQ. As expected, kinetics for activation and inactivation increased for both the mutant and wild type from 22–33°C. Unexpectedly, however, the relative persistent current did not change with increased temperature. Other, complex changes to gating kinetics were revealed for the ΔKPQ mutant at higher temperatures, such as an increased slow component of recovery from fast inactivation, as well as a depolarizing shift in $V_{1/2}$ of activation.

Understanding how disease-causing mutations, as well as wild-type channels, behave at physiological temperatures is important since even small changes to gating parameters from those observed at room temperature and at 37°C can have profound implications on conduction and refractoriness of the cardiac myocyte. More data collected at physiological temperatures will help increase the accuracy of cardiac action potential models, which are excellent tools in predicting arrhythmogenic effects due to perturbations to homeostatic conditions.

Concluding Remarks

Sodium channelopathies lead to disease through a great variety and combination of alterations to sodium currents, increasing the need for pharmacological therapies specific to genotype. The more we understand about the biophysical mechanisms underlying disease, the more rationally we can develop treatments. Although the mechanism for disease can vary, a significant portion of these channelopathies share a common property: sensitivity to temperature. This shared characteristic illustrates the importance of regulating body temperature and preventing fluctuations in temperature which exacerbate disease symptoms.
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