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Channelopathies from Mutations in the Cardiac Sodium Channel Protein Complex

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Abstract

The cardiac sodium current underlies excitability in heart, and inherited abnormalities of the proteins regulating and conducting this current cause inherited arrhythmia syndromes. This review focuses on inherited mutations in non-pore forming proteins of sodium channel complexes that cause cardiac arrhythmia, and the deduced mechanisms by which they affect function and dysfunction of the cardiac sodium current. Defining the structure and function of these complexes and how they are regulated will contribute to understanding the possible roles for this complex in normal and abnormal physiology and homeostasis.

Keywords

Macromolecular complex; Signaling complex; Interacting proteins; sodium current; SCN5A; Arrhythmia; Long QT syndrome; Brugada Syndrome; SIDS

1. Introduction and Background

1.1 I_{Na} in heart: excitability, arrhythmogenesis, and sodium-calcium homeostasis

Sodium current (I_{Na}) underlies excitability in cardiac ventricular and atrial myocytes and also in specialized conduction tissue including Purkinje cells. Peak I_{Na} is a large inward current responsible for rapid upstroke of the action potential (phase 0) and for conduction in working myocardium. The I_{Na} flowing just milliseconds after the peak, here called “early I_{Na} ”, decays rapidly but helps sustain the initial plateau (phase 1) during activation of the transient outward potassium current (I_{to}). I_{Na} then rapidly decays, but a late relatively small I_{Na} flowing tens to hundreds of milliseconds after the peak that is less than 1% of the peak

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Disclosures

None.

Conflict of interest

The authors declare no conflict of interest.

I_{Na} combines with the inward calcium current to sustain the plateau (phase 2) of the action potential. This late I_{Na} is sometimes called sustained or persistent I_{Na} but we prefer the more generic term late I_{Na} because late I_{Na} is often subject to decay and therefore not completely sustained or persistent. Increased late I_{Na} representing a “gain of function,” prolongs the action potential and produces a long QT interval on the ECG and is arrhythmogenic in part by causing early afterdepolarizations. Increased late I_{Na} is a cause of the broader category of the Long QT syndrome (LQTS) arrhythmia. Increased late I_{Na} also contributes to sodium loading of myocytes, and by secondarily decreasing extrusion of calcium by sodium/calcium exchange, causes calcium overload [4]. This has effects on both inotropy and lusitropy, and can be arrhythmogenic by producing delayed afterdepolarizations. Decreased peak and early I_{Na} represent a “loss of function,” slows conduction, and is arrhythmogenic by setting up reentry. It can also be arrhythmogenic by allowing for phase 1 repolarization in some myocytes and phase 2 reentry, a mechanism suggested for Brugada syndrome (BrS) [6]. Note that it is possible to have in the same tissue both a decrease in peak I_{Na} and an increase in late I_{Na} as seen in heart failure [8;9], demonstrating an apparent paradox that the terms “gain of function” and “loss of function” as applied to I_{Na} are not mutually exclusive. But broadly speaking, the SCN5A channelopathies can be classified as “gain of function” where late I_{Na} is increased or “loss of function” where peak and early I_{Na} are decreased.

1.2 I_{Na} flows through SCN5A; mutations in SCN5A cause channelopathies

The gene *Scn5a* encodes the subunit protein Nav1.5, also noted as SCN5A (non-italicized and capitalized to represent the protein) which is one of nine isoforms of voltage-dependent sodium channels [12]. It forms the pore through which flows most I_{Na} in the heart, “most” because other Nav α subunits carry some I_{Na} [14]. The structure/function of SCN5A has been well-studied and reviewed [16–18]. Mutations in *Scn5a* causing increased late I_{Na} have been implicated to cause LQT3 [20], and mutations causing decreased peak/early I_{Na} have been implicated to cause a number of syndromes, including BrS, idiopathic ventricular fibrillation (IVF), cardiac conduction disease (CCD), sick sinus syndrome (SSS), and familial atrial fibrillation (FAF) [23;24]. Mutations in SCN5A have also been associated with Sudden Infant Death Syndrome or SIDS [26], presumed but not proven to be from arrhythmia, and to be associated with dilated cardiomyopathy [29;30]. Channelopathies affecting SCN5A are not necessarily or exclusively associated with arrhythmia, nor in fact confined to the heart as in the case of a telethonin mutation associated with irritable bowel syndrome [31].

1.3 Sodium channel complexes (SCCs)

The SCN5A subunit participates in larger protein complexes, also called “macromolecular complex” [35] or “signaling complex” [36], which are composed of many different proteins [41–43] (Fig. 1). The proteins making up SCCs are linked to SCN5A directly or indirectly through intermediary proteins that regulate I_{Na} function and/or affect localization. These proteins have been variously called subunits, auxiliary proteins, accessory proteins, channel interacting proteins or ChIPs, adapter proteins, associated proteins, effector proteins, regulatory proteins, anchoring proteins, and scaffolding proteins depending in part on their roles in the complex but often more on author preference without clear definitions. For purposes of this review we will call them Sodium Channel Complex Proteins (SCCPs) and operationally define them as proteins that have a connection to SCN5A demonstrated by coimmunoprecipitation (co-IP) or other “pull-down” techniques (such as GST-fusion), either directly to SCN5A, or through intermediary connections. Mutations affecting eight SCCPs have been linked to SCN5A channelopathies (Table 1), but many more have not yet been directly implicated in channelopathies (Table 2). In many cases the sites and nature of the association and interaction have been identified (Table 1&2). Other proteins are candidates to be cardiac SCCPs because they have been shown to have one or more of the following: 1)

they affect SCN5A function, 2) they are SCCPs for non-cardiac isoforms of Nav, or attached to a known SCN5A SCCPs (but not yet shown to be part of the SCN5A complex by co-IP or other pull-down). These are listed in a Table S1 in the online supplement.

It is advisable to think of SCCs as a family of complexes, as at least two populations of SCCs in cardiac myocytes have been identified [45]. One is located at the myocyte lateral membrane with SNTA1 and a second one is located at the intercalated disc with SAP97/plakophilin-2 (PKP2). Delmar's group [47] has recently shown that the kinetic properties of I_{Na} adult rodent ventricular myocytes differed at the lateral membrane and intercalated disc, raising the possibility that differences in SCCPs at the different locations may be responsible for differences in kinetics. In addition some SCCPs may be other ion channels themselves, such as connexin 43 [49] and KIR2.1 [52]. In addition, it has long been suspected that multiple SCN5A subunits grouped together and interact with other SCN5A subunits within the complex because of cooperativity seen in gating [21;54]. More recently this interaction has been demonstrated [58], making it likely that more than one SCN5A subunits are in SCCs. Sodium channel isoforms other than SCN5A are found in heart (eg [59]), albeit at much lower levels than SCN5A. These are candidates for SCCPs in heart, but it has not yet been demonstrated that they reside in the same complexes as SCN5A. Surely SCN5A SCCs are indeed "complex" and heterogeneous in composition, localization, and function, and they may not be static in any of these qualities but could be dynamic in development, disease, and even normal physiology. In Figure 1 we depict three different putative SCN5A SCCs using both space filling models (top) and diagrams (below). Details of how the models were constructed and access to an interactive display of these models is provided in the online supplement.

1.4 Approaches to studying and understanding structure and function of the SCCs

For this review we have defined an SCCP as a protein that co-IPs with SCN5A, or as proteins showing a physical association by other means, for example by GST-fusion pull-down. By these criteria we have identified eight SCCPs where mutations in them are associated with arrhythmia (Table 1). Twenty-one additional SCCPs (Table 2) meet the SCCP association criteria but arrhythmia mutations in them are yet to be discovered. Co-localization experiments show proximity but do not prove attachment, therefore co-localization alone is not sufficient for our classification as SCCP. Functional characterization of I_{Na} has been done by a number of methods. Co-expression of the SCCP with SCN5A in *Xenopus* oocytes or heterologous transfected cells (eg HEK293 cells) is a usual first step. These models have the advantages of a minimalist system without other sodium channels or SCCPs, but it carries the disadvantage that one SCCP may depend upon the presence of other SCCPs not expressed in that cell type. Another model is targeted knockout of the putative SCCP in mice, or suppression of the SCCP in myocardial cell culture using inhibitory siRNAs. The effects of mutations in SCCPs can be studied by transgenic knock-ins, or overexpression of the mutated SCCP in myocardial cell culture. The development of induced pluripotent stem cells represents a significant advance and holds the promise of studying mutations from myocytes created directly from affected patients (see review [61]). This technique has been used to study mutations in SCN5A [64]. Finally, in some cases we cite data from studies of SCCPs with non-cardiac sodium channel isoforms, recognizing that SCCP interactions and effects may differ with different α subunits (eg, [66]). These different methods of study have advantages and limitations and may result in apparently conflicting results.

2. Arrhythmia syndromes associated with mutations in SCCPs

This review focuses on those SCCPs for which putative mutations have been identified in arrhythmia patients and for which dysfunctional I_{Na} supports a plausible arrhythmogenic

mechanism through either gain of function, loss of function, or both. Many of these SCCPs interact with other ion channels and may have multiple roles in cardiac myocytes, and thus the mutations may have additional arrhythmogenic mechanisms in addition to I_{Na} dysfunction.

2.1 $\beta 1$ subunit

Scn1b encodes the $\beta 1$ subunit of voltage-dependent sodium channels and is a member of the cell adhesion molecules of the immunoglobulin superfamily [68]. $\beta 1$, like its homologs $\beta 2$, $\beta 3$ and $\beta 4$, is composed of a single transmembrane spanning region with a short cytosolic C-terminus and an immunoglobulin domain in the extracellular N-terminus. It is expressed in cardiac and neuronal tissues ([22;72;73]) and is alternatively spliced to give a 218 aa $\beta 1$ or the 268 aa $\beta 1b$ splice variant [73], both of which are expressed in heart [13]. $\beta 1$ has been shown by co-IP to be associated with both the α subunit [3] as well as the scaffolding protein ankyrin-B [19], implicating at least two potential mechanisms of SCN5A modulation. The primary interaction site appears to be with a region of the P-loop of SCN5A in domain IV (DIVS5-S6) [1]. Phosphorylation status at Tyr(181) in $\beta 1$ appears important in the formation of the $\beta 1$ association with ankyrin-G in neural [81] and cardiac tissue [56;84]. A second primary interaction site on the SCN1A C-terminus has been described in neuronal sodium channels [86], but this has not yet been demonstrated for SCN5A. Interestingly, $\beta 1$ appears to be required for the interaction of α subunits [88] in the SCC.

I_{Na} modulation by $\beta 1$ in heterologous expression systems generally showed increased I_{Na} density and decreased late I_{Na} (Table 1). I_{Na} density was increased by $\beta 1$ in injected *Xenopus* oocytes [10] and in transfected HEK293 cells [5], suggesting a loss of $\beta 1$ function would cause decreased I_{Na} density. $\beta 1$ co-transfection decreased late I_{Na} in HEK293 cells [5] and tsA201 experiments [9]. Studies of $\beta 1$ effects on steady-state inactivation and activation (Table 1) have been contradictory, and generally do not account for decreased peak I_{Na} [7;91]. A $\beta 1$ knockout mouse [18] showed increased late I_{Na} and decreased I_{Na} density as suggested by the heterologous expression experiments, but did not report kinetic changes in I_{Na} activation and inactivation. In contrast to this and the experiments in heterologous cells [5;9]), RNA interference-based knockdown of $\beta 1$ in a canine model for heart failure [40;94] demonstrated no significant effect on peak I_{Na} density or kinetics, and late I_{Na} was decreased instead of increased. However, at the level of “clinical” phenotype in the surface EKG's of knockout mice [18] showed prolonged QT and RR intervals consistent with a loss of $\beta 1$ causing decreased peak I_{Na} and increased late I_{Na} .

Mutations in $\beta 1$ have been found in patients with BrS, CCD, and AF (Table 1) consistent with loss of function. Truncated $\beta 1b$ (p.Trp179X) and a missense mutation in $\beta 1$ (E87Q) were found in patients with BrS and CCD [13]. Both variants decreased I_{Na} density consistent with the expected loss of function mechanism for these arrhythmia syndromes. They failed to hyperpolarize activation or affect recovery, and the truncated $\beta 1b$ failed to shift inactivation [13]. The $\beta 1$ mutations R85H and D153N were found in patients with lone AF [22;87;92]. The patient with R85H demonstrated a distinctive right precordial ST elevation on surface EKG resembling BrS. In CHO cells D153N and R85H both decreased I_{Na} density compared to wild-type $\beta 1$ [22]. Interestingly, heterologous experiments predict a loss of function of $\beta 1$ would increase late I_{Na} but so far $\beta 1$ mutations have not been found in LQT. Rare R214Q variants in SCN1Bb have been linked to both BrS and SIDS [28] and modulated both SCN5A and Kv4.3 (I_{to}); this mutation has also been linked to lone AF [43].

2.2 $\beta 2$ subunit

The *Scn2b* gene encodes the sodium channel $\beta 2$ subunit protein that shares sequence homology and topology with the other sodium channel β subunits, and like $\beta 1$ has sequence homology to cell adhesion molecules [97]. $\beta 2$ is expressed in human adult heart in both atria and ventricle expressing more strongly in the epicardium [98]. $\beta 2$ is localized to the intercalated disks [59] and has been coimmunoprecipitated with SCN5A in mouse heart [3]. Moreover the cysteine residue participating in the SCN5A- $\beta 2$ intermolecular disulfide bond has been shown to be Cys26 on $\beta 2$, although the participating SCN5A residue has yet to be elucidated [34]. Mutating the disulfide bond residue at cys-26 decreased I_{Na} density when co-expressed with SCN1A [34]. $\beta 2$ may also act indirectly through ankyrin proteins [100] as shown for neuronal sodium channels, although this association was not found in another study [101].

When $\beta 2$ was co-expressed with neuronal α subunits in oocytes, I_{Na} density was increased without effects on kinetics [97]. In heterologous cells, however, hyperpolarizing shifts in both activation and inactivation were found that depended upon glycosylation [38] and the effects were additive to $\beta 1$ effects. RNA interference silencing of $\beta 2$ in normal and failing dog heart [40] caused no effect on peak I_{Na} density or kinetics, but late I_{Na} was increased (opposite the effect seen with $\beta 1$ silencing). Finally, a $\beta 2$ knockout mouse had primarily a neurological phenotype with seizures and reduced neuronal sodium channel density [102] but the effect on the heart and SCN5A, if any, was not described.

Two novel $\beta 2$ mutants R28Q and R28W were found in patients with lone AF [22] with R28W patients showing prolonged PR interval and right precordial ST elevation on surface EKG. In CHO cells, I_{Na} density was decreased and activation was shifted for R28G, and inactivation was positively shifted for R28W. Late I_{Na} was not affected [22]. Overall it appears loss or absence of $\beta 2$ decreased I_{Na} .

2.3 $\beta 3$ subunit

The *Scn3b* gene encodes a single isoform $\beta 3$ subunit that has homology to $\beta 1$, both containing myelin protein zero (MPZ) homology; $\beta 3$ shares 57 and 47% homology with $\beta 1$ and $\beta 2$, respectively [103]. $\beta 3$ expresses in human ventricle [57], human atria [55] in mice atria and ventricle [104], and in sheep ventricle and Purkinje (but not atria) [51]. Co-IP of $\beta 3$ with SCN5A has been demonstrated in HEK293 cells [105] and mouse myocardium [50]. By homology to $\beta 1$, a SCN5A association site is expected [103] but has not yet been demonstrated. Goldin has stated [48] “the $\beta 1$ and $\beta 3$ subunits are non-covalently attached and expressed in a complementary fashion, so that alpha subunits are associated with either $\beta 1$ or $\beta 3$ ”.

In oocytes, expression of $\beta 3$ with SCN5A increased I_{Na} density 3-fold over SCN5A alone, and accelerated recovery, and shifted SSI positively [51]. In heterologous cells, expression of $\beta 3$ with SCN5A increased I_{Na} density with $\beta 1$ present in tsA201 cells [57], and in HEK293 cells without $\beta 1$ [65], although some studies report no increase [60;106]. In HEK293 cells late I_{Na} was not significantly increased [65]. In SCN3B knockout mice, I_{Na} density was increased, the opposite of the expected results, SSI was negatively shifted [107], and sinoatrial and atrial conduction were slowed [50]. Although effects on kinetics are divergent in the various studies, the most consistent effect was that presence of the $\beta 3$ subunit increased I_{Na} density and absence reduced it (loss of function).

The $\beta 3$ mutation L10P was implicated in BrS (type 7) [57] and caused decreased I_{Na} density and decreased expression of SCN5A protein with variable effects on kinetics in transfected tsA201 cells. $\beta 3$ mutations R6K, L10P and M161T were found in familial atrial fibrillation [55] and all three caused decreased I_{Na} when expressed in CHO-5 cells. Identified in a

patient with idiopathic ventricular fibrillation, the mutant V54G decreased I_{Na} density and cell surface expression of SCN5A in HEK293 cells which were partially restored by co-expression with the $\beta 1$ subunit [63]. A $\beta 3$ mutation V36M found in a SIDS victim greatly decreased I_{Na} density in HEK293 cells and also significantly increased late I_{Na} relative to peak [65]. The $\beta 3$ mutant A130V from a patient with AF decreased I_{Na} density in a dominant negative fashion without a decrease in surface expression [60]. These $\beta 3$ mutants have a mixed influence (when co-expression with $\beta 1$ in heterologous models) on peak I_{Na} recovery and inactivation generally leading to a decrease sodium channel availability [55] through decreased channel expression at the surface [63].

2.4 $\beta 4$ subunit

$\beta 4$ is similar to $\beta 2$ in topology, sequence, and associations and joins SCN5A by a disulfide bond via unknown Cys sites. When co-expressed with NaV1.2 and NaV1.4 in tsA201 cells it caused a negative shift in activation [69]. This subunit has been studied mostly with neuronal sodium channels where it is important in the phenomenon of resurgent current [108]. $\beta 4$ is expressed in mouse ventricle [59] and co-IP's with SCN5A when expressed in HEK293 cells [109]. $\beta 4$ had minimal effects on kinetics, I_{Na} density or late I_{Na} in HEK293 cells when co-expressed with SCN5A. The mutation L179F found in a patient with LQT showed marked increase in late I_{Na} and was designated as a novel LQT, LQT10 [109]. A mutation S206L found in a case of SIDS also increased late I_{Na} and prolonged action potential duration when overexpressed in rat ventricular myocytes [65]. The detailed mechanisms for the effect on I_{Na} are unknown, but it appears mutations in the $\beta 4$ subunit are arrhythmogenic through an increase late I_{Na} .

2.5 Caveolin-3

Caveolin-3 (Cav3) is one of three caveolin isoform that are essential in forming cave-like structures called caveolae, which are nonclathrin coated vesicles. Cav-3 has been shown to regulate several ion currents in heart including I_{Na} [110]. Cav-3 is an oligomerized protein and shares features with Caveolin-1 including cytoplasmic N- and C-termini, W-W-like domain, highly conserved aromatic residues [111], a scaffolding domain essential for oligomerization, and palmitoylated cysteine residues at the C-terminus important for sterol interactions [112]. Whereas Caveolin-1 expression is ubiquitous, Cav-3 is restricted to striated muscle and increases with maturity in rat myocytes [113]. Cav-3 tends to localize to the lateral and t-tubular membranes in cardiac myocytes [114]. In transfected HEK293 cells [76] and in rat cardiac myocytes [84], Cav-3 co-IP's with SCN5A. The exact residues of association between Cav-3 and SCN5A are not yet known, but many other interaction sites on Cav-3 have been implicated in association with other signaling proteins such as adenylyl cyclase and β adrenergic receptors reflecting its importance as a structural member of the SCCP (eg [115])

Cav-3 appears to be important in mediating adrenergic upregulation of cardiac I_{Na} through G proteins [84]. Expression of wild-type Cav-3 with SCN5A in HEK293 cells had no effect on peak I_{Na} kinetics, or late I_{Na} [76;79]. Cav-3 mutants F97C and S141R, however, found in patients with LQTS, increased late I_{Na} in HEK293 cells [76] and these were designated LQT9. The Cav-3 mutants V14L, T78M, and L79R found in a SIDS cohort also showed increased late I_{Na} in transfected HEK293 cells [79]. Cav-3 is known to be an inhibitor of nNOS [116], and nitrosylation of SCN5A by nNOS has been shown to increase late I_{Na} [87]. The LQT9 Cav-3 mutation F97C increased late I_{Na} in HEK293 cells, increased late I_{Na} and action potential duration in cardiac myocytes, and also increased nitrosylation of SCN5A [77], suggesting that the mechanism for both LQT9 and LQT12 may be through direct SCN5A nitrosylation. Mutations in Cav-3 have also been linked to hypertrophic

cardiomyopathy (T64S) [117], although it is not clear the mechanism is through a channelopathy.

2.6 GPD1L

The gene *GPD1L* encodes the protein GPD1L; the letters stand for “glycerol 3 phosphate dehydrogenase 1 like” because it shares 84% homology with glycerol 3-phosphate dehydrogenase 1 (GPD1) [118]. *GPD1L* was first noted as part of mammalian gene sequence collection program [119], but the function or importance of GPD1L, was unknown. Mutations in *GPD1L* were linked to BrS in a large family [85], and the GPD1L mutation A280V [82] and the novel SIDS-associated mutation E83K [120] were both shown to decrease I_{Na} amplitude. A GST-fusion protein with GPD1L pulled down SCN5A in transfected HEK293 cells suggesting association with SCN5A [80]. The related gene *GPD1* encodes a NAD-dependent cytosolic enzyme that is an important link between the glycolytic pathway and triglyceride synthesis, catalyzing the reversible conversion of glycerol-3-phosphate (G3P) to dihydroxyacetone phosphate. GPD1L was also shown to have this activity in transfected HEK293 cells and the A280V mutant GPD1L showed significantly decreased enzymatic activity [80]. Decreased GPD1L activity would locally increase substrate G3P and by “mass action” feed the PKC-mediated phosphorylation of SCN5A at serine residue 1503 (S1503) where such phosphorylation is known to decrease I_{Na} [121]. In support of this hypothesis the mutation induced decrease in I_{Na} was abrogated by pharmacological blockers of the G3P-PKA pathway and was also abrogated by co-expression with the PKC phosphorylation deficient mutant SCN5A-S-1503A [80]. An alternative, or perhaps supplementary pathway, may involve effects of the GPD1L mutants on NAD through PKC and reactive oxygen species, and NAD effects through PKA [122].

2.7 MOG1

The cytosolic multicopy suppressor of *gsp1* (MOG1) affects protein trafficking through Ran, a GTP binding nuclear protein [123]. MOG1 is found in neonatal mouse ventricles localized to the intercalated disks [89] and is highly expressed in heart [124]. Association with SCN5A was discovered through yeast two-hybrid screen and confirmed with GST-SCN5A DII–DIII linker pull-down [89]. Co-IP in lysates prepared from mouse neonatal cardiomyocytes also confirmed the association of MOG1 and SCN5A [89]. Heterologous expression in HEK293 cells and MOG1 overexpression in neonatal myocytes led to increased expression of SCN5A and peak I_{Na} [89;92]. siRNA interference knock-down of MOG1 impaired SCN5A trafficking [92]. Coexpression of SCN5A with the MOG1 mutant, E83D, discovered in a BrS patient showed marked reduction of I_{Na} and evidence of impaired trafficking when expressed in HEK293 cells and when over expressed in neonatal mouse myocytes [92] in a dominant negative fashion. MOG1 does not appear to affect SCN5A kinetics [92]. The MOG1 mutant loss of function appears to cause BrS by decreasing SCN5A trafficking to the cell surface and decreased peak I_{Na} .

2.8 Alpha-1-Syntrophin

The cytosolic scaffolding product of the *Snta1* gene is the rod-shaped alpha-1-syntrophin (SNTA1) protein. SNTA1 is part of the dystrophin glycoprotein complex and plays a role in linking proteins to the cytoskeleton. SNTA1 contains a PDZ (PSD-95, Disk-large and ZO-1) domain, two pleckstrin homology (PH) domains, and a syntrophin unique (SU) domain allowing for its role in multiple protein complex interactions including other syntrophins and dystrophin [125]. It is expressed in human heart [67] and is associated with SCN5A as shown by GST pull-down using a SCN5A C-terminus GST-fusion protein [87] expressed in transfected HEK293 cells and by co-IP from lysates prepared from mouse ventricles [67]. SNTA1 is suggested to associate via the PDZ domain to the three specific residues, Ser-Ile-

Val at the SCN5A distal end of the C-terminus. [67] and is localized to lateral membrane of cardiac myocytes [45].

Mutations in SNTA1 have been associated with LQTS (LQT12) [87] and SIDS [95] with a proposed mechanism of increased late I_{Na} . Based on the associations of nNOS, SNTA1, PMCA4b [93] and SNTA1 with SCN5A [67], the full complex (Fig. 1A) was demonstrated in mouse heart by co-IP [87]. The LQT12 mutation A390V in SNTA1 was shown to selectively disrupt association of PMCA4b, a plasma membrane calcium transporter and an nNOS inhibitor [126], from the complex, leading to increased direct nitrosylation of SCN5A and late I_{Na} [87]. This represents an example of how disruption of members of the SCC that link to SCN5A through intermediates can result in a channelopathy.

3. Other Cardiac SCCPs

Many cardiac SCCPs have been identified that modulate cardiac I_{Na} but mutations in these proteins that have yet to be discovered in patients with inherited arrhythmias of unknown origin. These SCCPs (listed in Table 2, with additional possible SCCPs discussed in the online supplement) are candidate genes for SCN5A channelopathies.

3.1 Ankyrin-G

The cytosolic protein product of *Ank3* ankyrin 3 or ankyrin-G, is a scaffolding protein that bridges ion channel complexes with the spectrin-actin cytoskeleton [127]. Ankyrin-G shares homology with other ankyrins in four functional domains: membrane-binding domain (MBD), spectrin-binding domain (SBD), death domain (DD) and a C-terminal regulatory domain (CTD) [128]. Ankyrin-G is expressed in the human heart and has been shown by co-IP to associate with SCN5A in both rat myocytes and transfected HEK293 cells. In cardiac myocytes ankyrin-G is localized to intercalated discs and T-tubule membranes [19]. The interaction with SCN5A occurs in a conserved 9-aa sequence in the cytosolic DII–III linker loop of SCN5A [129] through repeats 14–15 loop tops on ankyrin-G [15]. In rat cardiomyocytes with ankyrin-G expression reduced by siRNA, total SCN5A expression, plasma membrane localization, and I_{Na} were reduced, without an effect on kinetics [15]. A signaling complex consisting of CaM Kinase II linked to SCN5A by β_{IV} -spectrin and ankyrin-G (Fig. 1B,E) phosphorylates SCN5A at S571 [25] to increase late I_{Na} .

The other eight SCN5A SCCPs described above cause channelopathies by mutations within the SCCP. So far no mutations in ankyrin-g have been associated with arrhythmia. A mutation in SCN5A (E1053K) discovered in a patient with BrS disrupts association of SCN5A with ankyrin-G, resulting in decreased I_{Na} [19]. Interestingly, peak I_{Na} density was not affected, but steady-state inactivation was shifted in the hyperpolarizing direction, I_{Na} decay was accelerated, and recovery from inactivation was slowed [19] suggesting a kinetic loss of function at normal resting potentials in heart. As it was subsequently shown that the main effect of loss of ankyrin-G is decreased I_{Na} density without a change in kinetics [15], it may be that the E1053K mutation has effects unrelated to binding to ankyrin-G. Also LQT3 mutations nearby the S571 residues in SCN5A mimic phosphorylation and cause increased late I_{Na} [46], but to date no arrhythmia related mutations in the SCCPs of this complex have been identified.

3.2 Plakophilin 2

Plakophilin2 (PKP2) is a desmosomal protein, belonging to the armadillo family of proteins, important in the context of intercellular junctions in cardiomyocytes. Structurally they consist of a head domain, 9-armadillo repeat domains and a tail domain [130]. PKP2 was associated with Nav1.5 by Co-IP and immunolocalized to the intercalated disc in cardiac myocytes [90]. Knockdown of PKP2 in cultured cardiomyocytes altered the

properties of the I_{Na} and GST pull-down assays demonstrated that the head domain of PKP2 associates with Nav1.5 [90]. It is not known if this association is direct or indirect. Mutations in PKP2 have been implicated in arrhythmogenic right ventricular cardiomyopathy (ARVC) as part of the desmosome complex [131] also involving connexin43 [49]. Haploinsufficiency of PKP2 in mice downregulated I_{Na} and was associated with arrhythmia [132]. Similarly a mutant in the desmosome associated protein desmoglein-2 decreased I_{Na} density when overexpressed in mouse myocytes [62]. Decreased SCN5A and connexin43 protein was observed in tissue from a majority of ARVC patients with various mutations including PKP2 and other AVRC mutations, as well as from patients without a known AVRC mutation [133]. These effects on SCN5A in AVRC may be caused by a more general remodeling in the desmosome from mutations in a number of proteins, and may represent an SCN5A channelopathy intermediate between the more specific SCCP induced SCN5A channelopathies (Table 1), and the more general SCN5A channelopathy caused by electrical remodeling in heart failure [134].

3.3 Synapse Associated Protein-97

Synapse associated protein-97 (SAP97) is a scaffolding protein belonging to the membrane associated guanylate kinase (MAGUK) family of proteins. They have been shown to play an important role in assembling signaling complexes at the membrane due to their modular structure consisting of 3 PDZ domains, a guanylate kinase like (GK) domain, a hook domain and a SRC homology-3 (SH3) domain. Abriel and colleagues have reported that SAP97 associated with the extreme carboxyl-terminus (residues-S-I-V) of SCN5A by GST pull-down assays and also by Co-IP [45]. SAP97 has been shown to localize to the intercalated disc and T-tubules in cardiac myocytes and knockdown of SAP97 in HEK293 cells and cultured rat atrial and ventricular cardiomyocytes resulted in reduced I_{Na} density [45;52]. No known mutations in SAP97 have yet been linked to SCN5A channelopathies.

5. Comments and Conclusions

Channelopathies caused by mutations in SCCPs, although relatively rare clinically, are “natural” experiments that give insight into the structure function of SCCs. For example, a function for GPD1L was completely unknown until associated with SCN5A and BrS [82]. Conversely, work associating SNTA1 with SCN5A [67] led to the discovery of LQT12 [87] and further definition of a particular SCC (Fig. 1A,D). Genotype-negative inherited arrhythmia patients (patients for which the causative gene and mutations are unknown) remain an issue especially for BrS [135]. Moreover mutations in SCCPs causing channelopathies appear to be relatively common in SIDS [79] [65] [95] [94] [65], suggesting more SCCP channelopathy genes to be discovered in SIDS patients. In addition to discovering novel SCCPs and channelopathies, future research that defines the composition, localization, dynamic nature, and regulatory functions of the various classes of SCCs will provide insights into mechanisms of channelopathies associated with the even greater health problem that is acquired heart disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights for review

Sodium channel complex proteins (SCCPs) regulate the sodium current in the heart.

Inherited mutations in SCCPs cause abnormalities in channel function called channelopathies which underlie cardiac arrhythmia.

The literature for established and potential SCCPs is reviewed with a special focus on those SCCPs that have known arrhythmia causing mutations.

Figure 1.

SCCP representations of a Syntrophin Complex (A,D), a CaMKinase II Complex, (B,E), and a Caveolin-3 Complex (C,F). Top panels: Atomic scale resolution representations highlighting functionally associated sub-complexes. Bottom panels: Diagrams of the panels above. For construction of the top panels each protein (or if not available, a matched homologue) was accessed from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (<http://www.rcsb.org>). Assembly was done manually taking into account known interactions sites. These renditions are intended to give impressions of relative size, shapes, and attachments and do not represent any energy minimization or other rigorous modeling. Additional details are in the online supplement.

Table 1

Mutations in Cardiac Sodium Channel (SCN5A) Complex Proteins.

96Protein	Gene	Size (aa)	Connection on SCN5A	Association	Normal I _{Na} Effect	Human Mutant	Mutant Effect	Phenotype
β1, β1b	<i>Scn1b</i>	β1 218 β1b 268	1° DIV P-loop [11]	Co-IP [3]	Kinetics conflicting (↓) I _{Na} Late [5]	Trp179X	(Ø-) Activ., (Ø-) SSI, (Ø↑) I _{Na} Peak	BrS(type 5), CCD [13]
					(↑)RecR [7] (↑)I _{Na} Peak [10]	E87Q	(Ø-) Activ., (Ø↑) I _{Na} Peak	BrS (type 5), CCD [13]
						R85H	(+) Activ.,SSI, (Ø↑) I _{Na} Peak	FAF, (↑)R.ST [22]
β2	<i>Scn2b</i>	215	2° through Ankyrin G [19]	Co-IP [37]		D153N	(Ø↑) I _{Na} Peak	FAF [22]
						R214Q	(Ø↑) I _{Na} Peak [28]	BrS, FAF [28;32]
						R28Q	(+)Activ. (↓) I _{Na} Peak	FAF [22] (↑)PR, (↑)R.ST
β3	<i>Scn3b</i>	215	1° Disulfide Bond, Unknown site [34]	Co-IP [50]	Sialylation Status [38] (↑)Late Current [40]	R28W	(+) SSI, (+)Activ, (↓)I _{Na} Peak	FAF [13] (↑)PR, (↑)R.ST
						R6K, L10P and M161T	Mixed (↓)I _{Na} Peak, (-) SSI, (↓)RecR	FAF [55] BrS(type7) [57]
							(↓)I _{Na} Peak	FAF [60]
β4	<i>Scn4b</i>	228	1° Disulfide Bond, unknown site [69]	Co-IP [76]		V54G	(↓)I _{Na} Peak (↓)Trafficking	IVF [63], SIDS [65]
						V36M	(↓)I _{Na} Peak, (↑)I _{Na} Late,	SIDS [65]
						S206L L179F	(↑)I _{Na} Late, (↑)Window I _{Na}	SIDS [65] LQT(type 10) {Medeiros-Domingo, 2007 567/td}
Caveolin-3	<i>Cav3</i>	151	?	Co-IP [76]	Scaffolding (↓)I _{Na} Late [77]	F97C, S141R	(↑)I _{Na} Late, V14L, T78M, and L79R	LQT(type9) [76] (↑)I _{Na} Late, SIDS [79]
GPD1L	<i>Gpd1l</i>	351	?	GST-PD [80] (Ø) Co-IP [82]	(↑)I _{Na} by phosphorylation [80]	A280V E83K, I124V, R273C	(↓)I _{Na} Peak (↓)I _{Na} Peak (+)Activ. in 273C	BrS (type 2) [85] SIDS [82]
MOG1	<i>Rangrf</i>	218	1° linker DII-DIII [89]	GST-PD, Co-IP [89]	(↑)Surface Density, (↑)I _{Na} Peak [89]	E83D	(↓)I _{Na} Peak, (↓)Trafficking [89]	BrS (type 8) [92]
1 syntrophin	<i>Sinta1</i>	505	1° C-terminal S-I- V residues [67]	GST-PD, Co-IP [67] [87]	Scaffolding	A390V S287R, T372M, G460S	(↑)I _{Na} Peak, (↑)I _{Na} Late, (↑)I _{Na} Peak, (↑) I _{Na} Late, (+) SSI	LQT (type 12) [87] SIDS [95]

SCN5A structure: DI-DIV = Repeat Domains I-IV; S1-S6 = Membrane Spanning Segments 1-6; P-Loop = Pour lining loop. Abbreviations: Ø = A failure to; ° = Primary association with SCN5A; 2° = Secondary Association with SCN5A by way of intermediate protein; Co-IP = Coimmunoprecipitation; GST-PD-GST Pull Down; (↓) = Decrease in; (↑) = Increase in; (+) = Depolarizing shift;

(-)=Hyperpolarizing shift; Activ.=Activation; RecR= Recovery Rate; RefractPer.=Refractory Period; SSI=Steady State Inactivation; I_{Na} Peak= Peak Sodium Current; I_{Na} Late=Late Sodium Current
a.k.a. Persistent Current; BrS=Brugada Syndrome, FAF=Familial Atrial Fibrillation; IVF= Idiopathic Ventricular Fibrillation; LQT=Long QT Syndrome; SIDS=Sudden Infant Death Syndrome; QT=QT
interval on Surface EKG; R, ST=Right Precordial ST elevation

Table 2

Proposed Sodium Channel Complex Proteins.
Please refer to Table 1 for abbreviations.

Protein	Gene	Size (aa)	Connection on SCN5A	Association	Normal I _{Na} Effect
14-3-3 η	<i>Ywhab</i>	246	1° DI-II linker [2]	Co-IP [2]	(-)RecR, (-)SSI [2]
α Actinin 2	<i>Actn2</i>	894	1° DIII-IV linker [11]	Co-IP [11]	([†]) I _{Na} Peak [11]
Ankyrin G	<i>Ank3</i>	4377	DII-III linker, 14–15 tops Ankyrin G [15]	Co-IP [21]	([†])Surface Density ([†])I _{Na} Peak [21]
β 4 Spectrin	<i>Sptbn4</i>	2,564	1° Unknown site, 2° CAMKII-Delta [25] 2° Through Ankyrin G [27]	Co-IP [25]	([†])I _{Na} Peak, (+)SSI via Phos. S571 [25]
Calmodulin	<i>Calml</i>	152	1° C terminus IQ [33] 1° DIII-DIV linker [25] 1° EF Hand [32]	GST-PD [39] NMR [32]	(+)SSI [44]
CaMKinase II δ isoform 3	<i>Camk2d</i>	510	1° DI-II linker [46] 2° IV Spectrin [25]	Co-IP [25]	(-)SSI, Phos. @ S571S, 516, T594 ([†]) I _{Na} Late [46] ?
Connexin 40	<i>Gja5</i>	358	?	Co-Loc. [53]	([†]) I _{Na} Peak [49]
Connexin 43	<i>Gja1</i>	382	?	Co-IP [56]	([†]) I _{Na} Peak [62]
Desmoglein 2	<i>Dsg2</i>	1118	?	Co-IP [62]	([†])I _{Na} Peak [70]
Dystrophin	<i>Dmd</i>	3,685	1° Unknown site 2° Syntrophin [67]	Co-IP [70]	(+)SSI ([†]) RecR [75]
Fibroblast growth factor FGF12B, 13	<i>Fgfl2</i> <i>Fgfl3</i>	181	1° C terminus 2° Calmodulin [74]	GST-PD [78]	(+) Activ. [78]
λ 2 syntrophin	<i>Snig2</i>	539	1° C terminus, PDZ [78]	GST-PD [83]	([†]) I _{Na} Peak [83]
Nedd4-2	<i>Nedd4l</i>	975	1° C terminus PY [83]	GST, Co-IP [87]	([†])I _{Na} Late [87]
nNOS	<i>Nos1</i>	1434	2° α 1 syntrophin [87]	Co-IP [90]	([†]) I _{Na} Peak (+)SSI [90]
Plakophilin-2	<i>Pkp2</i>	881	1° Unknown site [90]	GST-PD [93] Co-IP [87]	(-) I _{Na} Late [87]
PMCA4b	<i>Atp2b4</i>	1241	2° α 1 syntrophin [93]	Co-IP {Petitprez, 2011 PETITPREZ2011/id}	([†]) I _{Na} Peak [45]
Disks large homolog 1 (SAP97)	<i>Dlg1</i>	904	1° C terminus PDZ [45]	Co-IP [31]	(-) Activ. [31]
Telethonin	<i>Tcap</i>	167	1° Unknown Site [31]	GST-PD [96]	(-)SSI [96]
PTPH1	<i>Ptpn3</i>	913	1° C terminus PDZ [96]	Co-IP [70]	(-) I _{Na} Peak [70]
Utrophin	<i>Utrn</i>	3433	2° α 1 syntrophin [70]	GST-PD [99]	
Zasp	<i>Ldb3</i>	727	1° Unknown Site		

Protein	Gene	Size (aa)	Connection on SCN5A	Association	Normal I _{Na} Effect
2° Telethonin [99]					