Roles and regulation of the cardiac sodium channel Na$_v$1.5: Recent insights from experimental studies

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Abstract

During the past decade, Na$_v$1.5, the main voltage-gated Na$^+$ channel in the heart, has been shown to be involved in many cardiac diseases. Genetic variants in the gene *SCN5A*, encoding Na$_v$1.5, have been linked to various cardiac phenotypes, such as the congenital and acquired long QT syndromes, Brugada syndrome, conduction slowing, sick sinus syndrome, atrial fibrillation, and even cases of dilated cardiomyopathy. This unexpected phenotypic diversity may reflect that Na$_v$1.5 is not only restricted to the initiation of the action potential and rapid cardiac conduction, but may also be involved in other, not-yet elucidated, functions. Despite the fact that our understanding of the regulation of expression, localization, and function of Na$_v$1.5 is deepening, we are still far from a comprehensive view. Much of our current knowledge has been obtained by carrying out experiments using "cellular expression systems", e.g. host cells expressing exogenous Na$_v$1.5. Although very informative, these techniques are limited, in that Na$_v$1.5 is not expressed in the physiological cellular environment of a cardiac cell. Recently, however, there have been several studies published which used approaches closer to "normal" or pathological physiology.

In an attempt to summarize recently published data, this article will review the phenotypes of genetically-modified mouse strains where Na$_v$1.5 expression and activity are directly or indirectly modified, as well as the regulation of Na$_v$1.5 function using native cardiac myocytes. Despite obvious limitations, the reviewed studies provide an overview of the complex multi-factorial and multi-protein regulation of Na$_v$1.5.
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1. Introduction

The voltage-gated Na$^+$ channel Na$_v$1.5 initiates the cardiac action potential (AP) of the "working" myocardium, is essential for conduction of the electrical impulse, and is also known to control the AP duration [1]. More recently, its contribution to the sino-atrial node pacemaker function has also been demonstrated [2]. Na$_v$1.5 is the principal Na$^+$ channel isoform expressed in cardiomyocytes. Other isoforms of the Na$_v$ family are also expressed in the heart, although their contribution to the cardiac physiology is still poorly understood [3]. In terms of its biophysical properties, Na$_v$1.5 can be found in three states: *closed* at resting membrane potential (about −85 mV), *open* upon depolarization, and in

an *inactivated* non-conductive state. The transition between these states depends mainly on transmembrane voltage difference, time, and temperature. Recovery from the inactivated state occurs during repolarisation of the membrane during diastole. Fast depolarization of the myocyte (upstroke of the AP) and conduction both depend on the availability of Na$_v$1.5 (i.e. amount of channels in the closed state) [4]. Entry into the inactivated state is very fast (<1 ms) and for most channels, this inactivated state is very stable. It has been shown, however, that Na$_v$1.5 channels may re-open, thus generating a depolarizing inward current after the plateau phase of the AP [5]. This phenomenon may underlie the shortening of the AP duration caused by tetrodotoxin observed in Purkinje fibres and myocytes [6].

Since 1995, more than 170 naturally-occurring genetic variants in *SCN5A* (see www.fsm.it/cardmoc/), the gene coding for Na$_v$1.5, have been linked to cardiac disorders,

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such as congenital and drug-acquired long QT syndromes (LQTS), Brugada syndrome (BrS), conduction disorders, sudden infant death syndrome (SIDS), sinus dysfunction, atrial fibrillation [7,8], and dilated cardiomyopathy [9]. This long list of phenotypes may suggest that $Na_v1.5$ is not only restricted to the initiation and conduction of the AP, but may also be related to other more subtle functions.

$Na_v1.5$ is a glycosylated membrane protein with a molecular weight of ~220 kDa and consists of 2015 or 2016 amino acids (depending on the splice variant) [10]. The protein is made up of four homologous domains (DI to DIV, Fig. 1), each with six transmembrane segments (S1 to S6). Three intracellular linker loops and both the N-and C-termini of the channel are cytoplasmic. The four S4 transmembrane segments are involved in activation gating of the channel (Fig. 1, in green), while a cluster of three hydrophobic amino acids in the III-IV linker, Ile-Phe-Met (IFM), are involved in fast inactivation gating (Fig. 1, red box). In cardiac cells, $Na_v1.5$ associates with partner proteins which may be anchoring/adaptor proteins, enzymes which interact with and modify the channel, and proteins modulating the biophysical properties of $Na_v1.5$ upon binding (reviewed in [11]). $Na_v1.5$ also interacts with β-subunits (~30–35 kDa, β1 to β4-subunits, Fig. 1): one transmembrane-domain proteins involved in different aspects of $Na_v1.5$ function [12].

The precise localization of $Na_v1.5$ in cardiac cells is somewhat controversial. It is recognized that a pool of $Na_v1.5$ is located at the specialized cell-cell junctions known as the intercalated disks [13,14]. The localization of $Na_v1.5$ in lateral membranes and t-tubules, however, is still debated [14–17]. In dog myocytes, Na_v channels (most likely $Na_v1.5$) have also been observed in the endoplasmic reticulum [18].

During recent years, our understanding of the regulation of expression, localization, function and role of $Na_v1.5$ in

diseases has grown rapidly. A large amount of this knowledge has been obtained by carrying out experiments using "cellular expression systems" (host cells) such as Xenopus oocytes and human embryonic kidney (HEK)-293 cells which "heterologously" express wild-type and mutant $Na_v1.5$ [19]. Although very informative, these techniques are limited by the fact that the sodium channel protein is not processed in its physiological cellular context. Studies using approaches closer to the "normal" or pathological physiology have recently been published. The scope of this review is restricted to the discussion of recent findings obtained by (1) investigating the phenotypes of genetically-modified mouse strains, where $Na_v1.5$ is directly or indirectly modified, and (2) studying the regulation of $Na_v1.5$ function using "homologous" expression of the channel in cardiac cells. For prior studies there are several excellent review articles [20–23].

2. Mouse models with altered function of $Na_v1.5$

Despite initial scepticism related to the fact that the cardiac electrophysiological characteristics of the mouse are strikingly different than those of humans [24], important and informative $Na_v1.5$-related studies using genetically-modified mouse models have recently been published.

2.1. $Na_v1.5$ knock-out mouse studies

Homozygous knock-out (KO) *Scn5a* mouse embryos die during mid-gestation, most likely due to the severe cardiac malformations identified [25]. This helps illustrate the essential role of $Na_v1.5$ in cardiac development. As one might expect, heterozygous KO mice (*Scn5a+/−*) mainly display slow atrial, atrio-ventricular (AV), and intra-ventricular
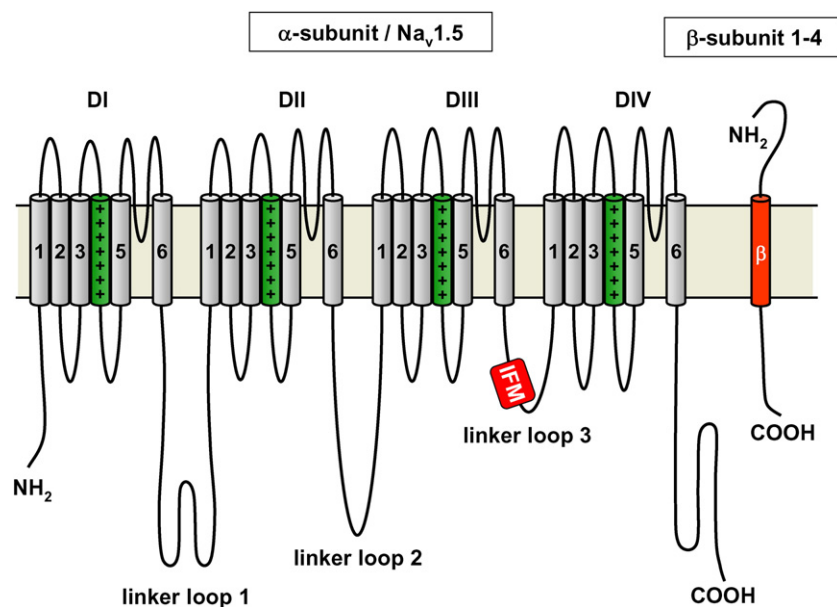

Fig. 1. Schematic membrane topology of $Na_v1.5$, the pore-forming subunit of the cardiac sodium channel and its associated β-subunits.

conduction, as well as increased inducibility of ventricular arrhythmias [25]. Following this initial report [25], several studies investigating the consequences of decreased $Na_v1.5$ expression were published. Royer and co-workers [26] reported that *Scn5a+/−* mice display an age-dependent deterioration of the global conduction properties associated with the manifestation of fibrosis in the ventricular myocardium. In certain aspects, this phenotype mimics Lenègre-Lev disease, a progressive cardiac conduction disorder [27]. These findings raised the question as to whether fibrosis might be the consequence of a loss of function of $Na_v1.5$, as recently suggested in humans in the context of BrS [28,29]. Fibrosis, as well as disruption of the cellular distribution of connexin-43, seems to underlie this age-dependent conduction slowing [30]. Recent evidence [31] shows that the slowed AV conduction in *Scn5a+/−* mice [26] depends on the expression of $Na_v1.5$ in AV node input and output regions. Finally, the role of $Na_v1.5$ in modulating sinus node automaticity has also been demonstrated in *Scn5a+/−* mice [32]. Altogether, these studies underline the importance of the Nav1.5-mediated inward current in cardiac automaticity and conduction.

2.2. $Na_v1.5$ knock-in or transgenic mouse models mimicking human diseases

The generation of knock-in (KI) or transgenic mice harbouring mutant *SCN5A* alleles is a powerful method for examining the consequences of a human *SCN5A* mutation in a physiological context. Three such mouse lines that have been recently produced are discussed below.

2.2.1. SCN5A mutation Δ1505-1507KPQ

Most of the mutations of $Na_v1.5$ found in congenital LQTS patients (defining the LQTS type 3) alter the fast inactivation process of the channel [33], thereby inducing a persistent inward depolarizing current during the AP plateau phase. Consequently, the repolarisation of the AP is delayed and leads to congenital LQTS. The deletion of three amino acids (Δ1505-1507KPQ) of $Na_v1.5$ was the first *SCN5A* mutation which lead to a Nav1.5 persistent current [34]. In 2001, the first KI ΔKPQ mouse model was generated [35]. This study confirmed that in myocytes, LQT-3 mutant $Na_v1.5$ channels generate a persistent current and prolong the AP; while in *vivo* the expression of mutant channels, increase the QT interval duration of the ECG. Increased arrhythmogenicity, illustrated by spontaneous polymorphic ventricular tachycardia (VT), was also reported [35]. More recently, a similar ΔKPQ mouse line was created [36] and the results obtained were consistent with the previous study [35]. These two mouse lines allowed the investigation of anti-arrhythmic procedures and drugs. Results showed that, consistent with clinical data, β-blockers failed [36]; whereas mexiletine (Na$_v$ blocker) and rapid pacing could suppress VT [37]. It is mainly thought that the LQT-3 arrhythmogenic phenotype depends on early after-depolarisations (EAD) caused by the prolonged AP [38], however, in a recent study

[39] the occurrence of so-called delayed after-depolarisations (i.e. after full repolarisation of the AP) was clearly demonstrated in myocytes of the ΔKPQ mouse. This finding suggests that this additional mechanism may contribute to the severity of arrhythmias seen in LQT-3. The LQT-3 genotype, although less frequent, is often more lethal [40]. Finally, a puzzling finding of these two first studies [35,36] is that the I_{Na} recorded from individual KI myocytes was about 2-fold larger that wild-type controls. This was not the case, however, in another recent study [39]. The reason for this discrepancy remains to be clarified.

2.2.2. SCN5A mutation 1795insD

SCN5A mutations lead not only to many different pathologic phenotypes [9], but also to combinations of these phenotypes, known as "overlap syndromes" [41–43]. The reasons for these overlapping phenotypes are not completely understood. It is thought, however, that at a slow heart rate the late-current dependent AP prolongation is responsible for LQTS, whereas at a high heart rate conduction slowing (because of reduced availability of Nav1.5) underlies the BrS phenotype. Remme and co-workers [44] recently generated a KI mouse line expressing the 1795insD *SCN5A* mutation (insertion of an aspartic acid in locus 1795, 1798 in mouse *Scn5a*), which has been shown to cause both LQTS and BrS in one large Dutch family [43]. The detailed phenotyping of this mouse line showed that expression of the 1795insD channel recapitulates many of the pathological features seen in patients, such as: bradycardia, conduction slowing because of decreased I_{Na}, and delayed repolarisation caused by the mutation-induced persistent I_{Na}. This study clearly illustrates the benefits of mouse models for the study of specific human arrhythmias resulting from $Na_v1.5$ dysfunction.

2.2.3. SCN5A mutation N1325S

The *SCN5A* mutation N1325S causes LQT-3 syndrome [45], and like many similar mutations, leads to an increased persistent current [46]. The generation of a transgenic mouse line enabling demonstration of the phenotypes of LQTS, VT, and sudden death [46], permitted researchers to investigate alternative arrhythmogenic mechanisms [45]. Experiments using isolated myocytes and performing AP recordings showed that, at heart rates with cycle length ≤ 500 ms, AP alternans and instability (i.e. large variability in AP duration) were clearly observed [45]. Interestingly, the Ca^{2+} channel blocker verapamil reduced this AP instability, suggesting an involvement of intracellular Ca^{2+} dysregulation in this phenomenon. Altogether, these studies illustrate that the mechanisms underlying arrhythmogenicity in LQT-3 may be multifold and mutation dependent.

2.3. Dystrophin-deficient mice

Similar to other ion channels, $Na_v1.5$ has been reported to be part of the dystrophin multi-protein complex [47]. Recently,

using a "pull-down" approach, our group showed that $Na_v1.5$ is interacting via adaptor proteins, called syntrophins, with dystrophin [48]. This interaction depends on the PDZ domain-binding motif on the C-terminus of $Na_v1.5$ [11]. In dystrophin-deficient mice (mdx5cv), an animal model of Duchenne muscular dystrophy, $Na_v1.5$ protein level was reduced in ventricular lysates, leading to reduced cellular I_{Na} and conduction defects that were documented via ECGs. The detailed mechanisms by which a lack of dystrophin reduces the expression of $Na_v1.5$ protein without altering the mRNA level [48], remain to be explored. Whether the decreased expression of $Na_v1.5$ plays a direct role in, or is the consequence of, the degenerative cardiomyopathy seen in Duchenne patients is still unanswered. Another intriguing question raised by these findings is related to the fact that dystrophin has been shown to be absent from the intercalated disks of human [49] and rat [50] cardiomyocytes, where a fraction of $Na_v1.5$ is clearly present [14]. These observations suggest that at least two pools of $Na_v1.5$ channels co-exist at the plasma membrane of cardiac cells. One pool, localized in lateral membranes, may belong to the dystrophin complex; whereas another pool resides at the intercalated disks. In the latter pool, the PDZ domain-binding motif of $Na_v1.5$ may be associated with other proteins, either via syntrophin (e.g. utrophin) or other PDZ-domain proteins. Baba and co-workers [16] recently showed that dog cardiomyocytes isolated from infarcted zones displayed a reduced I_{Na} density, associated with a marked loss of the $Na_v1.5$ staining in the lateral membranes only. In contrast, the $Na_v1.5$ staining remained unchanged in the intercalated disks. This leads one to believe that this lateral dystrophin-dependent pool of $Na_v1.5$ may be more sensitive to specific pathological insults (in this case ischemia) than the intercalated-disk pool.

2.4. Ca^{2+}/calmodulin-dependent protein kinase II

Phosphorylation of $Na_v1.5$ by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) has recently been reported [51]. CaMKII is a widely expressed serine/threonine kinase which transduces intracellular Ca^{2+} increase into phosphorylation of multiple proteins, including ion channels [52]. Importantly, CaMKIIδc has been shown to be up-regulated in human and animal heart failure models (reviewed in [53]). Wagner and co-workers [51] showed that CaMKIIδc is co-localized and can be co-immunoprecipited with $Na_v1.5$ in cardiac tissues, although the mode of interaction has not yet been investigated. Over-expression of CaMKIIδc in rabbit myocytes and transgenic mice altered several biophysical properties of $Na_v1.5$. In summary, CaMKII-dependent phosphorylation of $Na_v1.5$ shifted the steady-state inactivation curve towards negative voltages in a Ca^{2+}-dependent manner, slowed the recovery from inactivation, and markedly increased the persistent current (as seen with LQT-3 mutant $Na_v1.5$ channels). Overall, at rapid heart rate, these alterations decrease the availability of I_{Na}; while at slower heart rate, the persistent current-dependent prolongation of repolarisation predominates. Interestingly, this biophysical phenotype is similar to the one seen in patients and mice carrying the 1795insD mutation (see 2.2.2). These results are supported by the observation that VT could be generated in mice over-expressing CaMKII while showing signs of heart failure [51]. Overall, it is likely that CaMKII plays a prominent role in heart failure-dependent arrhythmias involving $Na_v1.5$. However, more direct evidence is needed in order to confirm this hypothesis.

2.5. Transgenic over-expression of $Na_v1.5$

What are the consequences of over-expressing $Na_v1.5$? Despite the fact that the cardiac $Na_v1.5$ transcript and protein levels were increased by about 10-fold in a *SCN5A* transgenic mouse line [54], no increase of I_{Na} and AP duration of atrial and ventricular cells was observed. These surprising findings suggest that there may be intrinsic mechanisms avoiding "overloading" of the cardiac cell membrane with $Na_v1.5$. Does it mean that cardiac cells are able to "sense" the number of $Na_v1.5$ at the cell membrane? Or, is the cell membrane under normal conditions "saturated" with $Na_v1.5$? These are open questions that merit further study. Finally, these $Na_v1.5$ over-expressing mice displayed a very mild shortening of the P-wave and PQ-interval duration [54]; a finding that is not easily explained due to the lack of effects at the cellular level.

2.6. Transgenic mice over-expressing the protein phosphatase calcineurin

Protein phosphatase calcineurin plays a central role in cardiac hypertrophy [55]. Interestingly, progressive conduction defects leading to complete cardiac conduction block, associated with episodes of VT, were observed in transgenic mice over-expressing a constitutive active form of calcineurin [56]. This phenotype was later shown to be due to a profound reduction of I_{Na} [57]. This I_{Na} decrease was age-dependent in that at 50 days of age, the recorded current was virtually abolished. Guo and co-workers [57] addressed the issue of how mice can survive with such a small I_{Na} in their discussion. This strong I_{Na} down-regulation can also be obtained by infecting neonatal myocytes with adenovirus encoding a constitutive active form of calcineurin [57]. Interestingly, there was clear evidence that intracellular Ca^{2+} homeostasis was involved in this process since thapsigargin, ryanodine and the Ca^{2+} chelating agent AM-BAPTA were all successful in counteracting the effects of calcineurin over-expression. Involvement of PKC in this process was also suggested because bisindolylmaleimide I antagonized the effects of calcineurin, a finding that is consistent with previous observations showing PKC-dependent down-regulation of I_{Na} [58]. The biochemical expression of $Na_v1.5$ was not altered in calcineurin-mice; whereas non-stationary noise analysis (a biophysical approach to evaluate the number of channels active at the cell surface) clearly showed a reduced density of Na^+ channels [57]. These *in vivo* results indicate that

intracellular Ca^{2+} and PKC are involved in regulation of $Na_v1.5$ trafficking, but the detailed molecular mechanisms are not yet understood.

2.7. Transgenic mice over-expressing Snail, a transcription factor

The Snail genes are transcription factors involved in the regulation of expression of many genes; in particular, many of the proteins of the cell-cell junctions are repressed by Snail [59]. In a mouse model of cardiac-specific over-expression of Snail, the phenotype of severe dilated cardiomyopathy and significant conduction defects was observed [60]. The I_{Na} recorded in the cardiomyocytes of these transgenic hearts was only about 10% of the wild-type current. The authors also showed that Snail protein is capable of interacting with the promoter region of SCN5A resulting in repression of expression in a heterologous expression system. Although suggested to be the case in humans [61], it remains to be determined whether the decreased expression of $Na_v1.5$ is the cause of the dilated cardiomyopathy seen in this mouse model.

3. Molecular regulation of $Na_v1.5$ in cardiomyocytes

In the following sections, recent results regarding molecular aspects of $Na_v1.5$ regulation in native cardiomyocytes are discussed.

3.1. Ankyrin proteins bind to and regulate $Na_v1.5$

Ankyrin proteins, encoded by three distinct genes ANK1-3, anchor membrane proteins to the actin and spectrin cytoskeleton [62]. In the heart, both ankyrin-B (encoded by ANK2) and ankyrin-G (encoded by ANK3) are expressed [62], and both are reported to be involved in different types of cardiac arrhythmias. In humans, many genetic variants of ANK2 are linked to diverse pathological phenotypes such as LQTS type 4 [63], drug-induced LQTS, and sudden cardiac death, giving rise to the "ankyrin-B cardiac syndrome" [64]. However, there is no direct evidence that $Na_v1.5$ is regulated by ankyrin-B, even though it was reported that in neonatal mouse cardiomyocytes KO for ankyrin-B, cardiac Na^+ channels display late openings similar to the ones seen in $Na_v1.5$ LQT-3 mutant channels [65]. In contrast, ankyrin-G directly interacts with a specific ankyrin-binding motif of the linker loop between domain II and III [66]. Mohler and co-workers described a BrS patient with a SCN5A mutation (E1053 K) in this motif which disrupted the interaction between $Na_v1.5$ and ankyrin-B [17]. Using an elegant technique involving lentiviral vectors, the authors [17] were able to express tagged $Na_v1.5$ channels in adult rat myocytes. Whereas wild-type channels trafficked normally to the intercalated disks and lateral membranes, E1053 K channels remained in the cytoplasm of infected myocytes [17]. These results suggest that ankyrin-G may not only act as an anchoring protein for

$Na_v1.5$, but that it may also participate in its correct trafficking and sorting. These interesting finding are not yet fully understood and demand further study.

3.2. Role of caveolin-3 in regulating $Na_v1.5$

Caveolin-3 is an important component of small invaginations of the plasma membrane called caveolae, in which a variety of signalling molecules and ion channels are enriched [67]. CAV3, the gene encoding caveolin-3, is known to be mutated in neuromuscular diseases such as limb-girdle muscular dystrophy and rippling-muscle disease [68], and more recently in patients with congenital LQTS (LQT-9) [69] and SIDS [70]. $Na_v1.5$ can be co-immunoprecipitated with caveolin-3 from native tissue [71], and also when co-expressed in HEK293 cells [69]. Furthermore, both proteins are co-localized [69,71,72]. Interestingly, dystrophin is also a component of caveolae [73]. This raises the possibility that the interaction with $Na_v1.5$ may be indirect via proteins of the dystrophin multi-protein complex, which are mainly expressed in the lateral membranes. The role of the $Na_v1.5$/caveolin-3 interaction is somewhat puzzling. Co-expression of $Na_v1.5$ with the mutants of caveolin-3 found in LQTS and SIDS patients significantly increased the persistent late current in HEK293 cells, a molecular phenotype consistent with the clinical features [69,70]. In rat cardiac myocytes, however, a robust rapid increase of I_{Na} in response to β-adrenergic stimulation by isoproterenol, (PKA-independent due to the presence of a PKA inhibitor) was completely abolished by anti-caveolin 3 antibodies dialysed into the myocytes [71]. Shibata and co-workers [72] proposed that a pool of $Na_v1.5$ channels are located in caveolar compartments that are not in contact with the extracellular side of the myocytes. β-adrenergic stimulation, in a PKA-independent but Gαs-dependent manner, opens the caveolae and thus increases the number of $Na_v1.5$ channels at the cell surface. This attractive model demands further study at the molecular level, since the role of caveolin-3 and its interaction with $Na_v1.5$ remains unclear.

Finally, in transgenic mice with a Duchenne muscle dystrophy phenotype where caveolin-3 is over-expressed, a decrease in dystrophin and its associated proteins are observed not only in muscle but also in the heart [74]. The cardiac phenotype of these mice is characterized by a fibrotic cardiomyopathy with prolongation of the QRS interval similar to the heterozygous SCN5A-KO mice. Altogether, these results illustrate the important, but not yet completely understood, role of caveolar $Na_v1.5$.

3.3. Na_v β-subunits regulating $Na_v1.5$

Na_v channels have been shown to be associated with regulatory β-subunits [12], one-segment transmembrane proteins (Fig. 1). Four genes encoding voltage-gated sodium channel β-subunits (β1−β4) are found in the human genome. The roles of these β-subunits are multiple and

often, controversial (as is the case for $Na_v1.5$ — reviewed in [12]). This problem may be due to the fact that groups have been testing the β-subunits in different types of cellular expression systems. Among the different functions of β-subunits, Isom's group showed that when using mouse total cardiac or cardiomyocyte lysates [75] β1 and β2 subunits associate with ankyrin-G and ankyrin-B. It was previously shown that the β1/ankyrin interactions are regulated by the phosphorylation state of a tyrosine residue located in the intracellular part of the protein [76]. Using a phosphospecific antibody, it was shown that tyrosine-phosphorylated β1 are located at the intercalated disks, and that non-phosphorylated proteins are in the lateral membranes [75]. In addition, $Na_v1.5$ channels of the intercalated disk-pool were shown to not only colocalize with phosphotyrosin-β1, but also with connexin-43 (a well-known intercalated disk protein) and N-cadherin [75]. A more detailed discussion of this potential multiprotein complex can be found in [12].

Thus far, *in vivo* evidence for a Na_v β-subunit-dependent regulation of $Na_v1.5$ is scarce, but the findings reported in two recent studies suggest it plays an important role. In a large family with congenital LQTS, a mutation in the gene coding for β4 Na_v subunit was found to co-segregate with the long QT phenotype [77]. In HEK293 cells, co-expression of mutant β4 significantly increased the Nav1.5-dependent persistent current, consistent with the LQTS phenotype [77]. In another study investigating the phenotype of β1 null mice, it was reported that they exhibit a significantly longer QT interval compared to wild-type mice [78]. Further investigation of this interesting phenotype is pending.

3.4. Protein kinase A (PKA)-dependent regulation of $Na_v1.5$

In cardiac cells, PKA is mainly activated upon β-adrenergic receptor stimulation, leading to an increase in intracellular cyclic AMP [79]. The role of this pathway in regulating $Na_v1.5$, respectively I_{Na}, has been studied for many years (reviewed in [80]), resulting in many published discordant results. In more recent studies [81–84], it has been consistently reported that upon PKA activation an acute time-dependent increase of I_{Na} was observed, with slight or no alterations in its biophysical characteristics. Boyden's group [81] published the only recent study using cardioiomyocytes to investigate this phenomenon. By incubating dog cardiomyocytes with a PKA-activating cocktail (membrane-permeable cAMP, IBMX and forskolin), the authors observed a 30% increase in I_{Na}; whereas cells close to the infarcted zone showed a reduced increase. Chloroquine pre-treatment blunted this PKA-dependent increase, leading the authors to conclude that part of these effects depend on "vesicular trafficking" [81]. PKA-dependent enhancement of trafficking of $Na_v1.5$ can also be monitored in HEK293 cells expressing GFP-tagged channels [82]. A detailed analysis using these cells suggested the presence of a sub-membrane population of $Na_v1.5$ redistributing into the plasma membrane upon PKA activation. The authors proposed that the location of these channels beneath the membrane and their PKA-dependent translocation may be consistent with the opening of "silent" caveolae hypothesis [72] (see 3.2). Although very appealing, the molecular aspects of the PKA-dependent trafficking model as well as the effects of adrenergic stimulation in regulating I_{Na} are still largely unknown.

3.5. Tyrosine phosphorylation of $Na_v1.5$

Functional regulation of ion channels via tyrosine phosphorylation has been shown to be very important [85]. In two recent studies [86,87], tyrosine phosphorylation of native cardiac $Na_v1.5$ was demonstrated using anti-phosphotyrosine antibodies probing immunoprecipitated $Na_v1.5$. Moreover, it was found that isolated rat cardiomyocytes showed increased I_{Na} upon stimulation by epidermal growth factor receptor, a receptor tyrosine kinase. This response was potentiated by orthovanadate, a tyrosine phosphatase inhibitor [87]. Interestingly Ahern and co-workers [86] reported that in HEK293 cells the tyrosine kinase Fyn modulated several biophysical properties of $Na_v1.5$. In addition, our group recently reported [88] that protein tyrosine phosphatase PTPH1 interacts with the same PDZ-domain binding motif of $Na_v1.5$ (as described for syntrophin proteins-see above). It is possible that the proper function of $Na_v1.5$ is dependent on the balance between protein tyrosine kinase and phosphatase activities in cardiac cells. However, the physiological relevance of these observations remains to be fully determined.

3.6. Regulation of $Na_v1.5$ by the adenosine monophosphate-activated protein kinase (AMPK)

AMPK is a serine/threonine protein kinase involved in sensing the metabolic status of the cell [89]. Several recent studies (reviewed in [90]) demonstrated that mutations in the γ2 regulatory subunit of AMPK lead to a complex cardiac phenotype displaying, alone or in combination, ventricular pre-excitation (Wolff–Parkinson–White syndrome), conduction defect, and cardiac hypertrophy. Light and co-workers over-expressed a constitutive active form of AMPK in rat ventricular myocytes [91] and observed, among other alterations, a significant increase of the persistent I_{Na} with a prolongation of the AP leading to EADs. Whether AMPK-dependent regulation of $Na_v1.5$ can be seen *in vivo* has yet to be studied.

4. Conclusions and perspectives

Since the beginning of cellular electrophysiology studies, the cardiac I_{Na} has been the focus of many scientists and laboratories [92]. During the past decade, this field has received a strong impetus due to the description of many naturally-occurring mutations in the $Na_v1.5$ gene which lead to severe cardiac disease. Initially, most of these studies were performed using "expression systems", and in many cases

the mutation-induced alterations of $Na_v1.5$ were easily elucidated and extrapolated to their clinical phenotypes. This approach, however, is not without limitations. For instance, although the BrS E1053K mutant channels displayed no trafficking defect in HEK293 cells, they were not routed to the cell membrane in cardiomyocytes [17]. Another illustrative example is the case of the BrS mutation T1620M; depending on the expression system used (oocytes vs. HEK293 cells), the molecular phenotypes were found to be contradictory to one another [93]. We are now in a new era where there is an important effort undertaken to develop experimental models that more closely mimic human physiological condition. Two main approaches have been used, the results of which have been reviewed in this article. These studies have been investigating genetically-modified mouse models and used sophisticated cell biology techniques applicable to isolated cardiomyocytes. Both techniques remain very labour intensive, time consuming, and are mastered by only a few laboratories. It is without doubt, however, that many of the questions remaining about the role and regulation of $Na_v1.5$ will only be adequately addressed by using such modern genetic, molecular, and cell biology techniques. A few of the unsolved issues are for example: the physiological relevance of the proteins shown to associate and regulate $Na_v1.5$ in mammalian cell lines, i.e. fibroblast growth factor homologous factor 1B [94], 14-3-3 protein [95], calmodulin [96,97], and Nedd4 ubiquitin ligases [98]; and also the possibility that $Na_v1.5$ is part of different multi-protein complexes located at the intercalated disks or the lateral membranes. It can be forseen that these future studies will help elucidate the multiple and complex roles of $Na_v1.5$ in cardiac physiology and disease.

Acknowledgments

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