$Na_v1.5$ dysfunction in acquired cardiac disease

Jesse B. de Bruin, Marti F.A. Bierhuizen

Abstract

Alterations in the expression and/or function of the cardiac sodium channel $Na_v1.5$ have been frequently observed in acquired cardiac disease. As mutations in the human gene encoding $Na_v1.5$, *SCN5A*, have been associated with abnormal cardiac electrophysiology, conduction problems and lethal arrhythmias[1], the changes in $Na_v1.5$ observed in acquired cardiac disease may contribute to the increased risk for sudden cardiac death. The underlying molecular mechanisms involved in these changes, however, are poorly understood.

$Na_v1.5$ is part of a multiprotein complex and as such its function is not only determined by $Na_v1.5$ expression itself but also by e.g. auxiliary β-subunits, components of the cytoskeleton, extracellular matrix proteins, regulatory phosphatases and kinases, glycosylation status and by trafficking proteins. Disruption of the integrity of this protein complex in pathological conditions may lead to alterations in sodium current (I_{Na}) density. It is important to understand how disruption of any participant of this multiprotein complex influences $Na_v1.5$ expression and/or function and how this impairs cardiac function.

In this review, first the changes observed in $Na_v1.5$ expression and/or function in acquired cardiac disease will be summarized. Then molecular factors will be highlighted whose altered expression affects cardiac $Na_v1.5$ expression and electrophysiology *in vivo*. Finally, the consequences of these observations will be discussed.

Introduction

The cardiac sodium channel is a member of the voltage-dependent family of sodium channels, whose main function is to conduct sodium ions (Na^+) through the cell's plasma membrane. It consists of the transmembrane pore forming α-subunit $Na_v1.5$ and auxiliary β subunits, but is also part of a much larger multiprotein complex. In normal heart function, sodium channels are responsible for depolarization of the cell membrane potential, making it less negative. Depolarization results from the influx of cations, like Na^+ through different types of Na^+ channels and Ca^{2+} through Ca^{2+} channels. Efflux of K^+ and influx of Cl^- inhibits depolarization. In cardiomyocytes a large enough depolarization may result in an action potential (AP). Repolarization refers to the change in cell membrane potential that returns the membrane to a negative value after depolarization of an AP. Repolarization is mostly caused by the efflux of K^+ out of the cells.

An AP is a precise balance between sodium, potassium and calcium currents. Long it was believed that alterations in potassium conductance were the major cause underlying lethal arrhytmias, but considerable evidence demonstrated that sodium is involved as well. The importance of sodium channels for arrhythmias is emphasized by research on inherited mutations in the gene encoding $Na_v1.5$ (S*CN5A*), causing e.g. long QT syndrome type 3[2] and Brugada syndrome[3]. Sodium channel dysfunction also occurs in acquired cardiac pathological conditions, such as atrial fibrillation (AF), myocardial ischemia (MI), and heart failure (HF) (Table 1 and references therein). The underlying

mechanism for sodium channel dysfunction in acquired cardiac disease is poorly understood and remains to be clarified.

In this review, we will first discuss the normal structure, electrophysiology and regulation of $Na_v1.5$. Then the expressional and electrophysiological changes in $Na_v1.5$ observed in acquired cardiac disease will be summarized. In this regard we will focus on changes in expression of $Na_v1.5$, I_{Na} current density, ECG parameters, I_{Na} kinetics and post-transcriptional regulation in 2 different experimental models: human explanted heart tissue and animal models of cardiac disease. Finally, molecular factors will be discussed whose altered expression affects cardiac $Na_v1.5$ expression and electrophysiology *in vivo*.

Cardiac sodium channel $Na_v1.5$ structure, electrophysiology, regulation and consequences of alterations in channel properties.

The $Na_v1.5$ α-subunit is encoded by the S*CN5A* gene in humans, located on chromosome 3p21. The $Na_v1.5$ α-subunit consists of four homologous transmembrane domains DI-DIV, linked by three intracellular loops (IDI-II, IDII-III, and IDIII-IV). Each domain consists of six transmembrane segments, termed S1-S6. The S4 segment is involved in activation, while the intracellular IDIII-IV loop and the C-terminal domain are involved in fast inactivation of the channel.

Action potential (AP)

Voltage-gated sodium channels play an important role in APs. Voltage-gated sodium channels have three types of functional states: fast activation (open), fast inactivation (closed) and recovery from inactivation (closed). If these channels open during a change in the cell membrane potential (phase 0 of action potential), influx of Na^+ down their electrochemical gradient further depolarizes the cell. This increase in sodium current slows down as the membrane potential approaches the equilibrium potential for Na^+. The cell also has the ability to quickly close the channels, thereby inactivating the influx of Na^+ (phase 1) and causing Na^+ current to decay. The cell has the ability to fast inactivate due to a tethered plug formed by domains III and IV of the alpha subunit, that blocks the inside of the channel a few milliseconds after depolarization. This brief period of rapid repolarization is followed by a period of slow recovery (phase 2, plateau). The plateau phase is followed by a period of more rapid repolarization (phase 3), which restores the membrane potential to the resting potential (phase 4). An example of an AP with the corresponding ion currents is depicted in figure 1. In addition to normal fast inactivation an additional closed “recovery from inactivation” state exists, in which channels recover from the inactivation state. The period no action potential is possible is called the refractory period or responsiveness of the channel.

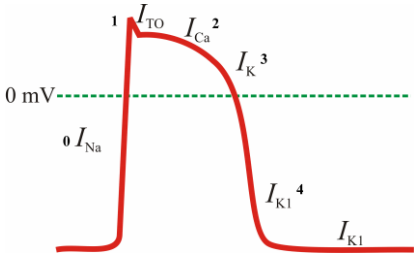

Figure 1: Ventricular action potential with contributions of the different ion channels. The upstroke of the action potential (phase 0) is caused by the influx of Na^+. The peak is caused by inactivating the influx of Na^+ (phase 1) and activating and inactivating of K^+ current. The plateau (phase 2) is caused by Ca^{2+} currents followed by a period of more rapid repolarization (phase 3) by two other K^+ currents, which restores the membrane potential to the resting potential (phase 4). Adapted from http://avivabio.com/products/tutorephys.php.

Sodium current (I_{Na})

Normally the majority of Na^+ channels opens transiently and quickly inactivates during the AP upstroke, causing a peak transient current, I_{NaT}. I_{NaT} is not only responsible for fast depolarization, but is also a determining factor for conduction velocity. A small fraction of the sodium current remains present during the AP plateau, carrying the so called late or persistent Na^+ current (I_{NaL}). Although the late sodium current is very small compared to the I_{NaT}, its contribution to total sodium current is substantial because the current is available throughout the AP plateau. Acquired heart disease is frequently associated with reduced I_{NaT} and/or increased I_{NaL}. Reduction in peak current density can be caused by a shift in the voltage-dependence of activation or inactivation, or by a combination of both. Alternatively, decreased I_{NaT} can also be caused by alteration in number of sodium channels. Characterizing the kinetics of I_{Na} or $Na_v1.5$ expression level could give more detail in the exact cause of the observed change in I_{NaT}.

Conduction velocity (ϑ)

Cardiac heart disease is often associated with conduction slowing. Myocardial conduction velocity (θ) is dependent on two factors: membrane excitability and passive tissue resistivity. Membrane excitability is dependent on the action potential upstroke determined by the fast sodium current (I_{NaT}), where resistivity is dependent on intra-, extra-, and intercellular resistances, determined by the extent of e.g. gap-junctional communication or amount of connective tissue. Decreased tissue excitability caused by reduction of the cardiac sodium current (I_{Na}) could be a possible mechanism for conduction slowing and arrhythmia in pathological conditions.

Steady-state activation

To measure the effect of potential on I_{NaT}, often the maximum rate of depolarization (V_{max} or dV/dt) during phase 0 is used. The relationship between V_{max} and membrane potential is called responsiveness. The voltage-dependence of activation curves of the sodium channel characterizes the responsiveness. An example of a normal activation relationship is depicted by curve C and D in figure 2. A parallel shift to the left, (curve D to curve C), results from improvement of responsiveness. A reduction in responsiveness will result in a parallel shift to the right (curve C to curce D). Shift to the right of the voltage-dependence of steady-state activation curve will lead to a decrease in I_{NaT} (loss-of-function), because for a given membrane potential, fewer sodium channels are activated. A hyperpolarizing shift to the left of the voltage-dependence of channel activation

allows the channel to be activated by smaller than normal depolarization, thus enhancing the activity of $Na_v1.5$ (gain-of-function).

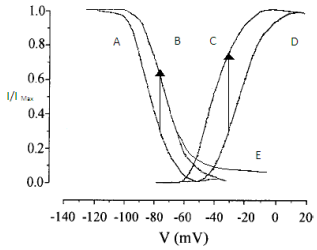

Figure 2: Voltage-dependence of activation and voltage-dependence of inactivation curves. A hyperpolarizing shift to the left of the voltage-dependence of channel activation (curve D to curve C) causes a gain-of-function. A depolarizing shift to the right (curve A to curve B) of the voltage-dependence of steady-state inactivation causes a gain-of-function or slower inactivation of the sodium channel. Curve E demonstrates an impaired recovery from inactivation of the Na^+ channel, thereby creating I_{NaL}.

Steady-state inactivation

Incomplete repolarization is considered one of the major contributors to reentry in arrhythmia. Changes found in literature of the voltage steady-state curves of inactivation were quantified by making use of the half-maximal voltage (measured as time to decay 50% of inward current), $V_{1/2}$ and the maximum slope factor of the curve, k of the Bolzmann function equation. Shift to the left of the voltage-dependence of steady-state inactivation curve (figure 2, curve B to curve A) will lead to a decrease in I_{NaT}, because for a given membrane potential, fewer sodium channels are still active (loss-of-function). Enhanced inactivation could lead to increased excitability. A depolarizing shift to the right (figure 2, curve A to curve B) of the voltage-dependence of steady-state inactivation curve, or slower inactivation of the sodium channel, will lead to an increase in "window" or late sodium current (I_{NaL}).

Recovery from inactivation

Slower inactivation of the sodium channel could not only be influenced by change in the voltage-dependence of Na channel inactivation, but also by impaired recovery from inactivation of the Na channel. This latter effect would be visible as an increase in V_{max} during steady-state level (Figure 2, curve E) and supposed to be another possible mechanism to explain the observed sustained I_{NaL} in acquired heart disease. Slower recovery from inactivation (figure 3) could contribute to the loss-of-function in I_{Na} demonstrated in acquired heart disease. Recovery from inactivation was quantified by making use of the time constant of current decay (τ).

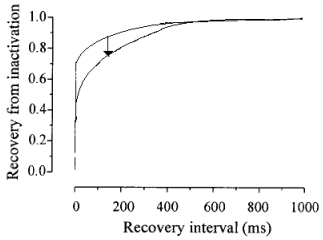

Figure 3. Prolongation of time-dependence of recovery from inactivation.

ECG

An electrocardiogram is a useful tool to assess changes in cardiac action potential morphology and membrane currents. Comparisons between the transmembrane potentials and the surface record show that phase V_{max} of the ventricular action potential corresponds with the QRS complex, plateau of the ventricular action potential with the S-T segment, and terminal repolarization with the T wave.[4] Increased I_{NaL}, leading to action potential prolongation, will become visible as prolonged QT-intervals on ECGs and conduction slowing as a prolonged QRS complex.

Regulatory factors that influence $Na_v1.5$ expression and function

As described previously, a decrease in functional sodium channels could decrease I_{Na} density. There are a number of possibilities that may explain the functional down-regulation of the sodium channel, such as a reduced transcription, reduced translation, altered membrane trafficking, changed subunit assembly, altered post-translational modification or increased degradation of $Na_v1.5$.
An altered expression of the major inward sodium channel gene (S*CN5A*) in heart failure could explain the found changes in I_{Na} density. Transcription factor TBX5 is known to directly regulates *SCN5A*, thereby decreasing Nav1.5 protein levels. Shang[5] and several other studies reviewed by Rook et al[6], have shown that not all S*CN5A* transcripts efficiently translate into the $Na_v1.5$ protein. As $Na_v1.5$ is located in specialized domains within the cardiomyocytes, impaired trafficking and adhesion could be another potential clarification. Known proteins that interacts with $Na_v1.5$ in the intercalated disc are ankyrin-G, SAP-97, Connexin43, Desmoglein2 and plakophilin-2[7]. Additionally, there are a number of post-translational modifications and regulatory factors that are known to influence $Na_v1.5$ expression and function, such as glycosylation and phosphorylation. An alternative explanation for the altered availability could be found in the turnover to $Na_v1.5$. The $Na_v1.5$ C-terminus contains a PY-motif that is known to bind Nedd4/Nedd4-like ubiquitin-protein ligases (Nedd4-2). $Na_v1.5$ current density was decreased by 65% upon Nedd4-2 co-transfection, whereas the PY-motif mutant $Na_v1.5$ was unaffected[8].

Cardiac sodium channel $Na_v1.5$ in acquired human cardiac disease

In human acquired cardiac disease, contradictory results have been reported with respect to alterations in $Na_v1.5$/S*CN5A* expression and I_{Na} properties (Table 1). I_{NaT} has been reported as decreased in HF ventricular cardiomyocytes[9], increased in HF ventricular cardiomyocytes[10] or was not changed at all in HF atrial cardiomyocytes[11]. This is in contrast to studies on animals that demonstrate consistently a decrease in I_{NaT} (Table 2) in acquired diseased hearts. Change in current density can be caused by a shift in the voltage-dependence of activation or inactivation, a reduction in expression or a combination of these parameters. Characterizing the kinetics of I_{Na}, therefore, could give more detail in the exact cause of the found reduction in I_{NaT}. Unfortunately, no significant differences in steady-state activation[9,11] , slower inactivation[9] of the sodium channel or change in responsiveness[12] were observed in failing human hearts, suggesting that the decrease in I_{NaT} is caused by alteration in number of sodium channels.
Total number of functional sodium channels is dependent on S*CN5A* expression and translation. To assess changes in S*CN5A* gene expression, the S*CN5A* mRNA levels in diseased and healthy explanted

human hearts were compared. Valdivia et al[9], showed no significant differences in S*CN5A* mRNA levels in ventricular cells obtained from explanted failing human hearts as compared to normal hearts. These results were consistent with results from Kääb et al[13]. However, also reduced mRNA levels have been found in failing human hearts[5,14]. The group of Borlak and colleagues[14] even reported a 50% reduction of S*CN5A* gene expression in human explanted hearts from patients that suffered from ischemic or dilatative cardiomyopathy. An important finding also was that no change in S*CN5A* mRNA expression was found in assist device-supported hearts, suggesting that S*CN5A* mRNA expression is directly regulated by pressure load and stretch force. Stretched cardiomyocytes have been demonstrated to possess increased sodium current and increased arrhythmia vulnerability[15]. Similar findings of a reduced S*CN5A* expression of 24.7 % were found by Shang and colleagues[5] in humans. In addition, they identified 3 new C-terminal S*CN5A* mRNA spicing variants. These 3 RNA variants, designated as E28B, E28C and E28D, were shown to encode non functional truncated Na^+ channels. Patients with HF showed a 24.7% reduction in the full length E28A variant mRNA and a 14.2-fold and 3.8-fold increase in E28C and E28D, respectively. Therefore, S*CN5A* RNA isoform switching may potentially underlie the observed reduction in I_{Na} density in heart failure. So far, however, these results have not been confirmed yet. Moreover, these new splicing variants could not explain all the pathological changes found in acquired human and animal cardiac diseases, because these splicing variants were only detected in humans and not in rats and mice. Another factor that could influence conduction includes change in distribution of $Na_v1.5$ in the heart. An increase in $Na_v1.5$ expression was shown from epi- to endocardium. Unfortunately, there is no difference found in transmural $Na_v1.5$ expression gradient and $Na_v1.5$ protein level in either the endo-, mid-, and epicardium of left ventricles between HF and normal hearts[11].

Unaltered expression does not necessarily means an unaltered protein level. Although altered translation could be a promising theory to explain the reduction of I_{Na} density, only one article focused on confirming this theory in humans. Western blotting on protein extracted from ventricular samples of human HF patients[5], revealed a 62.8% reduction in protein. The reduction in $Na_v1.5$ protein expression is associated with a 24.7% reduction in SCN5A E28A RNA expression, suggesting a transcriptional mechanism.

I_{NaL} in cardiomyocytes is found in both normal and failing human hearts, but is more abundant in failing hearts[9,16,17]. This I_{NaL} may delay repolarization and prolong action potential duration, thereby potentially involved in generating lethal arrhythmias. But the mechanisms leading to the I_{NaL} increase in HF and slower closure of the channel are not clear. Breakup of the cardiomyocyte cytoskelet with cytochalasin D has shown to increase late sodium currents[18]. Down-regulation of structural proteins or other part of the part of the $Na_v1.5$ macromolecular complex could be a possible theory in explaining late Na^+ current.

Cardiac sodium channel $Na_v1.5$ in pathophysiological animal models of cardiac disease

Several research groups have demonstrated that I_{NaT} is decreased in model of HF in dogs[9,19], but also in dog model of AF[20] and MI[21]. Only one study has shown no difference in I_{NaT} density in HF model of dogs[22]. The found decrease in action potential upstroke is a dependent factor for the found conduction slowing in acquired cardiac disease, as also found in diverse animal models. Data from a dog model of HF[23] and rabbit model of HF[24] showed an increase in QRS complex duration. An increase in QRS duration reflects a decrease in V_{max} or conduction velocity. Conduction slowing is also visible as the found prolongation of the action potential duration at 90% repolarization in multiple animal models[22,25,26,27,28]. Clarification for the decreased I_{NaT} could be found in altered voltage-dependent activation or inactivation. Looking to the results of previous research in dog models of HF[9,19,29,30], dog model of MI[21] and rabbit model of HF[31], no significant differences in steady-state activation were observed. The majority of research in models of acquired heart disease showed no change in slower inactivation of the sodium channel. Steady-state inactivation was unaltered in a dog mode of HF[9,19,22,30], rabbit model of HF[31] and dog model of AF[20]. As the channel gating characteristics in both human as in animal models are identical between normal and diseased hearts, an alternative clarification should be found in the number of active sodium channels.

$Na_v1.5$/*SCN5A* expression/function was also assessed in multiple animal models of cardiac disease. In comparison with the results obtained with explanted human hearts, animal models also yielded inconsistent results in *SCN5A* mRNA levels. Valdivia et al[9] showed no significant differences in mRNA levels in ventricular cardiomyocytes from a canine pacing model of heart failure. Similar results were also found in another dog model of HF[19] and in a post-MI model of rat[27]. The only contradictory result comes from a dog model of AF[32] showing reduced *SCN5A* mRNA concentration by 42% after 42 days of atrial tachycardia. Clarification for the found difference may be the difference in heart disease, but more research need to be done to be able to conclude that there is no difference mRNA concentrations in AF hearts.

Although altered translation could be a promising theory to explain the reduction of I_{Na} density, only a few groups have tried to confirm this theory. To quantify changes in α-subunit protein levels, western blot studies were performed on cardiac proteins isolated from a model of HF[19], AF[32] and post-MI[27]. In a dog model of AF[32], a significant reduction in α-subunit protein was correlated with corresponding changes in mRNA levels and I_{Na} density. This result suggests that the reduction in I_{Na} density is caused by transcriptional modifications. In contrast, only a reduction in α-subunit protein and not in mRNA levels was found in a dog model of HF[19], suggesting an altered translation.

I_{NaL} in cardiomyocytes is found failing hearts in models of HF in dogs[9,17,29] and in the left atrium of rabbits[28]. This I_{NaL} may delay repolarization and prolong action potential duration, thereby potentially involved in generating lethal arrhythmias. But the mechanisms leading to the I_{NaL} increase in HF and slower closure of the channel are not clear. Slower recovery from inactivation could be a possible mechanism to explain the observed sustained I_{NaL} in acquired heart disease. Although, the τ does not differ in a dog model of HF[22] and rabbit model of HF[31], significant slowing of recovery is detected in a rat and dog model of myocardial infarction[21,27] and in a dog model of AF[20]. Another possible suggestion is that other proteins other than the channel itself, as part of macromolecular complex, are involved in I_{NaL}.

Potential factors underlying altered cardiac sodium channel $Na_v1.5$ expression and function

Since $Na_v1.5$ is part of a multiprotein complex in cardiomyocytes, its expression and function may be influenced by participants of this complex. Alternatively, $Na_v1.5$ expression and function may be affected by post-translational modifications, such as glycosylation and phosphorylation, or other cellular components. The use of transgenic mice is therefore a strong tool to investigate the effect of individual structural proteins on the function of the sodium channel. In this paragraph, *in vivo* studies will be highlighted in which $Na_v1.5$ expression and/or function has changed because of an alteration in expression of such factors. The results of these investigations on transgenic mouse models are summarized in table 3.

Glycosylation

The presence of N-linked oligosacharide chains on the α-subunit became evident by its interaction with immobilized wheat germ agglutinin, a carbohydrate-recognizing protein, and by reduction of its apparent molecular weight after treatment with neuraminidase or N-glycanase.[33] The group of Ufret-Vincenty[34] investigated the hypotheses whether the changes in I_{Na} are caused by incomplete glycosylation during post-translational processing of the Na^+ channel protein during HF. To test this hypothesis the muscle LIM protein knockout mouse ($MLP^{-/-}$) was used as model of HF caused by sialic acid deficiency.

The α-subunit of the Na^+ channel in the $MLP^{-/-}$ heart had a lower average molecular weight than the control heart, thereby suggesting that $Na_v1.5$ $MLP^{-/-}$ hearts were less heavily glycosylated. $MLP^{-/-}$ hearts demonstrated a decrease in I_{Na}, negative shift of steady-state inactivation of I_{Na} and impaired recovery from inactivation compared with controls. These electrophysiological changes contributed to longer action potentials and a higher probability for early after depolarizations. Exposing $MLP^{-/-}$ and control cardiomyocytes to neuraminidase, an enzyme known to remove sialic acid residues, resulted in an increase in conductivity in control cells but not in $MLP^{-/-}$ cells. Thereby suggesting that the observed changes in I_{Na} and electrophysiology in $MLP^{-/-}$ hearts were probably caused by reduced sialylation of $Na_v1.5$ during post-translational processing[34].

Calcineurin (CnA)

CnA is a calcium-activated serine/threonine phosphatase that dephosphorylates nuclear factor of activated T cells 3 (NFAT3). Dephosphorylated NFAT3 translocates into the nucleus to activate the hypertrophic transcription factors GATA binding protein 4 (GATA4) and myocyte enhancing factor-2 (MEF2). Transgenic mice overexpressing continuously active CnA in the heart (MHC-CnA mice) were shown to have a decreased I_{Na} density, abnormal conduction and an increased vulnerability for arrhythmias[35,36]. The decrease in I_{Na} could be clarified by the observed decrease in $Na_v1.5$ protein expression[35]. However, these results were contradicted by another study showing no differences in $Na_v1.5$ protein expression[36]. Similarly as shown by Maltsev et al[22→30], rescue of calcium homeostasis with ryanodine, BABPTA-AM and thrapsigargin resulted in recovery of the reduction in I_{Na} in MHC-CnA hearts[36]. In addition, the same study showed that treatment with PKC inhibitor bisindolylmaleimide I, rescued the reduction in I_{Na} and dV/dt_{max} as well. Another intriguing aspect is

that the CnA pathway is upregulated in HF[37], but a direct correlation with $Na_v1.5$ expression is still lacking.

Ca^{2+}/calmodulin-dependent kinase II (CaMKII)

The expression of another important Ca^{2+}-induced modulator of $Na_v1.5$, i.e. CaMKII, was found to be increased in HF[38] and over-expression of CaMKII causes lethal arrhythmias[39]. CaMKII is a serine/threonine kinase able to phosphorylate multiple substrates, including $Na_v1.5$. Wagner et al[40], showed that adenovirus-mediated overexpession of CaMKII in rabbit myocytes and transgenic overexpressing of CaMKII in mice resulted in a hyperpolarizing shift in activation, increased slow inactivation, slow recovery from inactivation and increase in I_{NaL} in a Ca^{2+} dependent manner. This I_{NaL} is confirmed by Aiba et al[38], demonstrated in a model of guinea pig ventricular cardiomyocytes in the absence or presence of CaMKII that CaMKII significantly increases I_{NaL} and that the addition of CaMKII inhibitors abolished the CaMKII-induced increase in I_{Na}.

Important information that Ca^{2+} has a direct influence on I_{Na} density came from Maltsev et al[30], demonstrating that long-term intracellular Ca^{2+} buffering with BAPTA-AM results in a partial recovery of I_{Na} density in cardiomyocytes from failing canine hearts. Calsini et al[31], demonstrated that elevated Ca^{2+} reduced I_{Na} density and dV/dt_{max}, but a change in kinetic properties under physiological conditions was not seen, thereby suggesting that these effects were due to permeation block. In addition, no difference was seen in I_{Na} density and gating between HF and control. Wingo et al[41], suggested that Ca^{2+} modulation of the sodium channel is independent of CaM, because a peptide antagonist of Ca-dependent CaM binding had the same enhancement of fast inactivation. A direct binding of Ca^{2+} through the C-terminal EF-hand of the rat sodium channel showed high affinity and was therefore proposed as alternative pathway.

Ankyrin

Sodium channel activity requires precise trafficking to specialized domains within the cardiomyocytes. It was demonstrated that the $Na_v1.5$ α-subunit preferably is located in the cell membrane of the intercalated disc and transverse tubules[42,59]. In recent years, it has become increasingly clear that ankyrin proteins interact with $Na_v1.5$ and regulate its trafficking to the cardiomyocyte cell surface. Ankyrin proteins are adaptor proteins that link membrane proteins to the cytoskeleton. They have been shown to play an important role in the membrane insertion and anchoring of $Na_v1.5$. Three ankyrin proteins have been identified, of which ankyrin-G and ankyrin-B are known to regulate $Na_v1.5$ in the heart. Ankyrin-B dysfunction is associated with ankyrin-B arrhythmia syndrome, also called type 4 long QT syndrome (LQTS)[42]. In addition, ankyrin-B levels were shown to be significantly affected in a post-MI animal model of arrhythmias[43]. Mice lacking ankyrin-B expression in the heart showed a reduced I_{Na} density due to fewer functional Na^+ channels, a hyperpolarizing shift in voltage-dependent activation and inactivation and an increased I_{NaL} current, what could contribute to AP prolongation[44]. Nevertheless, these abnormal kinetics could not be ascribed to a change in $Na_v1.5$ protein expression and localization, because the group of Lowe et al[45] demonstrated that these were not altered in ankyrin-B deficient mice. On the other hand they reported that cardiomyocytes with reduced ankyrin-G expression displayed decreased $Na_v1.5$ expression, $Na_v1.5$ membrane targeting and I_{Na} density[45]. These data suggest that ankyrin-G is needed for normal $Na_v1.5$ trafficking and not ankyrin-B. Despite the available knowledge implicating

the importance of ankyrin in altered sodium channel function, little is known regarding altered expression or function of ankyrin in acquired cardiac diseases. Future experiments should determine if ankyrin is reduced in cardiac heart disease.

Plakophilin-2 / Desmoglein2

The desmosomal protein Plakophilin-2 (PKP2) is involved in linking cadherins to intermediate filaments in the cytoskeleton and coexists together with $Na_v1.5$ in the sodium multi-protein complex in the intercalated disc. Mutations in PKP2 has been associated with arrhytmias[46]. Loss of PKP2 expression via snRNA[47] and PKP2-heterozygous-null (PKP2-Hz) mice[7] demonstrated a decreased in I_{NaT}, decrease in conduction velocity no difference in voltage dependence of activation, a negative shift in steady-state inactivation and a slower recovery from inactivation.
Recently, another desmosomal protein, Desmoglein2 (Dsg2), has been found to interact with $Na_v1.5$ *in vivo*. Dsg2 is a calcium-binding transmembrane glycoprotein and belongs to the family of cadherins. Transgenic mice with heart-specific overexpression Dsg2 (Dsg2-N271S)[48] demonstrated an increase in conduction velocity and increase in arrhythmias. No no differences in the voltage dependencies of the activation and inactivation were detected in these mice. The found increase in conduction velocity should be clarified by the significantly lower AP upstroke velocity correlated with corresponding changes in I_{NaT}. It would be interesting to investigate whether other desmosomal proteins interact with $Na_v1.5$ and alter sodium channel function and whether PKP2 and Dsg2 are also down-regulated in acquired cardiac disease other than arrhythmias.

Syntrophin/dystrophin/Utrophin

Recently, syntrophins have been shown to interact with the last three residues (PDZ domain) of the $Na_v1.5$ C-terminus[49]. Syntrophins play an important role in mediating the link between the membrane-associated sodium channel $Na_v1.5$ and dystrophin[49]. Dystrophin is a cytoskeletal protein that connects the cytoskelet of primarily muscle cells to the surrounding extracellular matrix through the cell membrane. The mdx mouse, which lacks dystrophin, demonstrated a decrease in $Na_v1.5$ protein levels, a decrease in I_{NaT} and conduction slowing[49]. This effect was even increased in an utrophin dystrophin double knock out mice[50], thereby suggesting that Utrophin plays also a role in anchoring of $Na_v1.5$ in mdx mice. The found decrease in I_{NaT} could be clarified by the decrease in number of $Na_v1.5$, as there is a minimum until no effect on steady-state activation and inactivation. Disruption of this complex between dystrophin, utrophin and syntrophin, thereby destabilizing the sodium channel protein complex, could be an underlying cause of altered I_{Na} in cardiac heart disease, but no up- or down-regulation of these proteins were found in acquired cardiac diseases.

FKBP12

FK506 binding protein 12 (FKBP12) is a cis-trans prolyl isomerase that binds immunosuppressant tacrolimus (FK506) and belonging to the immunophilin protein family, which play a role in immunoregulation and protein folding and trafficking. FKBP12 overexpression transgenic (αMyHC-FKBP12) mice[51] demonstrated a significant reduction in I_{NaT}, increased I_{NaL}, slower conduct velocities, a positive shift of steady-state activation and inactivation to more depolarized potentials and slower recovery of I_{Na} and increase in cardiac arrhythmias and sudden cardiac death. $Na_v1.5$ was significantly reduced in FKBP12 overexpression transgenic (αMyHC-FKBP12) mice[51], suggesting that the reduction

in I_{Na} is partly attributable to lowered $Na_v1.5$ expression. On the other hand cardiomyocyte-restricted FKBP12 conditional knockout ($FKBP12^{f/f}$/αMyHC-Cre)[51] demonstrated an increase in I_{NaT} combined with a corresponding increase in upstroke velocity in phase 0. These results suggest FKBP12 plays an important role in sodium channel function. It is not known whether FKBP12 binds $Na_v1.5$ directly or interact in an indirect manner. That FKBP-tacrolimus complex also inhibit the previous descibed calcineurin[52] suggest an indirect effect. Future experiments should determine whether the effect of FKBP12 is indirectly caused by calcineurin and whether FKBP12 expression is altered in cardiac heart disease.

T-box transcription factor 5 (TBX5)

A possible clarification for the reduction in Scn5a expression could be found in the research of Arnolds et al[53]. This group demonstrated that removal of TBX5 from the cardiac conduction system in tamoxifen-inducible VCS-specific Cre BAC transgenic ($Tbx5^{minKCreERT2}$) mice shown to have conduction slowing and an increased vulnerability for arrhythmias. More interesting, $Na_v1.5$ expression is reduced in these mice, suggesting that TBX5 directly regulates SCN5A. In contrary, an assay measuring the effect of wild-type TBX5 and mutant G125 TBX5 showed no difference in expression levels of SCN5A[54]. It would be interesting to examine whether TBX5 is reduced in cardiac disease. It is necessary to continue to search for other transcription factors that are altered in cardiac diseases and directly or indirectly affect Scn5a expression.

RAS

Angiotensin-converting enzyme is part of the renin-angiotensin system and produces angiotensin II. Increased angiotensin is associated with increased risk in arrhythmias. To investigate the electrophysiological abnormalities that caused sudden cardiac death, electrophysionlogical changes were compared between a mouse model of RAS activation (ACE 8/8 mice) and wild-type mice[55]. ACE8/8 has shown to reduce $Na_v1.5$, Connexin 40 (Cx40) and Connexin 43 (Cx43) expression and reduces cardiac conduction. More interesting, I_{NaT} was not altered between ACE8/8 and wild-type, despite the reduction in $Na_v1.5$ expression. This suggests that although RAS reduces $Na_v1.5$ expression, $Na_v1.5$ protein levels were posttranscriptional compensated. The absence of change in $Na_v1.5$ protein and I_{NaT} also suggest that gab-junctions, such as Cx40 and Cx43 are mainly responsible for the found slow conduction and arrhythmias[55].

CD4C/HIV

Arrhythmias and alterations in cardiac electrical activity have been observed in AIDS patients. Grandy et al[56], investigated whether HIV had effect on I_{Na}, by using transgenic mice (CD4C/HIV mice) which exhibit similar symptoms as in AIDS patients. CD4C/HIV mice express the genes of the HIV-1 genome that are needed for the replication of the virus and the development of the disease. CD4C/HIV mice demonstrated a decrease in I_{NaT}, QRS prolongation and a decrease in action potential upstroke velocity. $Na_v1.5$ mRNA was unaltered between CD4C/HIV mice and control, suggesting that HIV does not alter SCN5A expression. Grandy et al[56] suggested, based on the found elevated levels of pro-inflammatory cytokines in CD4C/HIV mice and in AIDS patients, that elevated levels of cytokines can alter sodium current and conduction.

SCN3B

Voltage-gated sodium channels consists of the transmembrane pore forming α-subunit $Na_v1.5$ and one or more auxiliary β subunits. Six β-subunits have been identified encoded by 4 different genes; SCN1B, SCN2B, SCN3B and SCN4B. *Scn3b* knockout mice[59] has shown abnormal cardiac conduction properties and increased risk for arrhythmias. The negative shift in voltage-dependence of steady-state inactivation is consistent with the found decrease in I_{NaT}, although an increase in SCN5a mRNA was detected[59].

Conclusion and perspectives

By understanding the molecular basis of $Na_v1.5$ alterations in acquired cardiac disease may suggest novel therapeutic approaches for treatment of these life-threatening cardiac arrhythmias. By summarizing the different approaches, we would like to give an overview of the different but probably overlapping possible molecular mechanism underlying arrhythmias in acquired cardiac diseases. In general, we found that I_{NaT} is reduced in acquired heart disease and I_{NaI} increased, but does not change channel gating characteristics in both human as in animal models between normal and diseased hearts. This suggests that the differences in I_{NaT} were probably caused by reduction in $Na_v1.5$ protein expression or posttranslational modifications of the sodium multi-protein complex. In this review, we have aimed to find out whether transcriptional changes of the α-subunit of the sodium channel in acquired cardiac disease can explain the decrease in I_{NaT}. Some groups have studied the gene expression of *SCN5A*[9,19] in failing heart compared to normal heart by real-time PCR, but the results were inconsistent. We could not conclude with certainty that the level of $Na_v1.5$ mRNA in failing heart correlates with reduction in I_{na} density. A possible explanation for the inconsistent data could be found in the research of Zygmunt et al[57] This group shows that there were transmural regional differences in $Na_v1.5$ expression.

$Na_v1.5$ is part of a large multi-protein complex composed of e.g. components of the cytoskelet, trafficking proteins, extracellular matrix proteins, and auxiliary β subunits. Dysfunction of any member of this complex, therefore, has the potential to disrupt its function. This review has shown that disruption of part of this complex causes alterations in electrophysiology, similarly as in acquired cardiac diseases, and could be possibly relevant to the pathogenesis of arrhythmias. Moreover, there are also other molecular factors that influence $Na_v1.5$ function or expression, but are not part of the macromolecular $Na_v1.5$ complex, such as Tbx5[69→53], Nedd4-2[77→8] and RAS[25→13]. Up- or down-regulation of these proteins have been associated with altered $Na_v1.5$ expression and function. Therefore it is important to further study the expression of these proteins in acquired heart disease.

Despite the explosive amount of research in the last decade, the exact mechanisms for alteration in $Na_v1.5$ expression/function in acquired cardiac disease are still not understood. In addition, the inconsistency in results obtained makes it difficult to make hard conclusions. Clarification for the differences between groups has to be found in differences in recording conditions, species or tissue of origin. Additional work is needed to unravel exact mechanisms for alteration in $Na_v1.5$ expression/function in acquired cardiac disease.

Table 1. Changes in cardiac sodium $Na_v1.5$ expression and function in human acquired cardiac disease

Pathology/ Model	$Na_v1.5$ Protein	*SCN5A* RNA	I_{NaT}	I_{NaL}	Upstroke velocities	Total Na^+ influx	VD SSI	VD SSA	Recovery from inactivation	Remarks	Ref.
HF VT	nd	↔	nd	Nd	nd	nd	nd	nd	nd	Level of hH1 mRNA does not change in failing heart	13
HF VT	nd	↓ 50%	nd	Nd	nd	nd	nd	nd	nd	No significant change in expression with LVADs;	14
HF VC	nd	↔	↓ 57%	↑	nd	nd	↔	↔	nd		9
HF VC	nd	nd	nd	↑	nd	nd	nd	nd	nd	Inactivation and reactivation of I_{NaL} was found to be voltage-independent	16
HF VC	nd	nd	nd	↑	nd	↑ 53.6%	nd	nd	nd	TTX or STX-resistent I_{Na} targeted	17
HF VC	nd	nd	↑	↑ 58%	nd	nd	nd	nd	nd		10
HF-VT	↓ 62.8%	↓ 24.7 %	nd	Nd	nd	nd	nd	nd	nd	Upregulation C-terminal splicing variants E28C and E28D and downregulation E28B.	5
HF AC	↔	↔	↔	Nd	nd	nd	nd	↔	nd	Transmural $Na_v1.5$ expression gradient in HF is similar to normal hearts at both protein and RNA level.	11
HF VC	nd	nd	nd	Nd	dV/dt_{max} ↔	nd	nd	nd	nd	Both burst and scattered openings occured within range of take-off potential for EADs	12

Table 1: Summary of electrophysiological alterations in human cardiac sodium channel in acquired cardiac diseases. Abbreviation: HF, heart failure; SSI, steady-state inactivation; SSA, steady-state activation; VD, voltage-dependence; VT, ventricular tissue; VC, ventricular cardiomyocytes; AC, atrial cardiomyocytes; LVT, left ventricular tissue; nd, not determined; ↑, up-regulated; ↓, down-regulated; ↔, unaltered.

Table 2. Changes in cardiac sodium $Na_v1.5$ expression and function in animal models of cardiac disease

Pathology/ Model	Species	$Na_v1.5$ Protein	*SCN5A* mRNA	I_{NaT}	I_{NaL}	I_{Na} density (I_{Na}/C)	Upstroke velocities/ AP parameters	VD SSI	VD SSA	Recovery from inactivation	ECG para-meters	Arrhytmias	Remarks	Ref.
HF LVC	Dog	nd	Nd	nd	Nd	↑	↑ APD_{90}	nd	nd	Nd	nd	↑ EADs	AP duration was normalized by Na^+ blockers; Shift of the resting potential towards depolarization	25
AF	Dog	↓ 47%	↓	nd	Nd	↓ 52%	nd	nd	nd	Nd	nd	nd		32
HF VC	Dog	nd	↔	↓ 39%	↑	nd	nd	↔	↔	Nd	nd	nd		9
HF VC	Rabbit	nd	nd	nd	nd	2 fold ↑	APD ↑	nd	nd	nd	RR ↓	90% VT		26
HF VC + 1 µM Ca^{2+}	Dog	nd	nd	nd	↑	nd	nd	↑	↔	↓	nd	nd	Importance I_{NaL} underestimated because data were based on Ca^{2+} independent I_{NaL}	29
HF VC	Dog	nd	nd	nd	↑	↑ 53.6%	nd	nd	nd	nd	nd	nd		17
HF LVC	Dog	nd	nd	nd	Nd	↓	nd	↔	↔	nd	nd	nd	BAPTA-AM partially restored I_{Na} density; Long-term monotherapy with carvedilol restored I_{Na} density	30
HF LVC	Dog	↓30%	↔	↓	Nd	↓	Nd	↔	↔	nd	nd	nd	β1 and β2 subunit protein were unchanged; β1 mRNA unaltered	19
HF CV + Ca2+	Rabbit	nd	nd	nd	Nd	↓	↓ dV/dt_{max}	↔	↔	↔	nd	nd		31
Post-MI	Rat	↔	↔	↓ τ	↓ τ	nd	APD_{90} ↑	nd	nd	↓	nd	nd	Increase in gene expression was demonstrated in the NaCh Iα minor subtype, but not in the NaCh I subtype.	27
HF VC	Dog	nd	nd	nd	Nd	nd	dV/dt_{max} ↔	nd	nd	nd	33% ↑ QRS	nd	20% ↓(slower) conduction velocity	23
HF	Rabbit	nd	nd	nd	Nd	↔	nd	nd	nd	nd	QRS ↑; θT ↑, θL ↑, θTM ↔	nd	θ_T ↑, θ_L ↑, θ_{TM} ↔ Sodium current ↔ Cardiomyocytes width and length were increased 30% both	24
EBZ infarct zone cells	Dog	nd	nd	↓ 61%	Nd	nd	dV/dt_{max} ↔	↑	↔	↓	nd	nd		21
HF	Dog	nd	nd	↔	Nd	nd	↑APD_{90}; ↑APD_{50}	↔	nd	↔	nd	nd		22
AF	Dog	nd	nd	↓	Nd	↓	nd	↔	cAF ↔ nAF ↓	↓	nd	nd		20
LVC	Rabbit	nd	nd	nd	↑ LA ↔RA	↔	APD_{90} ↑	nd	nd	nd	nd	EAD ↑ LA	Research in LA myocytes; capacitance ↑ pF	28

Table 2: Summary of (electrophysiological) alterations in the cardiac sodium channel in animal models of cardiac disease. Abbreviations: HF, heart failure; AF, atrial fibrillation; SSI, steady-state inactivation; SSA, steady-state activation; VD, voltage-dependence; VT, ventricular tissue; VC, ventricular cardiomyocytes; AC, atrial cardiomyocytes; LVC, left ventricular cardiomyocytes; EBZ, epicardial border zone; nd, not determined; ↑, up-regulated; ↓, down-regulated; ↔, unaltered; APD, action potential duration.

Table 3: Potential factors underlying altered cardiac sodium channel expression/function

Pathology/ Model	$Na_v1.5$ Protein	*SCN5A* mRNA	I_{NaT}	I_{NaL}	I_{Na} density	Upstroke velocities/ AP parameters	VD SSI	VD SSA	Recovery inactivation	ECG parameters	Arrhytmias	Remarks	Ref.
HF/transgenic overexpression of calcineurin	↓	↓	Nd	nd	nd	Nd	nd	nd	nd	PQ ↑ QRS ↑ P wave ↑ RR ↑	↑	Discontinuous conduction and block are common; presumably transcriptional mechanism	35
HF/transgenic overexpression of calcineurin	↔	nd	↓	nd	nd	dVm/dt (phase 0) ↓	↔	nd	↔	QRS ↑ P wave ↔ RR ↔ QT ↔	↑heart blocks	Decreased number of functional channels	36
HF/CaMKIIδ_c- T mice;	↑	1.6 fold ↑ TG mice;	↑	↑	nd	APD_{90}↑	↓ in Ca^{2+}-dependent manner	↔	↓	QRS↑ QTc↑ PR↓	↑ (monomorphic and polymorphic VT)		40
mdx mice/ dystrophin knockout	↓ 50%	↔	↓ 29 %	nd	nd	nd	↔	↔	nd	P wave ↓ 19% QRS ↑ 18% P-wave ↔ ST interval ↔	nd	No hypertrophy, no different cel size and no fibrosis; Dystrophin was absent from the intercalated discs. Two distinct pools of $Na_v1.5$ channels co-exist	49
mdx/utrophin dystrophin double knockout mice	↓	nd	↓	nd	nd	APD_{90}↑; dVm/dr (phase 0) ↓	↓	↓	nd	nd	nd		50
(-/-) ankyrin-B knockout	↔*	nd	↓	↑	I_{NaT}↓ I_{NaL}↑	APD_{90}↑ dVm/dt (phase 0) ↔	↓	↓ (hyperpolarizing shift in $V_{0.5}$ for activation)	nd	QT interval ↔; QT interval ↑(with HR deceleration); RR ↓; T-wave ↔	nd	Lower current density was result of fewer functional Na^+ channels	44, 45*
HF/ muscle LIM protein knockout	↓	nd	↓ 35%	nd	nd	APD_{90}↑; dVm/dt (phase 0) ↓	↓	nd	↓	QT↑	↑ EADs	Incomplete glycosylation during post-translational processing contributes to Na^+ channel-dependent arrhythmogeneses in HF; Lower average molecular weight α-subunit	34
ACE 8/8 mice; angiotensin-converting enzyme Overexpression	↔	↓	↔	nd	nd	nd	↓	↑	nd	AV ↑ AH ↑ HV ↑	Ventricular tachycardia ↑		55
Tbx5^{minKCreERT2}	nd	↓	nd	nd	nd	nd	nd	nd	nd	PR ↑ QRS ↑ AH ↑	↑	conduction slowing	53
αMyHC-FKBP12	nd	↓	↓	↑	↓	dVm/dt (phase 0) ↔	↑	↑	↑	PR ↑ QRS ↑	38% sudden death		51
FKBP12$^{f/f}$/αMyHC-Cre	nd	nd	nd	nd	↑ 2 fold	dVm/dt (phase 0) ↑	nd	nd	nd	nd	nd		51
Scn3b$^{-/-}$ mice	nd	↑	↓	nd	nd	ADP90 ↔	nd	nd	↔	PR ↑ P wave ↑	Ventrical tachycardia ↑		59

PKP2-heterozygous-null (PKP2-Hz) mice	↔	nd	↓ ↓*	nd	nd	nd	↓ ↓*	↔	↑*	nd	nd	No changes in localization of NaV1.5	7, 47*
Dsg2-N271S mice	nd	nd	↓		nd	APD90 ↔; dVm/dt (phase 0) ↓	↔	↔	nd	QRS ↑	Arrhytmias ↑		48
CD4C/HIV mice	nd	↔	↓	↔	nd	dVm/dt (phase 0) ↓	nd	nd	nd	QRS ↑	nd	Interleukin-1*β* were elevated in HIV mice	56

Table 3: Overview of regulatory factors potentially underlying altered $Na_v1.5$ expression/function in acquired cardiac disease. Abbreviations: HF, heart failure; APD, action potential duration; SSI, steady-state inactivation; SSA, steady-state activation; VD, voltage-dependence; nd, not determined; ↑, up-regulated; ↓, down-regulated; ↔, unaltered.

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