Deranged Sodium to Sudden Death

Colleen E. Clancy[1*], Ye Chen-Izu[1], Donald M. Bers[1], Luiz Belardinelli[2], Penelope A. Boyden[3], Laszlo Csernoch[4], Sanda Despa[5], Bernard Fermini[6], Livia C. Hool[7], Leighton Izu[1], Robert S. Kass[3], W. Jonathan Lederer[8], William E. Louch[9], Christoph Maack[10], Alicia Matiazzi[11], Zuilin Qu[12], Sridharan Rajamani[2], Crystal M. Rippinger[1], Ole M. Sejersted [13], Brian O'Rourke[14], James N. Weiss[12], Andras Varro[15], and Antonio Zaza[16]

[1] Department of Pharmacology
University of California, Davis
Genome Building Rm 3503
Davis, CA 95616-8636

[2]Department of Biology
Cardiovascular Therapeutic Area
Gilead Sciences
Fremont, CA

[3] Department of Pharmacology
Columbia University College of Physicians and Surgeons
630 W. 168th St.
New York, NY 10032, USA

[4] University of Debrecen,
Faculty of Medicine
Department of Physiology
Debrecen H-4012, Hungary

[5]Department of Pharmacology and Nutritional Sciences
University of Kentucky College of Medicine
Lexington KY, USA

[6] Global Safety Pharmacology Division
Pfizer Incorporated

[7] Victor Chang Cardiac Research Institute Sydney
School of Anatomy, Physiology and Human Biology
The University of Western Australia
Crawley, WA, 6009
Australia

[8] Department of Physiology
University of Maryland
Baltimore, MD USA

[9]Institute for Experimental Medical Research,
Oslo University Hospital Ullevål
University of Oslo
Oslo, Norway

[10] Klinik für Innere Medizin III
Gebäude 40
Universitätsklinikum des Saarlandes
Kirrberger Straße
D-66421 Homburg/Saar

[11] National University of La Plata
Facultad de Ciencias Médicas
Provincia de Buenos Aires, Argentina

[12] Division of Cardiology
University of California, Los Angeles
Los Angeles, CA USA

[13] KG Jebsen Cardiac Research Center and Center for Heart Failure Research,
University of Oslo
Oslo, Norway

[14] Cellular and Molecular Medicine
School of Medicine
The Johns Hopkins University
Baltimore, MD USA

[15]University of Szeged,
Albert Szent-Györgyi Medical and Pharmaceutical Center
Department of Pharmacology and Pharmacotherapy
Szeged, Hungary

[16] Università degli Studi di Milano-Bicocca
Department of Biotechnology and Biosciences
Lombardy, Italy

[*] Correspondence: Colleen E. Clancy, Ph.D.
Department of Pharmacology
University of California, Davis
Genome Building Rm 3503
Davis, CA 95616-8636
Email: ceclancy@ucdavis.edu
Phone: 530-754-0254

INTRODUCTION

In February 2014, a consortium of scientists convened as part of the University of California Davis Cardiovascular Symposium to bring together experimental and mathematical modeling perspectives and discuss points of consensus and controversy on the topic of sodium in the heart [1]. This paper summarizes the topics of presentation and discussion from the Symposium, with a focus on aberrant sodium channels and abnormal sodium homeostasis in cardiac arrhythmias and therapy from cell to the whole heart.

DISRUPTION OF SODIUM HOMEOSTASIS IN CARDIAC DISEASE

Although normal cycling of intracellular Ca^{2+} in cardiac myocytes is often considered a critical indicator of mechanical functioning in the heart, the intracellular Na^+ concentration ($[Na^+]_i$) is tightly coupled to Ca^{2+} homeostasis and is an increasingly recognized modulating force of cellular excitability, frequency adaptation and cardiac contractility [2] [3] [4]. The direct coupling between intracellular Na^+ and Ca^{2+}concentrations is mediated via the Na^+/Ca^{2+} exchanger (NCX), which exchanges 3 Na^+ for each Ca^{2+}, and comprises the primary cellular extrusion mechanism for Ca^{2+}. The NCX can operate in both forward mode, during which it extrudes Ca^{2+}, or may promote Ca^{2+} influx when it operates in the 'reverse mode'~~"~~. The activity of NCX is sensitively tuned to changes in $[Na^+]_i$~~,~~ so that a millimolar increase in the concentration of Na^+_i, resulting from increases in heart rate, sympathetic tone or disease can result in changes to NCX activity that alter Ca^{2+} homeostasis leading to intracellular Ca^{2+} loading in both cellular and sarcoplasmic reticulum (SR) compartments [5]. The consequence of intracellular cardiac myocyte Ca^{2+} loading is stronger contraction [6], but if too much Ca^{2+} loading occurs, as in pathological states, there is potential for increased leak from the ~~higher~~ SR ~~Ca^{2+} load~~, which can result in spontaneous Ca^{2+} waves. If ~~the~~ Ca^{2+} waves are sufficiently large or persistent, the excess intracellular Ca^{2+} will be extruded via the NCX resulting in depolarizing current that may bring the cell to threshold Na channel activation, casing delayed afterdepolarizations (DADs) and arrhythmogenic triggered action potentials. For more detailed description of structural and functional determinants of NCX, please refer the white paper #3 (REF).

An additional important cellular mechanism for maintenance of $[Na^+]_i$~~.~~ homeostasis is the sodium-potassium ATPase (NKA), which uses energy derived from hydrolysis of an ATP molecule, allowing extrusion of three Na^+ ions in exchange for two K^+ ions. The NKA is half-maximally activated

between 10 - 22 mM $[Na^+]_i$ and 1 - 2 mM external K^+ , respectively [7]. Thus, at 4 mM normal extracellular K^+, NKA is ~ 70% saturated, with plenty of available ATP (5-10 mM) (half-maximal NKA activation is 80-150 μ M) [8]. The NKA is covered in detail in white paper #3 (REF).

Cardiac myocytes also contain Na transport mechanisms that promote simultaneous maintenance of $[Na^+]_i$ homeostasis and physiological pH including the sodium-hydrogen exchanger (NHE), which moves sodium into the cell in exchange for proton export [9]. The sodium-bicarbonate symporter (NBC) is also present in myocytes and acts as an additional mechanism to couple $[Na^+]_i$ homeostasis and pH [9]. In disease states, the importance of the coupling between multiple Na homeostatic mechanisms is evident. In ischemia, failure of ion homeostasis starts with an influx of Na^+ through ~~the Na^+/H^+ exchanger~~ (NHE) [10] in attempt to raise the acidified pH (through the extrusion of H^+). In ischemia/reperfusion injury, activation of ~~the N a$^+$/H$^+$ exchanger~~ (NHE) and ~~Na^+/HCO_3^- cotransporter~~ (NBC), a pathological increase in the persistent late Na current component, Na^+ entry through connexin hemichannels, and NKA inhibition results in reverse mode NCX activity that leads to Ca^{2+} overload [2]. During hypoxia, NHE from rabbit ventricular myocytes stimulated at 1 Hz accounted for 39% of the total Na^+ influx (as compared to 5% during normoxia) [11]. Inhibition of the NHE during ischemic episodes attenuated the rise in intracellular Na^+ [12,13]. Along with Na^+ influx via the NHE, a parallel decrease in energy production due to mitochondrial dysfunction and loss of ATP results in reduced Na^+ elimination through the Na^+/K^+ ATPase [14], which further augments intracellular Na^+.

Another key disease state marked by sodium dysregulation is heart failure (HF), where it has been shown that $[Na^+]_i$ is elevated in humans and in numerous animal models [15 6 16 17] and [18]. Elevation of $[Na^+]_i$ in HF may represent a compensatory adaptation that allows for an increase in Ca^{2+} influx via NCX, leading to improved contraction, as a type of physiological "digitalis".

While it is generally agreed upon that $[Na^+]_i$ is increased in many forms of heart ~~diceesease~~ disease, the specific pathways responsible for Na elevation are still a matter of controversy. Increased Na entry through Na channels and Na/H exchanger and reduced Na/K pump activity have been found in various animal models of disease. It could well be that the specific pathway is both species and model-dependent. For example, NKA expression is reduced in failing human myocardium [19], although mRNA levels are unchanged [20]. In the rat, mRNA and protein levels of the primary NKA-α_1 isoform are preserved in most HF models, whereas the protein levels of NKA-α_2 are apparently reduced,

while NKA-α_3 is increased [21]. In rabbit HF models all NKA isoforms have been shown to exhibit reduced protein expression in myocytes [22]. Clearly, a direct causative link between biochemical changes and function cannot be made because of potential confounding factors such as altered protein regulation, function or activity that are not measured with biochemical assays. An example is the differential regulation of NKA in HF, described in detail in White Paper #3 (REF).

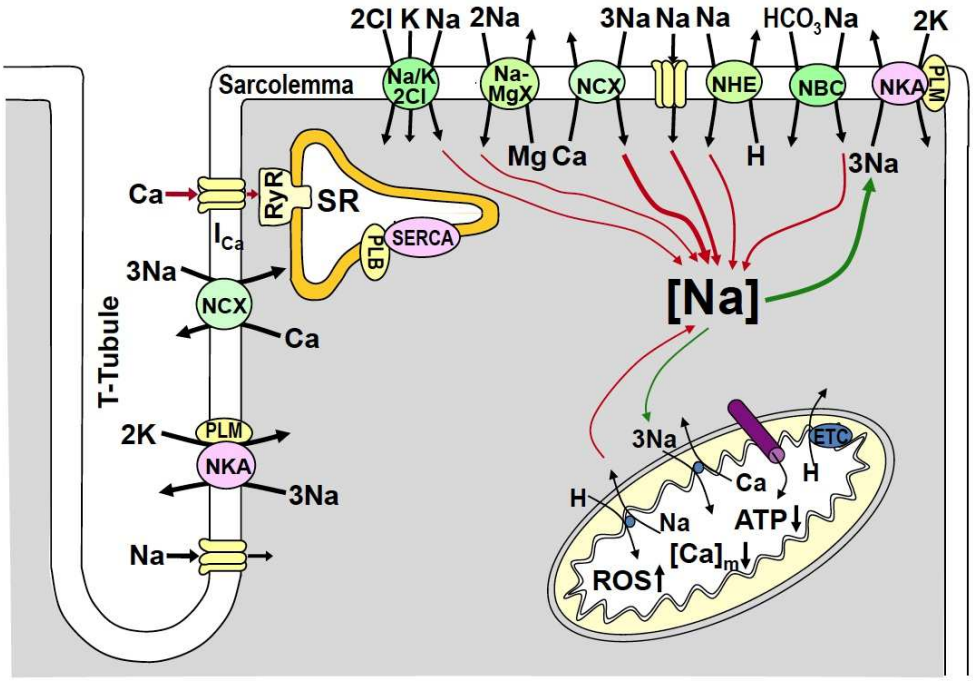

Figure 1: Schematic depiction of Na+ transport processes in the cardiac myocyte. From [2].

Larger Na^+ influx by other mechanisms has also been proposed to increase $[Na^+]_i$ in HF. For example, a TTX sensitive diastolic Na influx was observed to be upregulated in rabbits with pressure and volume-overload induced HF [15]. NHE upregulation has also been documented in HF [16]. Na^+ influx also occurs as a result of a gain of function of the Na^+ channel in the form of the non-inactivating late component of the Na^+current (I_{NaL}) that will be detailed in the following sections.

Elevated $[Na^+]_i$ is linked to disruptions in cardiac energetics and metabolism

Although increased $[Na^+]_i$ may improve the contractile function of the diseased heart, elevated $[Na^+]_i$ may have a pathological impact on cardiac metabolism and oxidative state. For example, the increase in $[Na^+]_i$ and reverse mode NCX mediated Ca^{2+} influx during the cardiac action potential is energetically less efficient than normal SR Ca^{2+} release and may contribute to a mismatch between energy supply and demand in the failing heart. [23]. Furthermore, it has been shown that when intracellular Ca^{2+} transients were triggered by NCX mediated Ca^{2+} entry, the efficiency of mitochondrial Ca^{2+} uptake was substantially reduced, suggesting reduced efficiency in the transport mechanism necessary to drive Ca^{2+}-induced stimulation of Krebs cycle processes [24]. An interesting point to consider and that needs to be clarified is whether this mitochondrial calcium uptake is rapid enough to track changes in intracellular sodium and calcium during systole and diastole.

In failing cardiac myocytes, increased $[Na^+]_i$ impairs energy supply-and-demand matching by promoting acceleration of mitochondrial Ca^{2+} efflux, via the mitochondrial Na^+/Ca^{2+} exchanger (NCLX), which extrudes Ca^{2+} from the mitochondria in exchange for Na^+ [25]. Increases in $[Na^+]_i$ have also been shown to cause an increase in mitochondrial H_2O_2 formation in normal and failing cardiac myocytes [26] that may additionally aggravate the Na^+-induced depletion of the antioxidative capacity and exacerbate oxidative stress in the failing heart [25].

ABERRATIONS IN SODIUM CHANNEL FUNCTION IN CARDIAC DISEASE

In addition to changes in $[Na^+]_i$ homeostatic mechanisms in the heart, changes to the distribution and function of cardiac Na channels have been linked to disease manifestation and progression in inherited and acquired cardiac arrhythmias. Either gain- and loss-of-function results, depending on the disease state, and both disruptions can result in dangerous proarrhythmic consequences arising from alterations in cardiac conduction and repolarization.

Loss of Na channel function

In the case of loss of Na channel function, either a result of disease-induced remodeling or as a result of drug application, reduced Na current can lead to insufficient cellular excitability to allow propagation of electrical waves, leading to a well-known precursor to reentrant arrhythmias - conduction block.

One instance of remodeling of Na channels that may play a critical role in arrhythmogenesis is in the infarct border zone where the electrical substrate is extensively remodeled compared to normal non-infarcted epicardium. The fact that progressive electrical remodeling that occurs in chronic disease states has been identified as a biomarker for sudden cardiac death, indicates the critical importance of revealing its mechanisms [27] [28]. Na^+ currents (as well as Ca^{2+}, and K^+ currents) in cells isolated from the epicardial border zone (EBZ) of 5-day infarcted hearts have been shown to have both altered current amplitudes and changes in kinetics [29] [30] [31] [32]. Within the reentrant circuit, two distinct cell regions have been identified, cells from the central common pathway of the circuit (IZc), and cells from the outer pathway on the other side of the line of block (outer pathway, IZo) [33]. Cells from both regions of the infarct zone regions had reduced Na^+ current density, but the cells from the IZo also exhibited slower Na^+ channel kinetics for time to peak and current decay [29]. These changes in Na channel function along with some observed changes to L-type Ca^{2+} currents give rise to electrical anisotropy that promotes stable lines of block within the zone [34]. These stable lines of block then allow for development of sustained reentrant excitation and stable ventricular tachycardias (VTs) in ~~the epicardial border zone~~ (EBZ).

If regional differences of ionic currents in cells of the EBZ are the mechanisms underlying the lines of block observed in the EBZ, then restoration of either the Na^+ channel or the L-type Ca^{2+} channel should be antiarrhythmic by disrupting the stability of the lines of block leading to termination of reentry. Indeed, recent studies [35] suggest that gene transfer mediated overexpression of the skeletal muscle sodium channel (SkM1), resulted in improved Na channel availability since SkM1 channels have positively shifted kinetics of inactivation rendering them primarily open at depolarized potentials at which cardiac Na channels are closed. SkM1 overexpression improved conduction and reduced the incidence of inducible VT/VF post-myocardial infarction [36]. Such approaches constitute the potential for new development of strategic interventions to restore electrical disruptions in the heart arising from electrically based remodeling.

Gain of Na channel function

Many recent works have focused on gain-of-function of the Na^+ current since a range of cardiac diseases are marked by pathological increases in the persistent late Na current component (late Na current, I_{NaL}) that follows the rapid transient activation of I_{Na}. I_{NaL} is upregulated in many pathologic conditions, such as in the failing and/or ischemic heart, in the heart exposed to oxidative stress, and in hearts of patients with congenital long QT3 syndromes ~~[37] [38] [39] [40] [41] [42] [43]~~ [37-43] Ca^{2+} dysregulation

results in pathological effects to promote ~~late I_{Na} (I_{NaL})~~ [44,45] via activation of CaMKII [46], increased mitochondrial oxidative phosphorylation [47] and consequent increased ROS [47,48]. Please see White Paper #2 (REF) for detailed coverage of Na channel regulation. Increased I_{NaL} leads to action potential prolongation, disruption of normal cellular repolarization, development of arrhythmia triggers, and propensity to ventricular arrhythmia. In heart failure, pharmacological targeting of I_{NaL} has been shown to result in: 1) normalization of repolarization; 2) decrease in beat-to-beat APD variability; and 3) improvement in Ca^{2+} handling and contractility [49-52].

At least three distinct alterations in $Na_V1.5$ gating have been shown to increase in I_{NaL} including window currents, differential gating modalities, and non-equilibrium gating. These mechanisms were initially revealed via detailed electrophysiological study of mutations in *SCN5A* that resulted in Long QT Type 3 Syndrome in patients. Window current describes the Na current that is measurable in the voltage range where the steady state inactivation curve and activation curve overlap [53-55]. The current can be observed within the "window" of voltage during cardiac repolarization or as a steady-state equilibrium current during voltage clamp. The window can be affected by changes to the activation and inactivation gating that result in expansion of the voltage range. In addition to mutations and polymorphisms, there have been a slew of physiological modulators identified such as Ca^{2+}, calmodulin, and phosphorylation (discussed in White Paper #2) that in normal physiology and in pathological conditions increase the size of the window [54].

The bursting of Na channels is a well-described gating mode where channels undergo a transient failure of channel inactivation. Maltsev and Undrovinas recorded I_{NaL} from heterologously expressed $Na_V1.5$ in the absence of other isoforms [40], showing that bursting channels were indeed of the same form as those underlying the transient inward current. Clancy et al. recorded and modeled the transitions from normally inactivating Na^+ channels to bursting channels in heterologously expressed single $Na_V1.5$ sodium channels. A computational model based on these rates was then used to predict the magnitude and rate dependence of I_{NaL} expected from ensemble currents [56].

Non-equilibrium gating describes another form of I_{NaL} that is not observed during typical voltage clamp depolarization protocols [57]. However, in response to a negative ramp current, a transient inward current is observed. The amplitude of the current if sensitively dependent on the rate of recovery from inactivation, where faster recovery or a shift in the voltage dependence of recovery from inactivation promotes the current. Just as for the window current, non-equlibrium Na current is affected by a

number of physiological modulators including calmodulin and phosphorylation (discussed in White Paper #2).

Intrinsic gating abnormalities of the cardiac Na^+ channel~~,~~ were reported by Maltsev and Undrovinas in their description of a novel, ultraslow inactivating Na current, I_{NaL}, in both normal and failing human hearts [39]. The same group also showed an increased density and slower inactivation kinetics of I_{NaL} [58] in chronic heart failure as compared to normal hearts. In single channels two modes of gating underlying late I_{Na} were observed, late scattered mode gating and burst mode of gating that had slower kinetics in failing human ventricular myocytes compared to normal ventricular myocytes [40]. Importantly, there were no differences in the unitary conductance of late Na^+ current between normal and failing human hearts, suggesting a single population of channels that are upregulated in HF [59].

LINKING Na^+ AND Na^+ CHANNEL ABNORMALITIES TO ARRHYTHMIAS

Disruptions in Na based processes in the heart foster arrhythmias by multiple mechanisms, depending on the specific perturbation to the Na^+-linked process. Of major benefit to revealing and understanding the mechanisms of Na based arrhythmias is the development of numerous new experimental techniques including examples such as targeted subcellular imaging [60-63], SR Ca^{2+} imaging [64], advances in electrophysiology [65], "cell-in-gel" and other techniques for mechanotransduction [66 67], mitochondrial imaging [68], stem cell technologies [69], just to name a few.

In addition to the developments in experimental techniques, there have been dramatic gains in accessibility of modern computing power, computational speed and reduction in computing cost. Recent advances have also been made in numerical techniques and computing~~[70,71] [72,73]~~ 70-73, the implementation of customizable solvers such as Continuity [74], modeling platforms like CHASTE and OpenCMISS [75,76], and infrastructures aimed at facilitating standardization, interoperability and dissemination of models (e.g CellML and FieldML)~~[75] [76] [77] [78] [79]~~ 75-79.

Mathematical models of cardiac physiology are widely used to complement experimental findings and clinical observations to improve understanding of cardiac electrical function in health and disease. Implementation of such models offers multiple advantages, especially that they enable exploration of high dimensional models to determine how their range of dynamical behaviors corresponds to that of

low dimensional models. Emergent behaviors can be mapped back to underlying parameters through component dissection, to reveal mechanisms of emergent behaviors, a function for which there is no efficient comparable experimental counterpart.

Experimental approaches and computational modeling and simulation are complementary methods to determine how abnormalities in Na processes at the level of the cell can cause emergent arrhythmias in the whole heart. An example is a hallmark arrhythmia trigger in human heart failure resulting from Ca^{2+}-induced ~~delayed afterdepolarizations (~~DADs~~)~~. When Na^{+} accumulation and overload occurs in cells, DADs arise because the resulting cytosolic Ca^{2+} accumulation via reverse-mode NCX may ultimately exceed Ca^{2+} efflux and precipitate Ca^{2+} overload. A pathological version of the Ca^{2+}-induced-Ca^{2+} release ensues, whereby spontaneous SR Ca^{2+} release leading to overloaded intracellular Ca^{2+} that is extruded by NCX, which may depolarize the cell sufficiently to activate Na channels leading to the emergence of ~~delayed afterdepolarizations (~~DADs~~)~~ and, if large enough, arrhythmogenic triggered action potentials. Because Na^{+} mediated Ca^{2+} overload does not occur uniformly in time or space, beat-to-beat variability in repolarization and emergent triggering early afterdepolarizations (EADs) and DADs occur unpredictably.

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DADs occurring in a single myocyte are an insufficient source of current to trigger a premature beat in the whole heart because the current generated in single cell is not enough to overcome the large electrotonically coupled downstream sink of tissue. Mathematical models have shown that DADs must occur simultaneously in many cells in order to generate an arrhythmia trigger [80].

In the case of loss of Na channel function as described in infarct border zone, a reduction in excitability at the cellular level emerges in coupled tissue as slowing of conduction velocity of the propagating depolarizing wave that drives cardiac excitation. Slow conduction can result in an increase in the "vulnerable window" to unidirectional block and, if the conditions are favorable, retrograde conduction, promoting reentrant arrhythmia in the organ [81, 82 81-83 84 85,86]. It is important to note that slow conduction is enough to prolong action potential duration (APD) at the cellular level and QT interval at the organ level as a result of the intrinsic dynamical properties of Na channels that give rise to the restitution relationship. The restitution relationship describes the correlation between APD and the preceding diastolic interval (DI). As the DI increases as a result of slow conduction, the subsequent AP will be relatively prolonged. If the D~~i~~I is sufficiently long and other anomalies are present, reductions in repolarization reserve occur and even triggered arrhythmias such as ~~early~~

~~afterdepolarizations (~~EADs~~)~~ may emerge [87 88 49 53 89]. Conversely, when the DI is very short, such as during rapid pacing or tachycardia and combined with other perturbations such as drugs or disease, the relationship between APD and DI may be very steep. In this situation, arrhythmogenic oscillation of the APD termed alternans can develop. All of these disruptions to normal cardiac electrical activity ~~Hi~~ promote development of reentrant arrhythmias and wavebreak causing fibrillation[90].

A gain of function of the Na channel during disease that results in an increase ~~L~~late Na^+ current has also been linked to arrhythmias associated with acquired diseases such as heart failure and post MI remodeling, due to their impact on action potential duration and repolarization abnormalities. Approximately 40% of chronic heart failure patients die due to sudden cardiac death, with ventricular tachycardia and fibrillation documented in 80% of patients [49,91,92]. Conditions and diseases that lead to an increased late I_{Na} exhibit electrical instability (due to afterdepolarizations, beat-to-beat variability in repolarization, ventricular arrhythmias), mechanical instability (impaired diastolic relaxation and ventricular wall tension, increased diastolic and decreased systolic force generation), as well as mitochondrial dysfunction [47]. This sets up a cascade leading to further ischemia and abnormal contraction in a pathological feedback loop. Failing canine ventricular myocytes with prolonged APs, Ca^{2+} transients and substantial diastolic Ca^{2+} accumulation leading to spontaneous Ca^{2+} release were shown to improve with TTX and ranolazine (a selective I_{NaL} blocker) [93-95]. These results are additional strong indication of the link between pathological I_{NaL} to the induction of deranged Ca^+ homeostasis at the cellular level. A subsequent study using human ventricular myocytes [39] similarly found improvement with TTX.

NEW THERAPEUTIC APPROACHES FOR NA LINKED ARRHYTHMIAS

As described above, both gain- and loss-of-function in the cardiac Na channel can result in dangerous proarrhythmic consequences by altering cardiac conduction and repolarization. Thus, the prospect of targeted pharmacological treatment to modify Na^+ based arrhythmias has fueled historical and recent pursuit of new drugs. However, the history of antiarrhythmic drug failures makes careful and reliable assessment of drug effects on cardiac rhythms a preclinical necessity to ensure safety and efficacy.

The difficulty in predicting drug effects on the electrical activity of the heart is clear from both the failure of large clinical trials to demonstrate drug safety for multiple antiarrhythmic drug classes (for example, the CAST [96] and SWORD [97] clinical trials), and from the market withdrawal of otherwise

promising drugs for treating cardiac dysrhythm, psychiatric disorders, gastrointestinal symptoms and infection following unexpected sudden cardiac death [98]. These events have resulted in a burdensome regulatory process for preclinical drugs that have prevented emergence of potentially therapeutic agents for clinical use.

The reasons that it has been so difficult to predict ion channel targeting drug effects on cardiac electrical activity are that most antiarrhythmic drugs have complex interactions with multiple channels, conformational state specificity, bioactive metabolites and neutral and charged drug fractions. Drugs alter the action potential waveform, which in turn affects drug potency. Thus, it is extremely difficult to know how intended antiarrhythmic drugs that primarily target ion channels will alter emergent electrical activity in the whole heart. Recently, the FDA and other stakeholders have suggested the potential implementation of a new paradigm for cardiotoxicity testing that includes implementation of complementary developments in computational modeling and simulation approaches and stem cell technologies [99].

Modeling and simulations for predictive pharmacology

Cardiac modeling and simulation has recently been utilized to investigate mechanisms of Na^+ channel blocking drugs that both reduce peak Na^+ current and that specifically target the late Na^+ current. A recent study by Cardona et al. investigated lidocaine effects in a multiscale computational model[100]. The authors demonstrated both anti-fibrillatory effects in normal tissue and predicted the potential for proarrhythmia with lidocaine during pathologies including acidosis and ischemia.

Moreno et al. [84] also implemented modeling and simulation approaches to investigate the mechanisms of failure of the once promising antiarrhythmic drug flecainide, the subject of the cardiac arrhythmia suppression trial (CAST), which, in the clinical trial startlingly showed increased mortality with flecainide over placebo. In the computational modeling and simulation study, the dynamical complexity of the drug kinetics was modeled for both charged and neutral drug fractions. After developing the drug-channel model, a simulation in cells first confirmed experimental findings: no overt proarrhythmic potential was ever observed at the cellular level [84]. *In tissue level simulations, the outcome was dramatically different.* Substantial use-dependent block by flecainide (an intrinsic dynamical property of channel block) was predicted in the model to result in failed impulse conduction, a higher dimensional phenomenon that emerged as a result of increased electrotonic load

in coupled tissue. Proarrhythmic conduction block led to development of tachycardia indicated by spiral wave reentry, which was verified experimentally [84]. This emergent phenomenon was linked back to the fundamental mechanism - the drug kinetics of unblock, identified as the basic mechanism of failure. Moreover, the study indicated that the kinetics of drug interactions for lidocaine promoted safety in higher dimensions as indicated by no reentrant arrhythmias in the presence of lidocaine in normal tissue.

Disease induced enhancement of late I_{Na} promotes the development of arrhythmogenic after-depolarizations, triggered arrhythmic activity, and torsades de pointes (TdP) in cardiac ventricular myocytes, cardiac tissue, and intact hearts [87 88 101 102 103]. Pharmacological targeting of I_{NaL} has been shown to improve cardiac electrical function in myocytes challenged by cardiac glycosides, hydrogen peroxide, pharmacological enhancement of late I_{Na}, and even with drugs that block hERG (I_{Kr}) and reduce repolarization reserve [104 105 106 88 101 41 102 42 49 107].

Recently, modeling and simulation have been used to probe and predict effects of the selective I_{NaL} inhibitor ranolazine in pathological situations [108]. Simulations of clinically relevant concentrations of drug were used to predict the cellular level effects of Na^+ channel blockade using both ranolazine and its active metabolites on hERG, which have potent blocking effects in the therapeutically relevant range. The model was used to predict if therapeutic effects of targeted pharmacological treatment by ranolazine prevailed over the unintended pathological block of hERG for normalizing arrhythmia triggers (EADs) in bradycardia-dependent arrhythmias in LQT3, as well tachyarrhythmogenic triggers arising from heart-failure induced remodeling (e.g. DADs). Model predictions suggested that acute targeting of late I_{Na} with ranolazine can be an effective therapeutic strategy in diverse arrhythmia provoking situations that arise from a common pathway of increased pathologic late I_{Na}.

Trenor et al. developed a tool for *in-silico* preclinical anti-arrhythmic drug safety assessment, that predicted the impact of I_{Kr}/I_{NaL} ratio of steady-state block of drug candidates on "torsadogenic" biomarkers that they defined as AP duration, triangulation, reverse rate-dependence, transmural dispersion of repolarization and electrocardiogram QT interval [109].

Although the studies described above included detailed descriptions of the kinetics of drug interactions with ion channels, it is important to note that even detailed kinetic models are phenomenological - for example, a Markov model of ion channel dynamics or drug channel interactions is a phenomenological

representation that greatly simplifies the underlying molecular quantum mechanics. In the studies described above, there is no way for example to predictively link atomic scale anomalies to higher order phenomenon, or to predict how structural perturbations might affect pharmacological effects in the whole heart. An important aspect of modeling and simulation as it relates to prediction of disease processes and pharmacology is choosing the level of detail in the model. There must be a match between the required complexity of the model and its predictive capacity, so as not to introduce unnecessary degrees of freedom that result in vastly over determined models. In other words, the modeler must be concerned with the issue of determining how to make the model "as simple as possible, but not simpler."

Stem cells for predictive pharmacology

The potential for personalized medicine *via* drug screening in patients' own induced pluripotent stem (hiPSC) cell-derived cardiomyocytes (hiPSC-CMs) [110] is another developing and exciting application at the interface of molecular and clinical information [111,112]. Patient-specific hiPSC-CMs containing unknown genotype profiles, or with known polymorphisms and/or mutations in cardiac ion channels can be used to qualitatively and quantitatively assess variability in drug responses [69]. Thus far, iPSC-CMs have been used to successfully model arrhythmic disorders, with excellent agreement between altered cardiac channel function and emergent electrophysiological phenotypes in the inherited long QT syndromes and catecholaminergic polymorphic ventricular tachycardia [112].

Terrenoire et al. recently demonstrated the usefulness of such an approach in a study where they derived iPSCs from a long QT syndrome patient with complex genetics [113]. They identified a *de novo* mutation in the SCN5A (F1473C) gene encoding the $Na_V1.5$, and a polymorphism (K897T) in KCNH2, the gene encoding hERG. Biophysics and molecular pharmacological analysis of ion channels expressed in iPSC-CMs demonstrated that the disease was primary consequence of the $Na_V1.5$ defect and was not influenced by the KCNH2 polymorphism. The mutation resulted in a gain-of-function in I_{NaL}, which resulted in delayed repolarization, a prolonged QT interval, and increased risk of arrhythmia. They also found a uniquely steep fast rate–dependent reduction in I_{NaL} that especially facilitated pharmacological inhibition by the Na channel blocker mexiletine. Of critical importance, the experiments revealed rate-dependent properties of ion currents and drug interactions that were unique to the patient's iPSC-CMs, and that were corroborated in a successful patient treatment regimen. This study is an example for the potential of iPSC-CMs approaches in developing patient-specific clinical regimens [113].

While the study described above focused on a gain of function perturbation in the Na channel, and its cellular level effects, iPSC-CMs can also be cultured in monolayer or grown on scaffolds to investigate patient specific metrics related to loss of Na channel function, especially conduction velocity. For example, measurements of voltage wavefronts in monolayers of iPSC-CMs via optical mapping ~~with~~ has recently been de~~e~~monstrated [114].

The potential for expansion of stem cell technologies in the cardiac therapeutic arena is vast [111,115]. For example, these cells may prove extremely useful to reveal some of the most basic variations in drug responses that might include the influence of sex, polytherapy, hormones, of course drug effects in the context of genetically based diseases. Examples include the apparent differential effects of hERG blockade in males and females, oral contraceptive effects on cardiac risk, or to determine the electrophysiological effects of beta-blockers in LQT-3 patients [116] [117] [118]. In order for stem cell~~s~~ technologies to enter the mainstream for screening and therapy, best practices are in development to improve maturity and homogeneity of electrical activity in iPSC-derived myocytes [119] [120].

SUMMARY

Understanding how disruption in cardiac Na^+-based processes leads to derangement in multiple cardiac components at the level of the cell and to then connect these perturbation to emergent behavior in the heart to cause is a critical area of research. The ubiquity of disruption of sodium channels and sodium homeostasis in cardiac disorders of excitability and mechanics emphasizes the importance of fundamental understanding of the associated mechanisms and disease processes to ultimately reveal new targets for human therapy.

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