

WHITE PAPER

Deranged sodium to sudden death

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Abstract In February 2014, a group of scientists convened as part of the University of California Davis Cardiovascular Symposium to bring together experimental and mathematical modelling perspectives and discuss points of consensus and controversy on the topic of sodium in the heart. This paper summarizes the topics of presentation and discussion from the symposium, with a focus on the role of aberrant sodium channels and abnormal sodium homeostasis in cardiac arrhythmias and pharmacotherapy from the subcellular scale to the whole heart. Two following papers focus on Na⁺ channel structure, function and regulation, and Na⁺/Ca²⁺ exchange and Na⁺/K⁺ ATPase. The UC Davis Cardiovascular Symposium is a biannual event that aims to bring together leading experts in subfields of cardiovascular biomedicine to focus on topics of importance to the field. The focus on Na⁺ in the 2014 symposium stemmed from the multitude of recent studies that point to the importance of maintaining Na⁺ homeostasis in the heart, as disruption of homeostatic processes are increasingly identified in cardiac disease states. 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 perturbations to emergent behaviour in the heart to cause disease is a critical area of research. The ubiquity of disruption of Na⁺ channels and Na⁺ homeostasis in cardiac disorders of excitability and mechanics emphasizes the importance of a fundamental understanding of the associated mechanisms and disease processes to ultimately reveal new targets for human therapy.

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Abbreviations AP, action potential; APD, action potential duration; DAD, delayed after-depolarization; EAD, early after-depolarization; EBZ, epicardial border zone; HF, heart failure; I_{Na} , Na^+ current; I_{NaL} , late Na^+ current; iPSC-CMs, induced pluripotent stem cell-derived cardiomyocytes; LQT3, long QT type 3 syndrome; NBC, sodium–bicarbonate symporter; NCX, sodium–calcium exchanger; NHE, sodium–hydrogen exchanger; NKA, sodium–potassium ATPase; ROS, reactive oxygen species; SR, sarcoplasmic reticulum.

Disruption of sodium homeostasis in cardiac disease

In the session on 'Disruption of Na^+ homeostasis, Sanda Despa, Brian O'Rourke, Donald Bers, Alicia Mattaizzi, William Louch, Christoph Maack and Sridharan Rajamani were presenters, discussion leaders and panelists. Below is a summary of the sessions and discussion.

Although it is well known that the normal cycling of intracellular Ca^{2+} in cardiac myocytes is critical for the normal electrical and mechanical functioning of the heart, the intracellular Na^+ concentration ($[Na^+]_i$) is tightly coupled to Ca^{2+} homeostasis and is increasingly recognized as a modulating force of cellular excitability, frequency adaptation and cardiac contractility (Faber & Rudy, 2000; Grandi *et al.* 2010; Despa & Bers, 2013; Fig. 1). The direct coupling between intracellular Na^+ and Ca^{2+} concentrations is mediated via the sodium–calcium exchanger (NCX), which exchanges 3 Na^+ for each Ca^{2+} , and comprises the primary cellular extrusion mechanism for Ca^{2+} (Blaustein & Lederer, 1999). The NCX can operate in both forward mode, during which it extrudes Ca^{2+} , or can mediate 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 $[Na^+]_i$ resulting from changes in the ion influx/efflux balance, 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 (Maack *et al.* 2006). A consequence of intracellular cardiac myocyte Ca^{2+} loading is stronger contraction (Pieske *et al.* 2002), but NCX-dependent Ca^{2+} loading may compromise the stability of the SR store, thus facilitating larger Ca^{2+} transients and/or spontaneous Ca^{2+} waves as in digitalis toxicity (Despa & Bers, 2013). If Ca^{2+} waves or transients are sufficiently large, the excess intracellular Ca^{2+} will be extruded via the NCX resulting in depolarizing current that may bring the membrane potential of the cell to the voltage threshold required for activation of the Na^+ channel, causing delayed after-depolarizations (DADs) and arrhythmogenically triggered action potentials (Despa & Bers, 2013). For a more detailed description of structural and functional determinants of NCX, please refer to the white paper no. 3 (Shattock *et al.* 2015).

An additional important cellular mechanism for the 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 and 22 mM $[Na^+]_i$ and between 1 and 2 mM external K^+ (Glitsch, 2001). 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) (Hilgemann *et al.* 1991). The regulation and function of NKA is covered in detail in white paper no. 3 (Shattock *et al.* 2015).

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 (Leem *et al.* 1999). The sodium–bicarbonate symporter (NBC) is also present in myocytes and acts as an additional mechanism to couple $[Na^+]_i$ homeostasis and pH (Leem *et al.* 1999).

In disease states, the importance of the proper coupling between multiple Na^+ homeostatic mechanisms is evident. In ischaemia, failure of ion homeostasis starts with an influx of Na^+ through the NHE (Eigel & Hadley, 1999) in an attempt to raise the acidified pH (through the extrusion of H^+). In ischaemia/reperfusion injury, activation of the NHE and NBC, a pathological increase in the persistent/late Na^+ current (I_{NaL}), Na^+ entry through connexin hemichannels (at least in isolated myocytes) (Kondo *et al.* 2000), and NKA inhibition result in reverse mode NCX activity that leads to Ca^{2+} overload (Despa & Bers, 2013). During hypoxia, NHE in rabbit ventricular myocytes has been shown to dominate total Na^+ influx (Bers *et al.* 2003). Inhibition of the NHE during ischaemic episodes attenuates the rise in intracellular $[Na^+]_i$ (Avkiran, 2003; Baetz *et al.* 2003). 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 (Hale *et al.* 2008), which further augments $[Na^+]_i$. The above examples show how disruptions to $[Na^+]_i$ homeostasis are evident in multiple disease states

and occur via distinct mechanisms that can lead to a pathological cascade through coupled Na^+ regulatory mechanisms that ultimately leads to disruptions in Ca^{2+} homeostasis.

Another key disease state marked by intracellular sodium dysregulation is heart failure (HF), where it has been shown that $[\text{Na}^+]_i$ is elevated in humans and numerous animal models (Despa *et al.* 2002; Pieske *et al.* 2002; Baartscheer *et al.* 2003; Schillinger *et al.* 2006; Louch *et al.* 2010). Elevation of $[\text{Na}^+]_i$ 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'. However, increased $[\text{Na}^+]_i$ has also been associated with diastolic dysfunction, as it impairs Ca^{2+} removal by NCX (Louch *et al.* 2010, 2012) and with energetic defects and oxidative stress (Kohlhaas *et al.* 2010; Bay *et al.* 2013; Nickel *et al.* 2014), of which the latter may contribute to the progression of the disease through inducing cell death and maladaptive remodelling (Nickel *et al.* 2014).

Question or controversy: sodium dysregulation

While there is little doubt that the sodium dysregulation is important in numerous cardiac pathologies, a detailed understanding of the precise mechanisms is missing.

Which are the critical pathways to Na^+ overload in each disease?

How does disease-linked remodelling alter ion homeostatic mechanisms?

Which disease-linked changes in Na^+ processes are compensatory?

While it is generally agreed upon that $[\text{Na}^+]_i$ is increased in many forms of heart disease, the specific pathways responsible for the increase in intracellular Na^+ in some diseases are still a matter of controversy. Increased Na^+ entry through Na^+ channels and NHE and decreased Na^+ extrusion by reduced NKA activity have been reported for 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 (Schwinger *et al.* 1999), although mRNA levels are unchanged (Allen *et al.* 1992). 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 (Verdonck *et al.* 2003). In rabbit HF models all NKA isoforms have been shown to exhibit reduced protein expression in myocytes (Bossuyt *et al.* 2005). 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 here is the differential regulation of NKA by phospholemman (PLM) in HF, described in detail in white paper no. 3 (Shattock *et al.* 2015). Moreover, there are important changes in localization of NKA isoforms during heart failure. Swift *et al.* showed altered expression patterns of NKA α_1 and NKA α_2 isoforms, which were linked to altered Ca^{2+} extrusion from cardiomyocytes and consequent changes in contractile parameters (Swift *et al.* 2008).

A large Na^+ influx via other mechanisms has also been proposed to increase $[\text{Na}^+]_i$ in HF. For example, a TTX-sensitive diastolic Na^+ influx (implicating involvement of the voltage-gated Na^+ channel, although the specific isoform is unclear) was observed to be upregulated in rabbits with pressure and volume overload-induced HF (Despa *et al.* 2002). NHE upregulation has also been documented in HF (Baartscheer *et al.* 2003). Acidosis was also shown to promote Na^+ loading in the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) knockout mouse (Li *et al.* 2012). Na^+ influx also occurs as a result of a gain of function of the Na^+ channel in the form of the non-inactivating component I_{NaL} that will be detailed in the following sections.

Question or controversy: Na^+ handling in disease

How do various isoforms of Na^+ transport processes contribute to function or disease?

How does modification to individual Na^+ transport isoforms alter function?

Do changes measured in animal models reflect human adaptive processes?

In summary, while dynamic beat-to-beat changes in $[\text{Na}^+]_i$ allow physiological adaptation in non-diseased cardiac myocytes, perturbations to Na^+ homeostasis occur through a variety of mechanisms in cardiac disease states. Disease-induced disruption to $[\text{Na}^+]_i$ maintenance occurs because the pumps and exchangers described above are unable to adequately compensate for pathological Na^+ loading without a consequent disruption in Ca^{2+} homeostasis. Not only does Ca^{2+} overload have the potential to disrupt electrical activity in the heart, it also has detrimental effects on cardiac energetics and metabolism.

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

Although increased $[\text{Na}^+]_i$ may improve the contractile function of the diseased heart, elevated $[\text{Na}^+]_i$ may have a pathological impact on cardiac metabolism and oxidative state. For example, the increase in $[\text{Na}^+]_i$ 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 (Weisser-Thomas *et al.* 2003). Furthermore, when intracellular Ca^{2+} transients were triggered by NCX-mediated Ca^{2+} entry, the efficiency of mitochondrial Ca^{2+} uptake is substantially reduced, suggesting reduced efficiency in the transport mechanism necessary (of NCX vs. Ca^{2+} current and SR Ca^{2+} release) to drive Ca^{2+} -induced stimulation of Krebs cycle processes (Kohlhaas & Maack, 2010). An interesting point to consider and that needs further clarification is what is the magnitude of mitochondrial Ca^{2+} uptake occurring on each heartbeat and how do kinetics of uptake, release and buffering impact cytoplasmic systolic and diastolic Ca^{2+} signals.

In failing cardiac myocytes, increased $[\text{Na}^+]_i$ impairs energy supply-and-demand matching by promoting acceleration of mitochondrial Ca^{2+} efflux, via the mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger (mNCE, or NCLX), which extrudes Ca^{2+} from the mitochondria in exchange for Na^+ (Liu & O'Rourke, 2008; Palty *et al.* 2010). This effect, together with the decreased amplitude of cytosolic Ca^{2+} transients in HF, blunts mitochondrial Ca^{2+} signalling to the Krebs cycle during increased work, resulting in oxidation of the pyridine nucleotide pool (NAD(P)H), thus further compromising NADH supply for oxidative phosphorylation (Liu & O'Rourke, 2008). Additionally, since NADPH is required to maintain matrix antioxidant pathway flux, its oxidation causes cellular overload of reactive oxygen species (ROS) (Kohlhaas *et al.* 2010; Liu *et al.* 2010). ROS accumulation then contributes to oxidative modification of Ca^{2+} handling and ion channel targets to promote arrhythmias. This cascade of failures, stemming from $[\text{Na}^+]_i$ overload, is thus hypothesized to provoke triggered arrhythmias (Liu *et al.* 2010), which, in the context of the altered electrophysiological substrate in HF, may induce sudden cardiac death (SCD). Interestingly, chronic inhibition of the mNCE during the induction of HF prevents these mitochondrial defects, and abrogates cardiac decompensation and sudden death, in a guinea pig model of HF/SCD (Liu *et al.* 2014).

Question or controversy: energetics and metabolism

How much mitochondrial Ca^{2+} uptake occurs on each heartbeat?

How do the kinetics of uptake, release and buffering influence cytoplasmic systolic and diastolic Ca^{2+} signals?

Abnormal sodium channel function in cardiac disease

In the session on 'Na⁺-induced arrhythmias: from the cellular level to the whole heart', Penelope James Weiss, Ole Sejersted, Jonathan Lederer, Zhilin Qu and Livia Hool

comprised the group of presenters, discussion leaders and panelists. A summary of the session and discussion is below.

In addition to changes in $[\text{Na}^+]_i$ homeostatic mechanisms in the heart, changes to the distribution and function of cardiac Na^+ channels have been linked to disease manifestation and progression of both inherited and acquired cardiac arrhythmias. Either gain or loss of Na^+ channel function can result, depending on the disease state, and both disruptions can lead to 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 as a result of disease-induced remodelling or as a result of drug application, reduced Na^+ current (I_{Na}) can cause insufficient cellular excitability to allow propagation of electrical waves, leading to a well-known requirement for some types of re-entrant arrhythmias: conduction block.

One instance of remodelling of Na^+ channels that plays a role in conduction block and arrhythmogenesis is in the infarct border zone where the electrical substrate is extensively remodelled compared to normal non-infarcted epicardium. The fact that progressive electrical remodelling that occurs in chronic disease states has been identified as a biomarker for sudden cardiac death indicates the critical importance of revealing its mechanisms (Gaudron *et al.* 2001; Goldberger *et al.* 2014). 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 (Lue & Boyden, 1992; Aggarwal & Boyden, 1995; Pu & Boyden, 1997; Dun *et al.* 2004) consistent with reduced cellular action potential (AP) amplitudes and upstroke velocity and prolonged post-repolarization refractoriness. Within the re-entrant 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; Baba *et al.* 2005). Cells from both 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 (Pu & Boyden, 1997). These changes in Na^+ channel function along with observed changes to L-type Ca^{2+} currents give rise to electrical anisotropy that promotes stable lines of block within the zone (Cabo & Boyden, 2003). These stable lines of block then allow for the development of sustained re-entrant excitation and stable ventricular tachycardias in the EBZ (Janse & Wit, 1989).

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 availability, and thereby normal function, should be antiarrhythmic by disrupting the stability of the lines of block leading to termination of re-entry. Indeed, recent studies (Coronel *et al.* 2010) suggest that gene transfer-mediated over-expression of the skeletal muscle sodium channel SkM1 resulted in improved Na^+ channel availability in cells of the EBZ because SkM1 channels have positively shifted kinetics of inactivation rendering them available to open at depolarized potentials at which cardiac Na^+ channels are not available. SkM1 overexpression improved conduction and reduced the incidence of inducible ventricular tachycardia/ventricular fibrillation (VT/VF) post-myocardial infarction (Boink *et al.* 2012). Such approaches constitute the potential for new development of strategic interventions to restore electrical disruptions in the heart arising from electrically based remodelling.

Question or controversy: remodelling in the infarct border zone

How can stability of functional lines of block be disrupted to promote the termination of re-entry?

Gain of Na^+ channel function. Recent work has focused on gain-of-function of the Na^+ current because 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 pathological conditions, such as in the failing and/or ischaemic heart, in the heart exposed to oxidative stress, and in hearts of patients with congenital long QT3 syndromes (Bennett *et al.* 1995; Wang *et al.* 1995; Maltsev *et al.* 1998a; Maltsev & Undrovinas, 2006; Song *et al.* 2006; Hund *et al.* 2008; Sossalla *et al.* 2010). Ca^{2+} dysregulation results in pathological effects to promote I_{NaL} (Mori *et al.* 2000; Wingo *et al.* 2004) via activation of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) (Wagner *et al.* 2006), increased mitochondrial oxidative phosphorylation (Belardinelli *et al.* 2006) and consequent increased ROS (Kohlhaas *et al.* 2010; Liu *et al.* 2010). Likewise increases in I_{NaL} , which may lead to an increase in intracellular Ca^{2+} can cause activation of CaMKII (Yao *et al.* 2011). Please see Chen-Izu *et al.* (2015) for detailed descriptions of Na^+ channel regulation. Enhanced 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) stabilization of repolarization; (2) decrease in beat-to-beat action potential duration (APD) variability; and (3) improvement in Ca^{2+} handling and contractility (Maltsev *et al.* 1998b; Undrovinas *et al.* 1999, 2006; Undrovinas & Maltsev, 2008).

At least three distinct alterations in $\text{Na}_V1.5$ (the cardiac isoform of the voltage gated Na channel) gating have been shown to increase I_{NaL} including the Na^+ 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 (LQT3) in patients. The window current describes the I_{Na} that is measurable in the voltage range where the steady-state inactivation curve and activation curve overlap (January & Riddle, 1989; Zeng & Rudy, 1995; Zaza *et al.* 2008). 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 widening of the voltage range. In addition to mutations and polymorphisms, there have been a number of physiological modulators identified including Ca^{2+} , calmodulin and phosphorylation (discussed in Chen-Izu *et al.* 2015) that can increase the voltage range of the window (Zeng & Rudy, 1995).

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 $\text{Na}_V1.5$ in the absence of other isoforms (Maltsev & Undrovinas, 2006), showing that bursting channels were indeed of the same form as those underlying the transient inward Na^+ current. Clancy *et al.* recorded and modelled the transitions from normally inactivating Na^+ channels to bursting channels in heterologously expressed single $\text{Na}_V1.5$ 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 (Clancy *et al.* 2002).

Non-equilibrium gating describes another form of I_{NaL} that is not observed during typical voltage clamp depolarization protocols (Clancy *et al.* 2003). However, in response to a negative ramp current, a transient inward Na^+ current is observed. The amplitude of the current is 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-equilibrium I_{Na} is affected by a number of physiological modulators including calmodulin and phosphorylation (Herren *et al.* 2013; Chen-Izu *et al.* 2015).

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 (Maltsev *et al.* 1998a). The same group also showed an increased density and slower inactivation kinetics of I_{NaL} (Maltsev *et al.* 2007) 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 non-failing ventricular myocytes (Maltsev & Undrovinas, 2006). Importantly, there were no differences in the unitary conductance of I_{NaL} current between normal and failing human hearts, suggesting a single population of channels in which this gating phenotype is upregulated in HF (Undrovinas *et al.* 2002).

Question or controversy: Na^+ channel defects

What are the disease-specific mechanisms leading to pathological increases in I_{NaL} ?

Are Na^+ currents measured from human 'non-failing' myocytes the same as those from 'normal' myocytes?

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 (Agarwal *et al.* 2011, 2014; Lederer *et al.* 2012; Despa *et al.* 2014), SR Ca^{2+} imaging (Wang *et al.* 2014), advances in electrophysiology (Horvath *et al.* 2013), 'cell-in-gel' and other techniques for mechanochemotransduction (Prosser *et al.* 2011; Jian *et al.* 2014), mitochondrial imaging (Zhou *et al.* 2014), and stem cell technologies (Matsa *et al.* 2014), just to name a few.

In addition to the advancement in experimental techniques, there have been significant 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 (Abramson *et al.* 2010; Rocha *et al.* 2011; Neic *et al.* 2012; Nivala *et al.* 2012), the implementation of customizable solvers such as Continuity, modelling platforms like CHASTE and OpenCMISS (Bernabeu *et al.* 2009; Bradley *et al.* 2011), and infrastructures aimed at facilitating standardization, interoperability and dissemination of models (e.g. CellML and FieldML; Bernabeu *et al.* 2009; Christie *et al.* 2009; Wimalaratne *et al.* 2009; Bradley *et al.* 2011; Quinn *et al.* 2011).

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: In particular they enable exploration of high-dimensional models to

determine how their range of dynamical behaviours corresponds to that of low-dimensional models. Emergent behaviours can be mapped back to underlying parameters through component dissection, to reveal mechanisms of emergent behaviours, a function for which there is no efficient comparable experimental counterpart.

Experimental approaches and computational modelling 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 DADs. When Na^+ accumulation and Ca^{2+} overload occurs in cells, DADs arise because the cytosolic Ca^{2+} accumulation may ultimately exceed Ca^{2+} efflux via NCX and precipitate Ca^{2+} overload. A pathological version of the Ca^{2+} -induced- Ca^{2+} release ensues, whereby there is spontaneous SR Ca^{2+} release leading to overloaded intracellular Ca^{2+} that is extruded by inward NCX current, which may depolarize the cell sufficiently to activate Na^+ channels leading to the emergence of DADs and, if large enough, arrhythmogenically 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 of early after-depolarizations (EADs) and DADs occur unpredictably.

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 a single cell is not enough to overcome the large electrotonically coupled downstream sink. Mathematical models and experiments in intact hearts have shown that DADs must occur simultaneously in many thousands of cells in order to generate an arrhythmia trigger (Hoeker *et al.* 2009; Xie *et al.* 2010; Myles *et al.* 2012). The precise mechanisms involved in the synchronization of DADs in many neighbouring cells remain to be resolved. However, there may be both local electrotonic (Sato *et al.* 2014) and Ca^{2+} handling effects, such as synchronous recovery of release channel function after the previous normal beat (Myles *et al.* 2012).

In the case of loss of Na^+ channel function as described in the 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 result in unidirectional block and, if the conditions are favourable, retrograde conduction, promoting re-entrant arrhythmia in the organ (Mines, 1914; Allesie *et al.* 1973; Starmer *et al.* 1991, 1993; Starmer, 2002; Moreno *et al.* 2011). It is important to note that slow conduction, when promoted via use-dependent inhibition of I_{Na} by

drugs for example, can dynamically prolong APD at the cellular level and QT interval at the organ level as a result of the intrinsic dynamical properties of Na^+ channels and drug-channel interactions 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 DI is sufficiently long and other anomalies are present, reductions in repolarization reserve occur and even triggered arrhythmias such as early EADs may emerge (January & Riddle, 1989; Boutjdir & el-Sherif, 1991; Sicouri *et al.* 1997; Undrovinas & Maltsev, 2008; Zaza *et al.* 2008). 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 promote development of re-entrant arrhythmias and wavebreak causing fibrillation (Weiss *et al.* 2005).

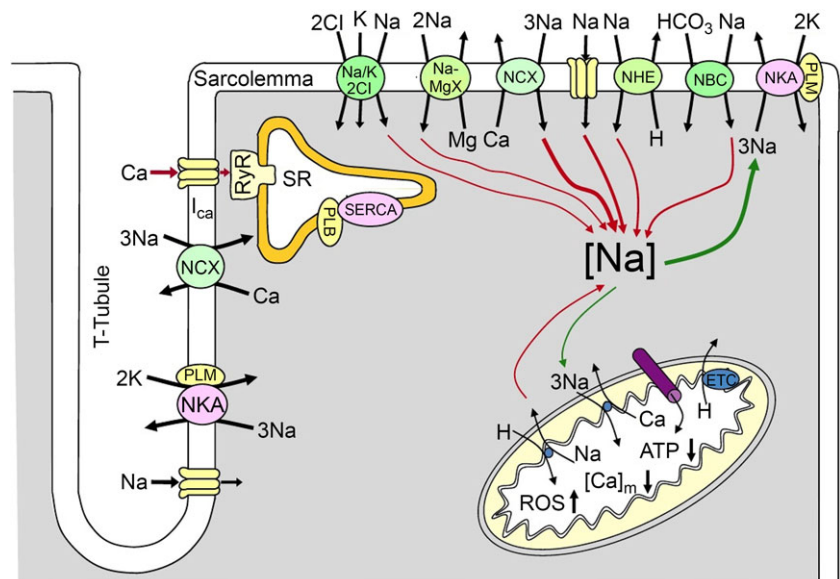
The other effect of loss of Na^+ channel function is slowed conduction that shortens the wavelength for re-entry. A slowing of the re-entrant wavefront acts to effectively increase the tissue size and may promote discordant alternans and allow induction and maintenance of arrhythmias (Weiss *et al.* 2011). Na^+ channels that are altered by disease or drugs may exhibit slowed recovery from inactivation, which changes the conduction velocity (CV) restitution (the relationship describing the dependency of CV on the inter-beat interval) which combined with APD restitution and/or Ca^{2+}

cycling instabilities promotes spatially discordant APD alternans to increase dispersion of refractoriness (Weiss *et al.* 2011; Sato & Clancy, 2013).

A gain of function of the Na^+ channel during disease that results in an increased I_{NaL} has also been linked to arrhythmias associated with acquired diseases such as heart failure and post-myocardial infarction remodelling, 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 (Nikolic *et al.* 1982; Bayes de Luna *et al.* 1989; Undrovinas & Maltsev, 2008). Conditions and diseases that lead to an increased I_{NaL} exhibit electrical instability (due to after-depolarizations, 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. This sets up a cascade leading to further ischaemia 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} inhibitor; Wasserstrom *et al.* 2009; Undrovinas *et al.* 2010; Antzelevitch *et al.* 2011). These results are additional strong indications of the link between pathological I_{NaL} to the induction of deranged Ca^{2+} homeostasis at the cellular level. A subsequent study using human ventricular myocytes (Maltsev *et al.* 1998a) similarly found improvement with TTX.

Figure 1. Schematic depiction of Na^+ transport processes in the cardiac myocyte

The figure is reproduced from Despa & Bers (2013). PLM, phospholemman; SERCA, sarco/endoplasmic reticulum Ca^{2+} -ATPase. $[\text{Ca}]_m$, mitochondrial Ca^{2+} ; ETC, electron transport chain; PLB, phospholamban; RyR, ryanodine receptor.



New therapeutic approaches for Na⁺-linked arrhythmias

In the session on 'Therapeutics', Robert Kass, Colleen Clancy, Luiz Belardinelli, András Varró, Lazlso Csernoch, Antonio Zaza, Bernard Fermini and Crystal Ripplinger presented and discussed current issues in the field. The section below summarizes these interactions.

As described above, both gain- and loss-of-function in the cardiac Na⁺ channel can result in dangerous proarrhythmic consequences by altering cardiac repolarization and conduction. Thus, the prospect of targeted pharmacological treatment to modify Na⁺-based arrhythmias has fuelled 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 (1989) and SWORD (Waldo *et al.* 1996) 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 (Drici & Barhanin, 2000). These events have resulted in a burdensome regulatory process for preclinical drugs that have discouraged the development and 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.

In the context of pharmacology that is now being developed to target pathologically enhanced I_{NaL} , it must be emphasized that selective I_{NaL} inhibition needs to be carefully determined (Szel *et al.* 2011; Belardinelli *et al.* 2013). In order to assess the specificity of I_{NaL} inhibition, the effect of a candidate drug needs to be thoroughly screened for its effects on both peak and late I_{Na} . Because peak I_{Na} may be differentially affected by drugs at various heart rates or diastolic voltages, the screening tests must include an array of assessments to ensure that selectivity of I_{NaL} versus peak I_{Na} persists under a variety of physiological conditions. For example, drugs that have relatively rapid unbinding kinetics may not exhibit obvious effects on peak I_{Na} unless the stimulation rate is sufficiently high.

Moreover, new drugs aimed at selective inhibition of I_{NaL} must be effective in cardiac disease states where hyperkalaemia, hypoxia, acidosis and ischaemia are likely.

Question or controversy: predicting therapy

How can critical determinants of drug effects in the whole heart be predicted from drug targets, kinetics and active metabolites?

Selective I_{NaL} inhibition needs to be clearly defined and careful determination of drug effects on peak I_{Na} needs to be considered at multiple stimulation frequencies.

Recently, the US Food and Drug Administration (FDA) and other stakeholders have suggested the potential implementation of a new pro-arrhythmia safety protocol that includes testing for the effects of drugs on multiple cardiac ion channels and integration of this information using computational modelling and simulation approaches, and stem cell technologies (Chi, 2013).

Modelling and simulations for predictive pharmacology.

Cardiac modelling and simulation has recently been utilized to investigate mechanisms of Na⁺ channel blocking drugs that both reduce peak Na⁺ current and that specifically target I_{NaL} . A recent study investigated lidocaine (lignocaine) effects in a multiscale computational model (Cardona *et al.* 2010). The authors demonstrated both anti-fibrillatory effects in normal tissue and predicted the potential for pro-arrhythmia with lidocaine during pathologies including acidosis and ischaemia, as has also been demonstrated experimentally due to differential effects of drugs on channels from the normal and infarct border zones (Pu *et al.* 1998).

Moreno *et al.* (2011) also implemented modelling 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 showed increased mortality with flecainide over placebo (CAST, 1989). In the computational modelling and simulation study, the dynamical complexity of the drug kinetics was modelled for both charged and neutral drug fractions. After developing the drug-channel model, a simulation in cells first confirmed experimental findings: no overt pro-arrhythmic potential was ever observed at the cellular level (Moreno *et al.* 2011). *In tissue level simulations, the outcome was very 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. Pro-arrhythmic conduction block led to development of tachycardia indicated by spiral wave re-entry, which was verified experimentally

(Moreno *et al.* 2011). 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 re-entrant arrhythmias in the presence of lidocaine in normal tissue.

Question or controversy: modelling and simulation for predictive therapy

It is still not possible to predictively link structural perturbations to pharmacological effects in the whole heart.

How to make the model, 'as simple as possible, but not simpler'?

Disease-induced enhancement of I_{NaL} promotes the development of arrhythmogenic after-depolarizations, triggered arrhythmic activity, and torsades de pointes in cardiac ventricular myocytes, cardiac tissue and intact hearts (Boutjdir & el-Sherif, 1991; Sicouri *et al.* 1997; Clancy & Rudy, 1999; Song *et al.* 2004; Wu *et al.* 2006). Pharmacological targeting of I_{NaL} has been shown to improve cardiac electrical function in myocytes challenged by cardiac glycosides, hydrogen peroxide, pharmacological enhancement of I_{NaL} , and even with drugs that block hERG channels (I_{Kr} ; the rapidly activating component of the delayed rectifier potassium current) and reduce repolarization reserve (Ver Donck *et al.* 1993; Haigney *et al.* 1994; Le Grand *et al.* 1995; Sicouri *et al.* 1997; Song *et al.* 2004, 2006; Wu *et al.* 2006; Undrovinas & Maltsev, 2008; Sossalla *et al.* 2010; Wu *et al.* 2011).

Recently, modelling and simulation have been used to probe and predict effects of the selective I_{NaL} inhibitor ranolazine in pathological situations (Moreno *et al.* 2013). 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 as tachyarrhythmogenic triggers arising from heart failure-induced remodelling (e.g. DADs). Model predictions suggested that acute targeting of I_{NaL} with ranolazine is an effective therapeutic strategy in diverse arrhythmia-provoking situations that arise from a common pathway of increased pathological I_{NaL} .

Trenor *et al.* developed a tool for *in silico* preclinical anti-arrhythmic drug safety assessment that predicted the impact of the $I_{\text{Kr}}/I_{\text{NaL}}$ 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 (Trenor *et al.* 2013).

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 mechanics. In the studies described above, it is difficult, if not impossible, to predictively link atomic scale anomalies to higher order phenomena, or to predict how structural perturbations might affect pharmacological effects in the whole heart. An important aspect of modelling and simulation as it relates to the 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 modeller must be concerned with the issue of determining how to, as Einstein suggested, 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 cell (hiPSC)-derived cardiomyocytes (hiPSC-CMs) (Braam *et al.* 2010) is another developing and exciting application at the interface of molecular and clinical information (Liang *et al.* 2013; Sallam *et al.* 2014). 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 (Matsa *et al.* 2014). 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 (Sallam *et al.* 2014). Indeed, the ion channel, Ca^{2+} handling and signalling in hiPSC-CMs still differ substantially from those in adult cardiac myocytes.

Question or controversy: patient-derived induced pluripotent stem cell (hiPSC)-derived cardiomyocytes

Ca^{2+} handling and signalling in hiPSC-CMs still differ substantially from those in adult cardiac myocytes.

Many challenges need to be overcome before stem cell technologies enter the mainstream for drug screening and therapy.

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 (Terrenoire *et al.* 2013). They identified a *de novo* mutation in the *SCN5A* (F1473C) gene encoding the $\text{Na}_V1.5$, and a polymorphism (K897T) in *KCNH2*, the gene encoding hERG. Biophysical and pharmacological analysis of ion channels expressed in iPSC-CMs demonstrated that the disease was a primary consequence of the $\text{Na}_V1.5$ 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 inhibitor 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 of the potential of iPSC-CM approaches in developing patient-specific clinical regimens (Terrenoire *et al.* 2013).

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 has recently been demonstrated (Chen *et al.* 2011).

The potential for expansion of stem cell technologies in the cardiac therapeutic arena is vast (Liang *et al.* 2013; Mordwinkin *et al.* 2013). 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 and 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 β -blockers in LQT3 patients (Kurokawa *et al.* 2008; Ahrens-Nicklas *et al.* 2009; Abu-Zeitone *et al.* 2014). However, many challenges still need to be overcome in order for stem cell technologies to enter the mainstream for screening and therapy. Toward this goal, best practices are in development to improve the maturity and homogeneity of electrical activity in iPSC-derived myocytes (Bett *et al.* 2013; Hulot *et al.* 2014).

Summary

In order to begin to translate the new findings on Na^+ derangement in the heart and its link to disease processes

to effective patient interventions, there must be a specific and concerted collaboration between the many scientific investigators at the bench, the computer terminal and the bedside. Such an effort would allow more immediate application of critical research findings and predictions to more rapidly improve outcomes for patients with cardiac disease states that are marked by disruptions in ion homeostasis. Consideration of patient-specific disease and therapeutics should be part of the consortium effort. A critical requirement for developing interdisciplinary efforts to promote understanding and knowledge among stakeholders in the field is to 'get them together in a room' for discussion and debate regarding the most critical aspects that need to be addressed. Indeed, the UC Davis Cardiovascular Symposium in 2014 on Na^+ in the heart aimed to do exactly this: bring together the world's topical experts to engage in scientific dialogue and debate to move the field forward.

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Additional information

Competing interests

None declared.

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