Pathophysiological mechanisms of arrhythmogenic right ventricular disorders
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CHAPTER 9

Synthesis

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The Brugada syndrome

The pathophysiological mechanism of the Brugada syndrome has been elusive. Two prominent schools of thought on this mechanism existed prior to the initiation of the research incorporated in this thesis. The first considered subepicardial abbreviation of the right ventricular action potential to be the pivotal mechanism.1 The second suggested that right ventricular activation delay underlies the Brugada syndrome.2 Direct testing of these hypotheses in patients had proven to be cumbersome. No ST-segment elevation was recorded in local electrograms in either endocardial3,4 or epicardial mapping studies5,6 indicating that the origin of the Brugada ECG pattern and thereby the substrate of the Brugada syndrome had been missed. In 2008, Nagase et al. did record ST-segment elevation in epicardial electrograms in a Brugada syndrome patient (figure 3C of the publication) but left this finding unmentioned.

Against this background, we had the opportunity to study the explanted, Langendorff-perfused heart of loss-of-function mutation carrier in SCN5A (G752R) undergoing cardiac transplantation for heart failure in dilated cardiomyopathy (Chapter 2). ST-segment elevation in a pseudo-ECG was provoked by the sodium channel blocker Ajmaline. We were unable to identify either early completion of repolarization or late activation as cause of the ST-segment elevation. ST-segment elevation in local electrograms was, however, accompanied by loss of the local activation signal at the basal right ventricular epicardium. The subepicardium at these sites of local ST-segment elevation was interspersed with adipose and fibrous tissue. Such discontinuities in the myocardium can influence conduction by creating non-conductive barriers. At sites where barriers cause a sudden tissue expansion more excitatory current is required for normal conduction and conduction will fail if the available excitatory current is insufficient (current-to-load mismatch).8,9 We hypothesized that excitation failure by current-to-load mismatch underlies the ST-segment elevation of the Brugada ECG pattern. The feasibility of this hypothesis was tested in Chapter 2 and 3. Excitation failure by current-to-load mismatch indeed caused ST-segment elevation on the pseudo-ECG of porcine 2D epicardial tissue preparations with artificial structural discontinuities. The success of conduction in this model depended on the width of the isthmuses and on the available sodium current which was reduced by Ajmaline and by increasing the pacing frequency (Chapter 3). In a computer model encompassing the heart and thorax, we demonstrated that structural discontinuities in the right ventricular subepicardium and reduced cardiac sodium current ($I_{Na}$) indeed results in the Brugada ECG pattern of which the ST-segment elevation was caused by excitation failure and negative T-wave by modest activation delay at neighbouring sites (Chapter 2). Remarkably, tissue discontinuities or sodium channel block in isolation did not have this effect.
**Current-to-load mismatch**

Current-to-load mismatch as a mechanism has been well studied as it influences conduction in many conditions and can initiate re-entrant arrhythmia under the right circumstances.\(^{10,11}\) It affects conduction at sites where the activation front encounters a sudden tissue expansion\(^9,12\) or a change in the myocardial fibre direction.\(^{13}\) In a normal heart, current-to-load mismatch conditions occurs at the transition between the sino-atrial node and the atrial myocardium\(^12\), at branching sites in the trabeculated atrial myocardium\(^13\) and at the Purkinje-muscular junction.\(^9\) Interestingly, conduction disorders at these sites (sinus exit block\(^14\), prolonged His-ventricular conduction times\(^15,16\), infra-Hisian and atrio-ventricular block\(^17\)) have been associated with loss-of-function mutations in \(SCN5A\). This indicates that clinical manifestations of \(SCN5A\) mutations via current-to-load mismatch are not limited to, as we hypothesized, the Brugada syndrome.

The conduction disturbances by current-to-load mismatch can initiate re-entrant arrhythmias. Sites of sudden tissue expansion leading to current-to-load mismatch are by definition asymmetrical. This means that the effect on conduction is asymmetrical as well and that conduction may fail in only one direction. This so-called unidirectional block is a prerequisite for the initiation of re-entrant arrhythmias.\(^{18}\) Unidirectional block can occur spontaneously if the load is sufficiently increased by a sudden tissue expansion\(^19\) or can be provoked by reduction of the myocardial excitability\(^8,9\) or premature stimulation.\(^{11,13}\) A classical clinical example of such conditions is the Wolff-Parkinson-White syndrome in which current-to-load mismatch causes unidirectional block at the junction between the accessory atrio-ventricular pathway and the myocardium which initiates atrioventricular circus movement (re-entrant) tachycardias.\(^{10,20}\)

**Modulators of the Brugada syndrome and current-to-load mismatch**

If current-to-load mismatch indeed is the causative pathophysiological mechanism of the Brugada syndrome it should explain not only the Brugada ECG pattern and the initiation of arrhythmias but also their characteristic modulation in patients.

The Brugada syndrome has many modulators of which the sodium current, \(I_{Na}^s\) appears to be the most important. Sodium channel blockers can provoke the Brugada ECG pattern in patients with a concealed form of the Brugada syndrome\(^{21}\) and are used in the diagnostic work-up of suspected patients.\(^22\) Furthermore, loss-of-function mutations in \(SCN5A\), encoding the pore-forming \(\alpha\)-subunit of the cardiac sodium channel, are by far the most common mutations encountered in Brugada syndrome patients\(^{23}\), although they can be identified in only 15-25% of patients.\(^{23,24}\) \(I_{Na}^s\) is the main depolarizing current in the ventricular myocardium and is crucial for normal conduction.\(^25\) The increased load in current-to-load mismatch conditions locally reduces the safety of conduction. This predisposes these sites to develop conduction block especially after reduction of \(I_{Na}^s\).\(^8,9\) Use-dependent sodium channel blockers
are particularly effective in causing conduction block under these conditions. An increased load has been demonstrated to prolong the open time of sodium channels in cable models. Use-dependent sodium channel blockers preferentially block channels in its open state and will be more effective when their open time is increased. Evidence for this effect can be found in the preferential slowing of conduction longitudinal (low resistance, high load) compared to transversal (high resistance, low load) to the fiber direction by use-dependent but not inactivated-state sodium channel blockers. Therefore, the association of loss-of-function mutations in SCN5A with the Brugada syndrome and its modulation by use-dependent sodium channel blockers, such as Ajmaline and Flecainide, is consistent with current-to-load mismatch being the underlying pathophysiological mechanism.

Other important modulators of the Brugada syndrome are the L-type calcium current ($I_{CaL}$) and the transient outward current ($I_{to}$). Enhancement of the $I_{CaL}$ by Isoproterenol and blockade of the $I_{to}$ by Quinidine can normalize the ECG and suppress ventricular arrhythmias in Brugada syndrome patients. Furthermore, mutations leading to a loss-of-function of the L-type calcium channel can be identified in 2-9% of patients. As a depolarizing current, $I_{CaL}$ can help sustain conduction in conditions in which the $I_{Na}$ alone is insufficient for conduction. Current-to-load mismatch is such a condition. In contrast, $I_{to}$ is a repolarizing current and its modulatory effect has been used in support of the hypothesis that the Brugada syndrome is a repolarization disorder. $I_{to}$ can, however, also influence conduction because the current available for conduction depends on the sum of all inward (depolarizing) and outward (repolarizing) currents during the early phase of the action potential. A reduction of $I_{to}$ has been shown to help maintain successful conduction between cardiomyocytes during conditions of increased resistance of coupling in simulations. In Chapter 3, we tested the hypothesis that $I_{CaL}$ and $I_{to}$ modulate the Brugada ECG pattern in the same model based on current-to-load mismatch as in Chapter 2. Indeed, an increase of $I_{CaL}$ and a decrease of $I_{to}$ enhanced conduction, prevented excitation failure and attenuated the Brugada ECG pattern. The modulation of the Brugada syndrome by Isoproterenol and Quinidine is therefore consistent with the hypothesis that current-to-load mismatch is its underlying pathophysiological mechanism.

Other well known modulators in the Brugada syndrome are febrile illness, vagal activity of the autonomic nervous system, and the eating of a copious meal. The effect of febrile illness appears to be directly temperature dependent as therapeutic hypothermia (32 °C) after resuscitation was associated with the disappearance of the Brugada ECG pattern in a patient with sudden cardiac arrest. A missense mutation in SCN5A has been demonstrated to affect gating of the sodium channel in a temperature sensitive manner and cause a loss-of-function at higher temperatures. The data, however, do not dismiss the hypothesized pathophysiological mechanisms of the Brugada syndrome as they all incorporate the modulatory effect of the cardiac sodium current. As indicated in Chapter 4, the mechanism by which the
autonomic nervous system modulates the Brugada syndrome is unknown. The Brugada syndrome has been associated with a reduced norepinephrine reuptake, but it is unclear whether this is a functional disorder or indicates local denervation. The mechanism by which the eating of a copious meal augments the Brugada syndrome is also unclear. A reduction in the extracellular potassium levels after a large intake of carbohydrates has be postulated to augment the features of the Brugada syndrome. However, no relation could be identified between the modest changes in the plasma potassium levels and provocation of the Brugada ECG pattern in patients after infusion of glucose and insulin.

Lastly, changes in the potassium concentration also appear to modulate the Brugada syndrome. Hypokalemia has been suggested to trigger arrhythmias in some patients. Additionally, hypokalemia by a low dietary potassium intake has been suggested to underlie the high prevalence in the north-east region of Thailand of sudden unexplained death syndrome, a condition shown to be identical to the Brugada syndrome. The Brugada ECG pattern has also been described in patients hyperkalemia in the setting of renal failure. Consistently with the hypothesis addressed in this thesis, a similar concentration dependent effect of potassium exists on conduction. This effect is the result of the influence of the extracellular potassium concentration on the resting membrane potential. Lowering the potassium concentration from 4.0 to 2.7 mM hyperpolarizes the resting membrane potential and increases the required excitatory current to reach threshold potential and thus slows conduction in Purkinje fibers. Increasing the potassium concentration from 4.0 to 7.0 mM also slows conduction. The effect of hyperkalemia is caused by depolarization of the resting membrane potential which inactivates cardiac sodium channels and reduces \( I_{Na} \). The same concentration dependent effect of potassium has been observed in current-to-load mismatch conditions at the Purkinje-ventricular junction. Current-to-load mismatch is therefore fully consistent with the modulation of the Brugada syndrome in patients.

**Structural abnormalities and the role of genetic background in the Brugada syndrome**

The hypothesis that current-to-load mismatch underlies the Brugada syndrome implies the presence of structural myocardial discontinuities. This seems to be in conflict with the widely held concept of the Brugada syndrome as a channelopathy and a functional heart disease, without structural abnormalities. In Chapter 4, we review the indications of structural abnormalities and the role of genetic background in the Brugada syndrome. We come to the conclusion that a mere reduction of \( I_{Na} \) is insufficient to cause the Brugada syndrome and that subtle signs of structural abnormalities are present in many patients. We suggest that subtle structural discontinuities are essential for the development of the Brugada syndrome and that loss-of-function mutations in \( SCN5A \) have the role of a modulator. These subtle structural
abnormalities are not detected by current non-invasive clinical techniques.

**Brugada syndrome and “J wave syndromes”**

In 2008, Haïssaguerre et al. demonstrated a high prevalence of J-point elevation in the inferolateral leads (termed early repolarization) in patients with idiopathic ventricular fibrillation (VF). This early repolarization in inferolateral leads can also be observed frequently (~11%) in Brugada syndrome patients. The apparent overlap between these patient groups prompted some to consider the pathophysiological mechanisms underlying the Brugada ECG pattern and early repolarization to be the same and to use a covering terminology for these conditions: “J wave syndromes”. In Chapter 5, the overlap between idiopathic VF victims with inferolateral J-point elevation and the Brugada syndrome is explored. The differences in the modulation of the ECG patterns in these patient groups by pharmacological agents argue against a single pathophysiological mechanism underlying both conditions.

**Recent developments in the Brugada syndrome**

In 2011, much awaited data on the epicardial substrate of the Brugada syndrome were published by Nademanee et al. Epicardial mapping identified low-voltage areas with fractionated electrograms at the anterior right ventricular outflow tract of 9 highly symptomatic patients. Fractionation of extracellular electrograms is caused by asynchronous conduction at the recording site due to structural abnormalities. Ablation of these sites successfully prevented arrhythmic events in all but one patient. The unipolar electrograms recorded at these locations strongly resembled those provoked by sodium channel blockade in Chapter 2. We were, however, not able to find such fractionated and late activation in either unipolar or bipolar electrograms (bipolar electrograms not shown). This raises the question whether these severe conduction abnormalities are present in all patients and underlie the Brugada ECG pattern or occur only in such highly symptomatic patients. Support for the selective presence in symptomatic patients can be found using signal averaged ECG studies in which late potentials were associated with arrhythmic events in the Brugada syndrome. Another important question relates to the cause of this delayed and fractionated activation described by Nademanee et al. Besides conduction block and conduction slowing by current-to-load mismatch other mechanisms can also cause regional impairment of conduction and fractionation of electrograms. Zigzag conduction in surviving muscle bundles after a myocardial infarction is such an example. The isolation of individual bundles in this setting can cause fractionation of electrograms and the increase in the activation path leads to activation delay even if the conduction velocity itself is normal. At normal conduction velocities $I_{CaL}$ lags behind the activation front and will not influence conduction. Activation delay by an increased length of the activation path is therefore not sensitive to modulation by $I_{CaL}$ in the
absence of regional conduction slowing by f.e. current-to-load mismatch at branching points of the myocardial bundles and is not consistent with the modulation of the Brugada syndrome by Isoproterenol. Electrical uncoupling can also cause significant activation delay and the $I_{CaL}$ can help maintain conduction at severe levels of uncoupling. The conduction velocity at such levels of uncoupling is, however, already severely reduced leaving little room for modulation of the conduction delay by $I_{CaL}$. Lastly, reduced excitability can cause activation delay by conduction slowing and is influenced by $I_{CaL}$. The level of conduction slowing by reduced excitability is however limited to $\sim 1/3$ of normal before conduction fails. As the large activation delay is generated over a short distance it cannot be explained by solely a local reduction in myocardial excitability. Furthermore, a homogeneous reduction in $I_{Na}$ or electrical coupling will homogenously slow conduction and will not result in fractionation of electrograms. Therefore, neither zigzag conduction, electrical uncoupling nor reduced excitability can on their own explain the features of the Brugada syndrome but may contribute to the observed subepicardial activation delay and the arrhythmogenic substrate in patients.

**Arrhythmogenic right ventricular cardiomyopathy**

In Chapter 6, we tested the hypothesis that pharmacological reduction of the mechanical load on myocyte-myocyte junction can prevent the development of arrhythmogenic right ventricular cardiomyopathy (ARVC) in the model of trained heterozygous plakoglobin-deficient mice. As shown previously, endurance training (swimming) causes right ventricular enlargement, activation delay and extrasystoles in this ARVC model. We demonstrated that pharmacological load reduction by nitric oxide donors and loop diuretics prevented right ventricular enlargement, conduction slowing and inducibility of ventricular arrhythmias, and preserves connexin43 expression after endurance training. The findings in our study support the concept that mechanical overload of the myocyte-myocyte junction causes ARVC. These results may on the long-run result in a pharmacological intervention study to slow the progression of ARVC in patients. There are however, several drawbacks to the extrapolation of the findings to the clinic beyond the obvious reservations of extrapolation of mouse models to patients. First of all, the load reduction in our model prevented the development of the ARVC phenotype. This is an interesting finding as ARVC can be a progressive disease in some patients. However, patients with advanced ARVC may not benefit from treatment as the fibrofatty replacement is likely irreversible. Secondly, mutations in desmosomal proteins have been identified in only $\sim 40\%$ of ARVC patients. It is uncertain to what extent the overload of myocyte-myocyte junctions contributes to ARVC in patients without these mutations. Nonetheless, our data are encouraging because to date no pharmacological treatment exists that slows progression of ARVC in patients.
In Chapter 7, the overlap of clinical features between the Brugada syndrome and ARVC is reviewed. The current diagnostic criteria of ARVC and Brugada syndrome exclude their coexistence in patients. All clinical features of the Brugada syndrome do, however, occur in a subgroup of ARVC patients. In acknowledgement of this overlap in pathophysiological mechanisms in these patients a broadening of the diagnostic criteria of the Brugada syndrome is proposed by the addition of a category: “Brugada features in the presence of underlying structural heart disease”.

**Energy consumption during ventricular fibrillation**

Lastly, in Chapter 8, we tested the hypothesis that VF increases the cardiac energy consumption during simulated cardiopulmonary resuscitations. In isolated, Langendorff-perfused porcine hearts, VF markedly increased the cardiac oxygen consumption during non-ischemic conditions (fixed coronary perfusion) and reduced the recovery of the creatinephosphate levels and contractility during simulated cardiopulmonary resuscitation (reduced coronary flow) after 7 minutes of simulated cardiac arrest by VF (VF, no coronary perfusion). Our data demonstrate a potential downside to the increased focus on minimizing interruptions of chest compression from the 2005 resuscitation guidelines onwards. The strategies used to minimize these interruptions increase the VF duration during cardiopulmonary resuscitations. This in turn will increase the myocardial energy consumption and may adversely affect the restoration of the myocardial energy state. Our results advocate the testing of resuscitation strategies that can immediately detect and defibrillate VF during CPR without interruption of chest compressions.
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