Combined reduction of intercellular coupling and membrane excitability differentially affects transverse and longitudinal cardiac conduction

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Aims Reduced excitability and gap junction expression are commonly found in electrically remodelled diseased hearts, but their contribution to slow conduction and arrhythmias is unclear. In this study, we have investigated the effect of isolated and combined reductions in membrane excitability and intercellular coupling on impulse propagation and arrhythmogeneity in genetically modified mice.

Methods and results Cx43 and Scn5a1798insD/+ heterozygous (HZ) mice were crossbred to create a mixed offspring: wild-type (WT, n = 15), Cx43 HZ (n = 14), Scn5a1798insD/+ (Scn5a) HZ (n = 17), and Cx43/Cx43 Scn5a1798insD/+ (Cx43/Cx43 Scn5a HZ (n = 15) mice. After ECG recording, epicardial activation mapping (208 recording sites) was performed on Langendorff-perfused hearts. Arrhythmia inducibility was tested by one to three premature stimuli and burst pacing. Conduction velocity longitudinal (CVL) and transverse (CVT) to fibre orientation and dispersion of conduction were determined during S1-S1 pacing (150 ms). Connexin43 (Cx43) and sodium channel Nav1.5 protein expression and myocardial tissue collagen content were determined by immunohistology. Compared with WT animals, P, QRS, and QTc intervals were prolonged in Scn5a HZ and Cx43/Cx43 Scn5a HZ, but not in Cx43 HZ animals. Scn5a HZ mice showed decreased CVL in right ventricle (RV) but not in left ventricle compared with WT. In the RV of Cx43/Cx43 Scn5a HZ, CVL was reduced, but CVT was not different from WT. Arrhythmia inducibility was low and not increased in either single- or double-mutant mice.

Conclusion Reduction of both electrical coupling and excitability results in normal conduction velocity parallel to fibre orientation but in pronounced conduction slowing transverse to fibre orientation in RV only, although this does not affect arrhythmogeneity.

KEYWORDS Gap junction; Electrical coupling; Excitability; Impulse conduction

1. Introduction

Cardiac impulse propagation is determined by three factors:1 (i) membrane excitability, controlled by voltage-gated sodium channels; (ii) electrical cell-to-cell coupling, mediated by gap junction proteins (connexins), and (iii) cardiac tissue architecture, i.e. cell size,2 myocardial fibre orientation,3 and interstitial fibrosis.4,5

In cardiac disease, these conduction parameters are often altered. Excitability was shown to be reduced by reduction of sodium channel expression and function up to 50%.6–8 Electrical coupling was reduced by a 30–50% decreased connexin 43 (Cx43) expression9–14 and intercellular collagen deposition (fibrosis) was increased from 5 up to 30%.15–17 Currently, it is largely unclear of how these parameters interact and modify impulse conduction, merely because several early studies have shown that the interaction between excitability and electrical coupling is complex. The concept of discontinuous conduction in the heart was defined by Spach and coworkers, who found variations in action potential upstroke velocity (Vmax), which were incompatible with continuous conduction analogous to the cable model.18 Vmax was dependent on the direction of conduction and, was paradoxically larger during slow conduction in the high resistive direction perpendicular to myocyte orientation. Vmax was lower parallel the long and low resistive axis of the myocytes.18 Parallel to the fibre

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axis, $V_{\text{max}}$ increased during electrical uncoupling\textsuperscript{19} while during reduction of the sodium current, $V_{\text{max}}$ and conduction velocity were reduced.\textsuperscript{20} Besides excitability of individual myocytes and axial and transverse electrical coupling, also the size and shape\textsuperscript{21} of heart cells and their passive membrane properties\textsuperscript{22} play a role in conduction velocity.

In the previously mentioned studies, electrical coupling or sodium channel conductance was modulated using pharmacological interventions, which are infamous for their lack of specificity. An example is the use of heptanol, which is not specific for junctional currents and inhibits many non-junctional currents, thereby changing active and passive membrane properties.\textsuperscript{23,24} The use of genetically modified mice is therefore an important tool to overcome this limitation and allows for specific and dissected downregulation of electrical coupling and excitability.

In the recent past, the consequences of altered determinants of impulse propagation have been studied through investigation of Scn5a haploinsufficient mouse models,\textsuperscript{4,25} Cx43 knockout mice,\textsuperscript{26–28} and experimental models of increased myocardial fibrosis.\textsuperscript{4,5,29} However, in these studies only a single determinant of cardiac impulse propagation was altered, whereas many myocardial pathological conditions are characterized by simultaneous remodelling of multiple conduction determinants. Interestingly, up to 30–50\% reduction in either Scn5a or Cx43 expression or increased interstitial fibrosis alone has been shown to result in only minor or no changes in impulse conduction or arrhythmogeneity.\textsuperscript{4,5,26} Thus, the heart appears capable of maintaining normal conduction velocities and electrical stability when single determinants of impulse conduction are abnormal, a phenomenon referred to as ‘conduction reserve’.\textsuperscript{30–32} In particular, conduction reserve has been shown to be lower in the right ventricle (RV) compared with left ventricle (LV).\textsuperscript{4,5,25,26}

In the current study, we hypothesized that simultaneous impairment of two determinants, i.e. reduction in both excitability and cell-to-cell coupling, is required to exhaust cardiac conduction reserve, thereby leading to slow conduction and arrhythmia vulnerability. Therefore, we investigated the combined effect of a clinically relevant 40\% reduction of excitability\textsuperscript{25} and 50\% reduction in Cx43-mediated gap-junctional coupling\textsuperscript{33} on impulse conduction and arrhythmogeneity, compared with wild-type animals, and to animals with either reduced coupling or excitability alone.

Our results indicate that isolated reduction of excitability, but not electrical coupling, results in decreased longitudinal conduction velocity ($CV_L$). Interestingly, attenuation of both excitability and coupling reduces transverse conduction ($CV_T$), but normalizes $CV_L$. Thus, the additive effect of co-reduction in excitability and coupling results in a mixed effect on impulse propagation, which may be of particular importance during pathophysiological conditions.

2. Methods

2.1 Animals

Two transgenic mouse lines were used in this study: Cx43\textsuperscript{cre-ER(T)}/flox mice\textsuperscript{33} and heterozygous Scn5a\textsuperscript{1798insD+/+} (Scn5a HZ) mice.\textsuperscript{25} Both transgenic mouse models were studied previously and described in detail elsewhere.\textsuperscript{25,26} Scn5a\textsuperscript{1798insD+/+} (Scn5a HZ) mice have an approximately 40\% reduction in peak sodium current.\textsuperscript{25} An intercross of the two lines was set up to create four groups of animals: (i) wild-type (WT) animals, Cx43\textsuperscript{floxflox}/Scn5a\textsuperscript{+/-} [n = 19 (7 males/12 females); age 16.2 ± 0.4 weeks]; (ii) animals solely carrying the Cx43 mutation, Cx43\textsuperscript{creER(T)/+}/Scn5a\textsuperscript{+/-} (Cx43 HZ) [n = 18 (7 males/11 females); age 16.5 ± 0.4 weeks]; (iii) animals solely carrying the Scn5a\textsuperscript{1798insD+} mutation, Cx43\textsuperscript{floxflox}/Scn5a\textsuperscript{1798insD+} (Scn5a HZ) [n = 18 (8 males/10 females); age 16.6 ± 0.6 weeks]; and (iv) animals carrying both mutations, Cx43\textsuperscript{creER(T)/floxd/+/+/+}/Scn5a\textsuperscript{1798insD+} (Cx43/Cx43 Scn5a HZ) [n = 18 (9 males/9 females) age; 17.3 ± 0.3 weeks]. Male-to-female ratio was not significantly different between the four groups ($\chi^2$-test P = 0.853) nor was age (two-way ANOVA; P = 0.377). The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the animal ethics review board of the University Medical Center Utrecht (approval number 2007.II.02.052).

2.2 ECG recording

In all animals, a three-lead ECG was recorded under light isoflurane anaesthesia, and analysed off-line using Chart 5 (ADInstruments) as described previously.\textsuperscript{44}

2.3 Preparation of hearts for Langendorff perfusion

After cervical dislocation, the heart was excorporated, canulated, and connected to a Langendorff perfusion set-up as described before.\textsuperscript{4,26} Hearts were submerged during the entire experiment in a perfusion medium (composition is specified elsewhere)\textsuperscript{37} of 37°C.

2.4 Recording of electrograms during Langendorff perfusion

Electrical recordings of RV and LV were made with a 208 points unipolar electrode (16 x 13 grid, spacing 500 µm) as described previously.\textsuperscript{4,26} The ventricles were stimulated from the centre of the grid at basic cycle length (BCL) of 150 ms. Electrograms were acquired using a 256-channel data acquisition system (Biosenmi, Amsterdam, The Netherlands). The effective refractory period (ERP), the longest coupling interval of the premature stimulus that failed to activate the entire heart, was determined for each ventricle separately. Every sixteenth basic stimulus was followed by one premature stimulus. Starting at 140 ms, the coupling interval of the premature stimulus was reduced in steps of 10 ms until ERP.\textsuperscript{26}

Susceptibility to arrhythmias was tested by applying 16 basic stimuli (BCL 150 ms) followed by three premature stimuli with an interval of ERP + 5 ms and by burst pacing if premature stimulation failed to induce arrhythmias. Two seconds of burst pacing was performed starting 20 ms above ERP down into steps of 5 ms until ventricular tachycardia/fibrillation (VT/VF) was induced or one-to-one capture was lost. Stimulation protocols were standardized and applied to all mice included in the study. Inducibility for arrhythmias was tested up to five times for each protocol. If arrhythmias occurred, the inducibility was verified by re-application of the same protocol.

2.5 Data analysis

The moment of maximal negative dV/dt in the unipolar electrograms was selected as the time of local activation and determined with custom-written software based on Matlab (The Mathworks Inc.).\textsuperscript{36} Activation times were used to construct activation maps. Conduction velocity parallel ($CV_P$) and perpendicular ($CV_T$) to fibre orientation were determined from the paced ($S_1-S_2$) activation maps. Activation times of at least three consecutive electrode terminals along lines perpendicular to intersecting isochronal lines
(1 ms) were used to calculate conduction velocities. Anisotropic ratio (AR) was defined as \(CV_L/CVT\). Dispersion of conduction was assessed for LV and RV using the method described by Lammers et al.37

### 2.6 Statistics

All parametric data are expressed as mean \(\pm\) SEM and were analysed using a two-way ANOVA. A Bonferroni post hoc test was performed only when significant differences were found between the four genotype groups in the two-way ANOVA. Non-parametric data were analysed by the \(\chi^2\) test. Probability values of \(P \leq 0.05\) were considered statistically significant. Data were analysed using SPSS 13.0 software.

### 2.7 Immunohistochemistry and histology

Following electrophysiological measurements, hearts were rapidly frozen in liquid nitrogen and stored at \(-80^\circ\text{C}\). Immunohistochemistry was performed using rabbit polyclonal antibodies raised against Cx43 (1:250; Zymed Laboratories), anti-Nav1.5 antibody specificity is described previously.5 In addition, total mouse heart homogenate was blotted together with lysate of HEK293 cells transfected with Scn5a. Anisotropic ratio was defined as \(CV_L/CVT\). Dispersion of conduction was assessed for L V and RV using the method described by Lammers et al.37

### 3. Results

#### 3.1 Electrophysiological characteristics

##### 3.1.1 Surface ECG

Table 1A summarizes surface ECG characteristics of the four experimental groups studied. Apart from the RR interval, all ECG parameters showed a significant difference between

<table>
<thead>
<tr>
<th>Table 1</th>
<th>ECG and electrophysiological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
</tr>
<tr>
<td>(A) ECG characteristics</td>
<td></td>
</tr>
<tr>
<td>Age (weeks)</td>
<td>13.1 (\pm) 0.36</td>
</tr>
<tr>
<td>RR interval (ms)</td>
<td>103.07 (\pm) 4.29</td>
</tr>
<tr>
<td>PR interval (ms)</td>
<td>32.29 (\pm) 0.77</td>
</tr>
<tr>
<td>P duration (ms)</td>
<td>9.3 (\pm) 0.38</td>
</tr>
<tr>
<td>QRS interval (ms)</td>
<td>9.32 (\pm) 0.18</td>
</tr>
<tr>
<td>QTc (ms)</td>
<td>70.68 (\pm) 1.92</td>
</tr>
<tr>
<td>(B) Electrophysiological characteristics</td>
<td></td>
</tr>
<tr>
<td>Stimulation threshold (V)</td>
<td>0.52 (\pm) 0.06</td>
</tr>
<tr>
<td>ERP (ms)</td>
<td>53.53 (\pm) 5.21</td>
</tr>
<tr>
<td>Longitudinal CV (cm/s)</td>
<td>54.19 (\pm) 2.63</td>
</tr>
<tr>
<td>Transverse CV (cm/s)</td>
<td>35.62 (\pm) 2.06</td>
</tr>
<tr>
<td>Anisotropic ratio</td>
<td>1.55 (\pm) 0.06</td>
</tr>
<tr>
<td>Wavelength (cm)</td>
<td>1.88 (\pm) 0.23</td>
</tr>
<tr>
<td>Dispersion median</td>
<td>1.26 (\pm) 0.08</td>
</tr>
<tr>
<td>Dispersion absolute</td>
<td>2.29 (\pm) 0.14</td>
</tr>
<tr>
<td>Dispersion index</td>
<td>1.84 (\pm) 0.08</td>
</tr>
<tr>
<td>Arrhythmogeneity (%)</td>
<td>5.9 (n = 1/17)</td>
</tr>
</tbody>
</table>

Bold values represent values with \(P < 0.05\). All \(P\)-values in ANOVA column are of two-way ANOVA. Exceptions are the \(P\)-values of arrhythmogeneity that is obtained by \(\chi^2\)-test. A Bonferroni post hoc test was performed only when significant differences were found between the four genotype groups in the two-way ANOVA. Non-parametric data were analysed by the \(\chi^2\) test. Probability values of \(P \leq 0.05\) were considered statistically significant. Data were analysed using SPSS 13.0 software.
the groups (P ANOVA < 0.05). The PR interval was significantly longer in the Cx43/Scn5a HZ animals, either compared with the WT (9.0% increase) as well as to Cx43 HZ animals (9.5% increase). Compared with WT animals, P-wave, QRs, and QTc intervals were all prolonged in both the Scn5a HZ (increase of 31.1%, 10.0%, and 13.5%, respectively) and Cx43/Scn5a HZ animals (increase of 34.2%, 14.3%, and 16.3%, respectively). P-wave duration and the QRs-interval of the Cx43/Scn5a HZ animals were both significantly prolonged when compared with the Cx43 HZ animals (23.4% and 12.9% prolongation, respectively), as was the P-wave duration of the Scn5a HZ animals compared with Cx43 HZ (19.9% prolongation).

### 3.1.2 Ventricular impulse conduction

Typical epicardial activation maps recorded during S1-S1 of RV and LV of the four experimental groups are depicted in Figure 1. Myocardial fibre orientation at the epicardium of RV and LV is indicated by the black double-headed arrows in the heart pictograms. Table 1 summarizes the electrophysiological characteristics of all groups. Significant differences of various parameters among the groups were preferentially found in RV. Stimulation threshold of LV in both groups of mice heterozygous for Scn5a1798insD/+(Scn5a HZ and Cx43/Scn5a HZ) was significantly higher compared with LV WT. RV stimulation threshold in these animals was not significantly different from the RV WT. RV ERP of Scn5a HZ animals was significantly longer than the Cx43 HZ group. In LV, conduction velocities (CVl, CVr), AR, and ERP were not significantly different between the four groups. In RV, however, CVl was reduced in both Scn5a HZ and Cx43/Scn5a HZ animals, albeit only significantly in the Scn5a HZ mice compared with both WT and Cx43 HZ mice.

![Typical epicardial activation maps of RV and LV of wild-type, Cx43-, Scn5a-, and Cx43/Scn5a HZ mice. Black arrows in the pictograms of LV and RV demonstrate myocardial fibre orientation. Ventricles are stimulated from the centre of the grid, allowing measurements of CVl (bold arrow) and CVr (dashed arrow). Isochrone interval: 1 ms. Red colour denotes earliest activation time, and blue latest.](image)

Transverse CV was not altered in Cx43 HZ animals compared with WT (35.62 ± 2.06 cm/s and 33.12 ± 3.07 cm/s, respectively). CVr was reduced both in the Scn5a HZ (28.04 ± 1.75 cm/s) and the Cx43/Scn5a HZ (23.87 ± 2.11 cm/s) RV, compared with WT animals; nevertheless, this reduction was only significant in the Cx43/Scn5a HZ RV. Both CVl and CVr of the Cx43 HZ RV did not differ from those of WT RV. AR was significantly increased in Cx43/Scn5a HZ RV compared with both Scn5a HZ and WT RV.

No significant differences were found in wavelength (λ = CVr × ERP) for both RV and LV between the four groups. Dispersion in conduction, determined by calculation of the maximal time delays between adjacent electrodes from the activation maps, was not significantly different between the four groups of both RV and LV.

### 3.1.3 Arrhythmogeneity

In LV, arrhythmias were induced in one Cx43 HZ heart only. No arrhythmias were induced in the LV of WT, Scn5a HZ or Cx43/Scn5a HZ animals [Table 1(B)]. In RV, arrhythmias were induced in one WT, four Cx43 HZ, two Scn5a HZ, and two Cx43/Scn5a HZ hearts [P = NS; χ² test; Table 1(B)]. Induced arrhythmias were either non-sustained (5–15 complexes) or sustained (>15 complexes) VTs.

### 3.2 Tissue characteristics

#### 3.2.1 Fibrosis

Picro Sirius Red staining showed homogenous and comparable distribution of interstitial fibrosis in all four groups both for RV and LV (Figure 2). No significant differences were observed in the amount of interstitial fibrosis between the four groups, neither for RV and LV (Table 2).
3.2.2 Expression and distribution of Cx43 and Nav1.5 protein

Double-labelling of Cx43 and dystrophin showed that Cx43 expression is clearly reduced in Cx43 HZ mice, compared with WT (Figure 3). Cx43 labelling is preferentially present at the intercalated discs, and to a lesser extent along the lateral borders of the cells. In the Scn5a HZ, the Cx43 expression and distribution were comparable to WT. In Scn5a HZ mice, immunolabelling of the cardiac sodium channel protein Nav1.5 showed reduced expression levels, but similar distribution compared with WT, in concordance with the haploinsufficiency (Figure 4). Nav1.5 expression in the Cx43 HZ was similar to the expression level in the WTs.

In the double heterozygous mice (Cx43/Scn5a HZ) Cx43 and Nav1.5 labelling intensity and distribution was highly comparable to Cx43 HZ and Scn5a HZ mice, respectively. This indicates that neither Cx43 expression, nor Nav1.5 expression is affected by the combined haploinsufficiency.

Table 2 Levels of interstitial fibrosis

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Cx43 HZ</th>
<th>Scn5a HZ</th>
<th>Cx43/Scn5a HZ</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV</td>
<td>1.52 ± 0.53</td>
<td>1.94 ± 0.24</td>
<td>1.63 ± 0.24</td>
<td>1.40 ± 0.17</td>
<td>0.264</td>
</tr>
<tr>
<td>LV</td>
<td>1.81 ± 0.57</td>
<td>1.43 ± 0.18</td>
<td>1.78 ± 0.5</td>
<td>1.09 ± 0.45</td>
<td>0.703</td>
</tr>
</tbody>
</table>

All P-values in ANOVA column are of two-way ANOVA.

*P < 0.05 between HZ groups to WT.

4. Discussion

In this study we investigated the electrophysiological characteristics of genetically modified mice with either 40% reduction of excitability, 50% reduction in Cx43-mediated gap-junctional coupling, or a combination of both. The main findings from these experiments are: (i) Scn5a heterozygous mice demonstrate prolongation of P-wave, QRS-, and QTc interval times. Combined with Cx43 deficiency, these ECG parameters were prolonged even further, in addition to an increased PR interval; (ii) Combined or isolated reduction of either excitability or coupling does not affect LV electrophysiological characteristics. Although RV conduction is not affected in the Cx43 HZ, RV CV_T is decreased in the Cx43/Scn5a HZ RV compared with Scn5a HZ. In contrast, RV CV_L is improved in Cx43/Scn5a HZ RV when compared with Scn5a HZ, indicating an agonistic effect on CV_T and an antagonistic effect on CV_L of Cx43 reduction on conduction in the background of reduced...
Mixed effect of reduced excitability and coupling

Figure 3  Cx43 immunohistochemical expression and distribution. Cx43 (green/FitC) and dystrophin (red/Texas Red) labelling of wild-type, Cx43-, Scn5a-, and Cx43/Scn5a HZ RV and LV tissue sections. All images were taken with similar exposure time. Bars in all figures: 50 μm.

Figure 4  Nav1.5 immunohistochemical expression and distribution. Nav1.5 (green/FitC) labelling of wild-type, Cx43-, Scn5a-, and Cx43/Scn5a HZ RV and LV tissue sections. Images taken with equal exposure time. Bars in all figures: 50 μm.
excitability; (iii) although electrophysiological properties were significantly altered in the RV of both Scn5a HZ and Cx43/Scn5a HZ mice, these changes were not sufficient to increase the vulnerability to arrhythmias.

4.1 Tissue characteristics of the genetically modified animals

The immunohistochemical staining in this study showed that haploinsufficiency for either Cx43 or Scn5a resulted in a concomitant homogeneous reduction in staining intensity for Cx43 or Nav1.5 protein, respectively. In case of the Cx43 haploinsufficient mice, this homogeneous reduction was previously reported and represented a reduction in Cx43 protein of approximately 50%. The reduction in Nav1.5 staining intensity in Scn5a \(/^{1998\text{insD}+/^+}\) mice correlated to an approximately 40% reduction of peak sodium current. Staining intensity and expression patterns of Cx43 and Nav1.5 in Cx43/Scn5a HZ mice were similar to that of mice haploinsufficient for either Cx43 or Scn5a. This lack of interaction between Cx43 and Nav1.5 protein expression was previously shown. Myocytes haploinsufficient for Cx43 had sodium currents that were indistinguishable from WTs and Nav1.5 staining intensity and pattern was identical. Similarly, Scn5a haploinsufficient mice were reported to have normal Cx43 staining intensity and expression pattern at young age (3–4 months, comparable to this study). At higher age (>12 months), levels of interstitial fibrosis were enhanced by Scn5a haploinsufficiency. Picro Sirus Red staining in this study showed that levels of fibrosis were low and similar in all groups in this study.

The immunohistochemical and histological experiments indicate that double haploinsufficient Cx43/Scn5a HZ mice are a representative combination of Scn5a and Cx43 haploinsufficient mice.

4.2 RV and LV conduction reserve

Cardiac impulse propagation is dependent upon three main factors, two of which are modified in this study: electrical coupling and excitability. The third factor, tissue architecture, was not modified in our model, since myocardial fibrosis was not increased. Cardiac tissue displays conduction reserve, allowing proper impulse propagation and reducing the risk on arrhythmogeneity if conduction parameters are slightly modified. Results of the current and previous studies show that under similar conditions conduction reserve of RV is less than that of LV, as indicated by the altered electrophysiological characteristics of RV, while those of LV of the genetically modified animals remained unaltered. Such differences might be explained by different basal expression levels of Cx43, Nav1.5, and/or collagen between LV and RV. We were not able to detect such differences in the current study. Interestingly, a recent study by Veeraraghavan and Poelzing showed that, in guinea pig, Nav1.5 and Kir2.1 protein expression were 18% and 12% lower, respectively, in RV compared with LV. They elegantly showed that under control conditions, \( I_{K1} \) heterogeneity is an important determinant of transverse impulse conduction: Lower \( I_{K1} \) corresponded to increased CVL. However, \( I_{Na} \) heterogeneity was dominant under circumstances of reduced sodium current. In our study, in contrast to guinea pig, CVL was higher in RV compared with LV and reduced sodium current (Scn5a HZ) resulted only in significant (25%) RV longitudinal conduction slowing. The role of \( I_{K1} \) and its interaction with \( I_{Na} \), Cx43 levels, and distribution and other factors, such as cell size or wall thickness on differences between conduction reserve between RV and LV is currently unknown in the mouse and requires further investigation.

4.3 Coupling, excitability, and refractoriness

Refractoriness is dependent on various factors: action potential duration, electrical coupling, and excitability. Previous studies have shown that reduced excitability results in prolongation of ERP. Reducing intercellular coupling by 50% on the other hand demonstrated a trend towards shorter ERP, but no effect at all. In the current study, RV ERP of Cx43 HZ animals tended to be shorter, whereas that of Scn5a HZ animals tended to be longer compared with WT animals, albeit not significant. However, the significant polarization of ERP of Cx43 HZ and Scn5a HZ animals demonstrates the different mechanism by which intercellular coupling and membrane excitability affect impulse propagation, safety factor, but also refractoriness.

4.4 Reduced cell-to-cell coupling and excitability impairs conduction and increased anisotropy

ECG recordings in Cx43 HZ mice showed that a 50% reduction in Cx43 expression does not alter electrocardiographic parameters. This is compatible with the unaltered electrophysiological characteristics of RV and LV in Cx43 HZ mice, which was also observed in previous studies performed in Cx43 heterozygous animals. In contrast to animals demonstrating reduced coupling, Scn5a HZ mice displaying 40% reduction of excitability showed marked electrocardiographic changes, compatible with the currently observed electrophysiological changes on epicardial mapping.

Although a 50% reduction in cell-to-cell coupling has no effect on electrocardiographic and electrophysiological characteristics of the Cx43 HZ mice under baseline conditions, reduced coupling seems to reduce conduction reserve. The moderate, non-significant transverse conduction slowing in heterozygous Scn5a mice is further decreased and becomes significant in mice demonstrating both reduced coupling and excitability. Interestingly, the effect of reduced cell-to-cell coupling on CVL is different. In the current experiments, longitudinal conduction was observed to be significantly reduced in Scn5a HZ mice. In contrast to CVL, additional reduction in electrical coupling to the substrate seems to attenuate the reduction in CVL. This might be explained by the distribution of Cx43 gap junctions, that are abundantly predisposed in the area of the intercalated disc, and are less frequently found in the lateral sarcosome. As the lateral sarcosome of the myocyte is less coupled than the intercalated disc, an equal reduction of Cx43, as is seen in this study, will influence the less coupled lateral sarcosome more, and affect CVL conduction more substantially. The lateral uncoupling of the cells may result in more axial current giving rise to a more moderate CVL reduction when both excitability and coupling are being reduced. This effect of paradoxical improvement of impulse conduction by partial uncoupling has been previously described by Rohr et al. These investigators showed that at tissue expansions, conduction block occurred because there is a mismatch between the amount of current.
delivered by the excited cells and the amount of current needed by the much larger amount of unexcited cells. During partial uncoupling at the discontinuity, this 'current-to-load' mismatch is reduced owing to reduction of the load by increase in the internal resistance at the expansion. Computer modelling studies showed that decreased intercellular coupling reduces 'current-to-load' mismatch, increases safety factor, and enhances dV/dt. The interplay between membrane factors and gap junction factors has a mixed effect on propagation and propagation safety, as it further inhibits conduction in the transverse direction of impulse propagation, but has a beneficial effect on the longitudinal impulse propagation, when compared with the effect of sole reduced excitability on impulse propagation.

4.5 Arrhythmogeneity

In mice with reduced excitability, decreased gap-junctional coupling, or a combination of both, the incidence of arrhythmia inducibility is negligible and not significantly different from WT animals. As the interplay between reduced coupling and excitability results in a comparable refractory period for the double-knockout and the WT animals, the occurrence of re-entry arrhythmias in this animal model is most likely prevented by the lack of sufficiently small wavelength.  

4.6 Conclusion

Reduced membrane excitability and coupling have a significant impact on conduction impairment and anisotropy. However, the additive effect of co-reduction in excitability and coupling results in a mixed effect on longitudinal and transverse impulse propagation, as compared with either Cx43 HZ or Scn5a HZ animals. This interplay between membrane excitability and electrical coupling may be of particular importance under pathological conditions in which one electrophysiological characteristic (reduced coupling) may compensate for another (reduced excitability) in terms of their opposite effects on propagation safety, refractoriness, and arrhythmogeneity.

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Conflict of Interest. None declared.

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