

# Mechanisms underlying increased right ventricular conduction sensitivity to flecainide challenge

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**Aims** The cardiac sodium current ( $I_{Na}$ ) is a major determinant of conduction. Mechanisms underlying regionally heterogeneous conduction slowing secondary to reduced  $I_{Na}$  in diseases such as the Brugada syndrome and heart failure remain incompletely understood. Right precordial electrophysiological manifestations during flecainide challenge suggest a decreased right ventricular depolarization reserve. We hypothesized that heterogeneous cardiac sodium channel (Na<sub>v</sub>1.5) distribution between ventricles causes interventricular depolarization heterogeneities.

**Methods and results** Western blotting analysis revealed Na<sub>v</sub>1.5, and Kir2.1 protein expressions were 18.2 and 12.0% lower, respectively, in the guinea pig right ventricle (RV) compared with the left ventricle (LV). Conduction velocity ( $\theta$ ) heterogeneities were quantified by optical mapping during LV or RV pacing. Although RV transverse  $\theta$  ( $\theta_T$ ) was significantly greater than LV  $\theta_T$  by  $33.09 \pm 1.38\%$  under control conditions, there were no differences in longitudinal  $\theta$ . During partial sodium channel blockade (flecainide, 0.5  $\mu$ M), RV  $\theta$  decreased by  $35.3 \pm 1.3\%$ , whereas LV  $\theta$  decreased by  $29.2 \pm 1.0\%$ . These data demonstrate that the RV has an increased conduction dependence on sodium channel availability. Partial blockade of the inward rectifier potassium current ( $I_{K1}$ ) by BaCl<sub>2</sub> (10  $\mu$ M) significantly increased  $\theta$  in both ventricles under control conditions. However, BaCl<sub>2</sub> only increased conduction dependence on sodium channel availability in the LV. This suggests that the LV may have an increased depolarization reserve compared with the RV, but the larger  $I_{K1}$  depresses control LV  $\theta$ .

**Conclusion** Interventricular  $I_{K1}$  heterogeneities may underlie conduction heterogeneities observed under control conditions. However, under conditions where  $I_{Na}$  is functionally reduced in disease or during pharmacological sodium channel blockade, the heterogeneity in Na<sub>v</sub>1.5 expression may become a significant determinant of conduction heterogeneities.

## 1. Introduction

Cardiac conduction velocity is determined by various factors including tissue excitability, gap junctional coupling, and cell geometry. Decreased tissue excitability secondary to inhibition of the cardiac sodium current ( $I_{Na}$ ) has been associated with slow and unsafe conduction and arrhythmogenesis under various conditions<sup>1,2</sup> such as Brugada syndrome (BrS),<sup>3–5</sup> acute myocardial ischaemia, and during treatment with class I anti-arrhythmic drugs.

In some cases, BrS has been linked to loss of function mutations in *SCN5A*,<sup>5,6</sup> the gene encoding for the pore-forming alpha subunit of the cardiac sodium channel (Na<sub>v</sub>1.5). The disease is characterized by electrophysiological manifestations in the right precordial leads and tachyarrhythmias that preferentially originate in the right ventricle (RV).<sup>4,7,8</sup> Although regional conduction heterogeneities in

diseases such as BrS have been previously demonstrated, the mechanisms underlying those heterogeneities remain unknown. Furthermore, clinical unmasking of BrS via sodium channel blockade<sup>9</sup> suggests that the underlying mechanism of interventricular depolarization heterogeneities is related to sodium channel availability.

Interventricular electrophysiological heterogeneities in the normal myocardium have been well established,<sup>10–13</sup> and it has been previously demonstrated that Na<sub>v</sub>1.5 is reduced in the RV compared with the left ventricle (LV) in sheep.<sup>14</sup> However, the functional consequences of interventricular Na<sub>v</sub>1.5 heterogeneities remain unknown. Therefore, we hypothesized that the mechanism of interventricular electrophysiological heterogeneity during loss of sodium channel function is caused by heterogeneous distribution of cardiac sodium channels, leading to preferential conduction slowing and decreased depolarization reserve in the RV.

In this study, we demonstrate that the RV exhibits greater conduction dependence on sodium channel blockade as a result of reduced Na<sub>v</sub>1.5 expression in the RV compared

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with the LV in guinea pig. Surprisingly, we also demonstrate that under normal conditions, heterogeneities in the inward rectifier potassium current ( $I_{K1}$ ) mask  $\text{Na}_v1.5$  heterogeneities during conduction.

## 2. Methods

This 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).

### 2.1 Western immunoblotting

Guinea pigs were anaesthetized [30 mg/kg pentobarbital sodium (Nembutal) ip], and their hearts were rapidly excised. The ventricles were divided into 12 parts—four each (anterior base, posterior base, anterior apex, and posterior apex) from the LV and RV and the septum. The samples were then snap-frozen, cut into small pieces, and homogenized by sonication into a whole-cell homogenate. Western immunoblotting was performed following a previously described procedure.<sup>15</sup> Briefly, the protein content in the whole-cell homogenates was assessed through a BCA assay, and equal amounts of proteins from the samples were electrophoresed on 4–12% Bis-Tris gels (BioRad, Hercules, CA, USA). The proteins were then transferred onto a nitrocellulose membrane. After treatment with a 5% casein solution to block non-specific binding of antibodies, the membrane was treated with primary antibody [rabbit anti- $\text{Na}_v1.5$  ( $n = 4$ )/anti-Kir2.1 polyclonal antibody ( $n = 3$ ) (Chemicon, Temecula, CA, USA)/goat polyclonal GAPDH antibody (Abcam, Cambridge, MA, USA) followed by goat anti-rabbit HRP-conjugated secondary antibody (JacksonImmuno, West Grove, PA, USA). The membrane was then treated with enzymatic chemiluminescence reagents, and the bands were visualized on autoradiography film.

Protein expression in the samples was quantified on the basis of the size and density of the bands.  $\text{Na}_v1.5$  and Kir2.1 expression levels were normalized to the regional GAPDH expression for inter-animal comparison.

### 2.2 Optical mapping

Guinea pigs were anaesthetized [30 mg/kg pentobarbital sodium (Nembutal) IP]. Their hearts were rapidly excised and perfused as Langendorff preparations (perfusion pressure 55 mmHg) with oxygenated (100%  $\text{O}_2$ ) Tyrode's solution at 36.5°C containing (mmol/L)  $\text{CaCl}_2$  2, NaCl 140, KCl 4.5, dextrose 10,  $\text{MgCl}_2$  1, HEPES 10 (pH 7.41). The right and left atria were excised to avoid competitive stimulation from the atria. Hearts were stained with the voltage-sensitive dye di-4-ANEPPS (15  $\mu\text{mol/L}$ ) by direct coronary perfusion for 10 min.

Conduction velocity ( $\theta$ ) was quantified by a previously described optical voltage mapping system.<sup>16</sup> Specifically, we used two SciMedia MiCam02 HS CCD cameras (SciMedia, Irvine, CA, USA) in a tandem lens configuration capable of resolving membrane potential changes as small as 2 mV with 1 ms temporal resolution from  $90 \times 60$  sites simultaneously. Following staining with the voltage-sensitive dye di-4-ANEPPS, the preparation was excited by three 60-LED light sources (RL5-A9018, Superbrightleds, St Louis, MO, USA) fitted with  $510 \pm 5$  nm filters (Chroma, Rockingham, VT, USA) and a 50 mm aspheric lens (Edmund Optics, Barrington, NJ, USA). Fluoresced light passed through a 150 mm achromatic (BK7/Flint, Ealing, Rocklin, CA, USA) lens, a 50 mm aspheric B270 crown glass lens (Edmund Optics), a 35 mm planoconvex BK7 lens (Edmund Optics), and a 610 nm LP filter (Newport, Irvine, CA, USA) before it was incident on the CCD array. The interpixel resolution was 0.184 mm in the x-direction (90 pixels) and 0.199 mm in the y-direction (60 pixels).

### 2.3 Optical action potential measurements

Motion was reduced by perfusion of 7.5 mM 2,3-butanedione monoxime (BDM). Hearts were stimulated with a unipolar silver wire placed on the anterior epicardial surface close to the equatorial plane of the ventricle being mapped at 1.5 times the stimulation threshold with a basic cycle length of 300 ms unless otherwise specified. Activation time was defined as the time of the maximum first derivative of the action potential as described previously.<sup>17</sup>

### 2.4 Conduction velocity measurements

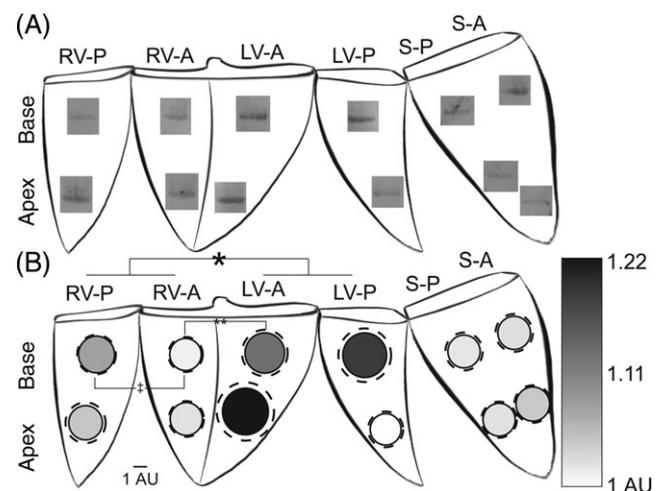
Activation times were fitted to a parabolic surface as described previously.<sup>18</sup> The gradient at each point was assigned a conduction velocity vector. The averaged conduction velocity vectors in the slow and fast axes of propagation ( $\pm 15^\circ$ ) are reported as they reflect transverse and longitudinal propagation.<sup>19</sup>

### 2.5 Interventions

The class IC sodium channel blocker flecainide was varied from 0 to 2  $\mu\text{M}$  to determine conduction dependence on sodium channel availability. A 10  $\mu\text{M}$  dose of  $\text{BaCl}_2$  was used to test the effect of partial  $I_{K1}$  blockade on conduction velocity ( $\theta$ ). To study the effects of hypokalaemia, we reduced the concentration of extracellular potassium in the perfusate from 4.5 to 3 mM.

### 2.6 Statistical analysis

Statistical analysis of the data was performed using a two-tailed Student's *t*-test for paired and unpaired data or a single factor ANOVA. A  $P < 0.05$  was considered statistically significant. All values are reported as mean  $\pm$  standard error unless otherwise noted.



**Figure 1** (A) Representative  $\text{Na}_v1.5$  bands from the 12 different regions of the heart that were tested. (B) Normalized  $\text{Na}_v1.5$  expression in the ventricles and the septum. The expression in each region is denoted by the radius and greyscale of the corresponding circle.  $\text{Na}_v1.5$  expression in the left ventricle was 18.2% greater than that in the right ventricle ( $*P < 0.05$ ), whereas no significant difference was observed between the right ventricle and the septum ( $P = \text{ns}$ ). Also the anterior basal right ventricle expressed less  $\text{Na}_v1.5$  than the posterior basal right ventricle ( $**P < 0.05$ ) and the anterior basal left ventricle ( $†P < 0.05$ ). RV, right ventricle; LV, left ventricle; S, septum; A, anterior; P, posterior.

### 3. Results

#### 3.1 Regional heterogeneities of $\text{Na}_v1.5$

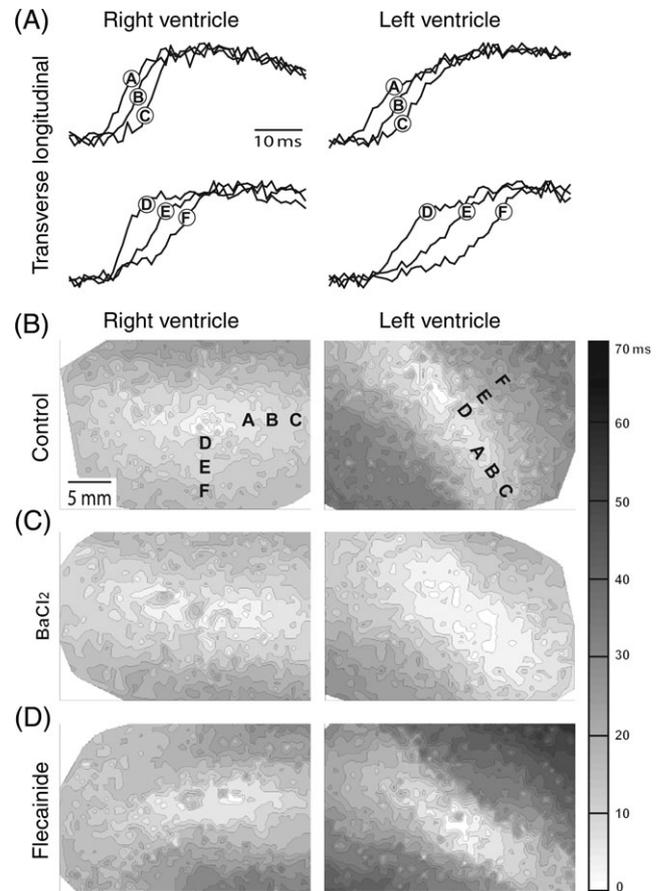
$\text{Na}_v1.5$  expression was quantified using western immunoblotting in homogenized tissue samples ( $n = 4$ ). *Figure 1A* shows representative bands corresponding to  $\text{Na}_v1.5$  protein expression from 12 regions of the heart. Visually, bands from the apical and basal regions of the anterior LV and the basal region of the posterior LV are denser than bands in the RV and septum, indicating greater  $\text{Na}_v1.5$  expression in these regions. Bands from the RV and septum appear to be similar, suggesting that the  $\text{Na}_v1.5$  expression in these regions may also be comparable. *Figure 1B* summarizes data from the four hearts tested, where the radii of the black-edged circles represent normalized  $\text{Na}_v1.5$  expression, and the dashed circles represent the standard error. Overall, there were no significant differences in  $\text{Na}_v1.5$  expression between the RV and the septum.  $\text{Na}_v1.5$  expression was significantly greater in the LV compared with the RV by 18.2%. In particular, anterior RV basal myocardium expressed significantly less  $\text{Na}_v1.5$  (18.8%) than the anterior basal region of the LV. Finally,  $\text{Na}_v1.5$  expression in the posterior RV base was significantly higher than the RV anterior base by 10.5%.

#### 3.2 Regional conduction velocity heterogeneities in the normal heart

Since protein quantity does not necessarily correlate to protein function, we measured conduction velocity ( $\theta$ ) in both ventricles precisely in regions with the lowest and greatest  $\text{Na}_v1.5$  expression levels in order to determine the effect of interventricular  $\text{Na}_v1.5$  expression heterogeneities. Conduction was quantified during pacing from the anterior epicardial surface of either the RV or LV. Upstrokes of epicardial action potentials from equally spaced sites along the longitudinal direction demonstrate uniform conduction velocity as evidenced by equal spacing between upstrokes in both the LV and RV (*Figure 2A*). However, faster RV transverse conduction relative to LV is evidenced by reduced temporal spacing of action potential upstrokes in the RV. Representative isochrone maps of activation from the two ventricles demonstrate elliptical spread of activation, consistent with the anisotropic spread of activation characteristic of cardiac tissue (*Figure 2B*). Also, the isochrone lines are more closely spaced perpendicular to the fibre direction, which indicates slower impulse propagation in the transverse direction.

The latest time of activation in the RV, as illustrated in *Figure 2B*, is earlier than that in the LV, and transverse isochrone spacing in the RV is greater than that in the LV, suggesting transverse RV  $\theta$  is greater than LV  $\theta$ . For all experiments over all cycle lengths tested, transverse conduction velocity ( $\theta_T$ ) was significantly greater in the RV compared with the LV ( $n = 4$ , *Figures 2A* and *B* and *3A*) by  $33.09 \pm 1.38\%$ . However, longitudinal conduction velocity ( $\theta_L$ ) was similar between the RV and LV (*Figures 2A* and *3A*) over all cycle lengths tested.

The anisotropic ratios for all experiments are summarized in *Figure 3B*. Anisotropy was significantly lower in the RV than in the LV at all cycle lengths ( $n = 4$ ), which is consistent with the more elliptical pattern of propagation observed in the LV (*Figure 2B*). Since there were no measurable



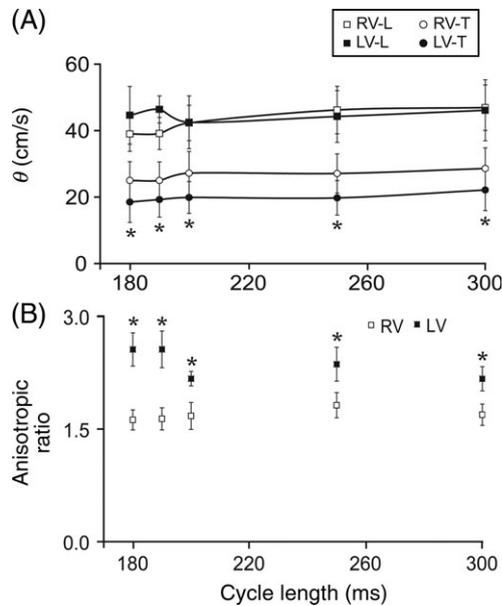
**Figure 2** (A) Action potentials from three equally spaced sites along both longitudinal and transverse directions of propagation are shown to demonstrate conduction delay. Representative isochrone maps of activation in the right and left ventricles. (B) At baseline, right ventricular  $\theta_T$  is greater than left ventricular  $\theta_T$ . (C) Partial  $I_{K1}$  blockade by  $\text{BaCl}_2$  significantly increases  $\theta$  in both ventricles. (D)  $I_{Na}$  blockade by  $0.5 \mu\text{M}$  flecainide decreases conduction velocity in both ventricles. Right ventricular  $\theta_T$  remains significantly greater than left ventricular  $\theta_T$ .

differences in  $\theta_L$  between the RV and LV at baseline and the anisotropic ratio did not change with any experimental intervention,  $\theta_T$  was chosen as the parameter for assessing conduction heterogeneities.

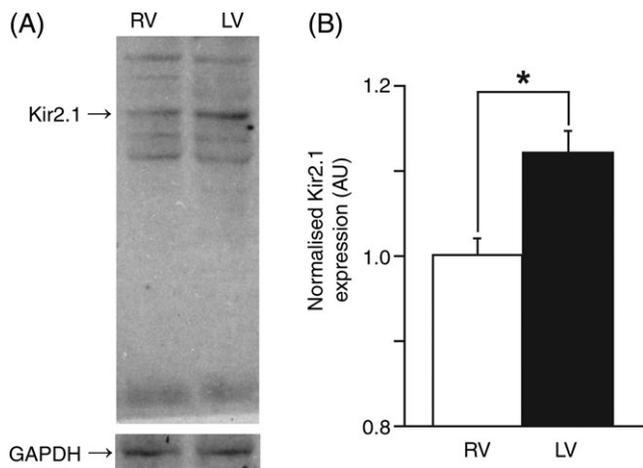
Importantly, greater RV  $\theta_T$  during control conditions is inconsistent with the lower  $\text{Na}_v1.5$  expression observed in the RV. These data suggest a possible role for an ion channel other than  $\text{Na}_v1.5$ , also heterogeneously distributed between the ventricles, in determining  $\theta$  heterogeneities under control conditions.

#### 3.3 The effect of $I_{K1}$ heterogeneities on conduction

Representative western immunoblots in *Figure 4A* indicate reduced density of expression of Kir2.1, the principal ventricular pore-forming subunit of the inward-rectifying potassium channel,<sup>20</sup> in the RV relative to the LV. GAPDH expression was not significantly different between the RV and LV as illustrated in representative bands (*Figure 4A*). Therefore GAPDH was used as an additional control. For all experiments, Kir2.1 protein density was lower by  $12.0 \pm 1.5\%$  ( $n = 3$ ) (*Figure 4B*) in the RV compared with the LV. Further, it has been previously shown that peak  $I_{K1}$  current in the guinea pig RV is lower than in the LV.<sup>21</sup> In order to

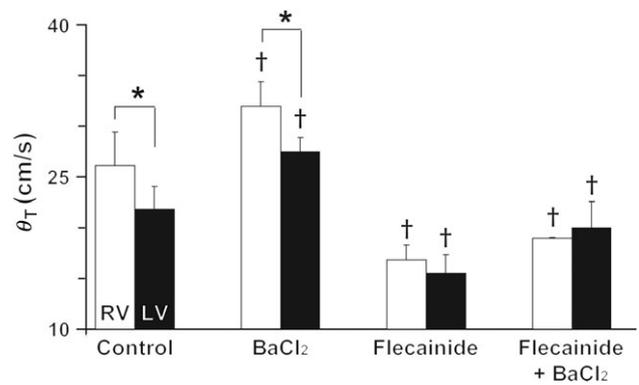


**Figure 3** (A)  $\theta_L$  and  $\theta_T$  in the right and left ventricles during control conditions for different pacing cycle lengths. Right ventricular  $\theta_T$  was significantly greater than left ventricular  $\theta_T$  for all cycle lengths ( $^*P < 0.05$ ). (B) Anisotropic ratio did not change significantly in either ventricle over the range of cycle lengths tested relative to baseline values ( $P = ns$ ). Interestingly, the right ventricle had a significantly lower anisotropic ratio than the left ventricle at all cycle lengths tested ( $^*P < 0.05$ ).



**Figure 4** (A) A representative western blot showing Kir2.1 expression in the right and left ventricles is shown along with corresponding GAPDH control bands. (B) Normalized Kir2.1 expression in the right ventricle (white bar) was  $12.0 \pm 1.5\%$  ( $^*P < 0.05$ ) lower relative to that in the left ventricle (black bar).

determine the influence of  $I_{K1}$  on conduction,  $10 \mu\text{M}$   $\text{BaCl}_2^{22}$  was perfused. Representative isochrone maps of activation in the presence of  $\text{BaCl}_2$  are presented in Figure 2C. The latest time of activation is decreased in both ventricles relative to control, whereas the spacing between isochrones is increased, demonstrating an increase in  $\theta$ . Over all experiments,  $\text{BaCl}_2$  increased  $\theta_T$  from  $26.1 \pm 3.3$  to  $32.0 \pm 2.4$  cm/s in the RV and from  $21.9 \pm 2.2$  to  $27.5 \pm 2.9$  cm/s in the LV ( $n = 4$ , Figure 5).



**Figure 5** Blockade of  $I_{K1}$  by  $\text{BaCl}_2$  caused a significant increase in  $\theta_T$  in both ventricles relative to control ( $^*P < 0.05$ ). Right ventricular  $\theta_T$  was significantly greater than left ventricular  $\theta_T$  both in the presence and absence of  $\text{BaCl}_2$  ( $^*P < 0.05$ ) (averaged over both flecainide and  $\text{BaCl}_2$  groups). Flecainide ( $0.5 \mu\text{M}$ ) caused  $\theta_T$  to significantly decrease in both ventricles ( $^*P < 0.05$ ). However,  $\text{BaCl}_2$  did not have a significant effect on  $\theta_T$  in the presence of  $0.5 \mu\text{M}$  flecainide ( $P = ns$ ). The difference between right ventricular and left ventricular  $\theta_T$  did not attain statistical significance in the presence of flecainide.

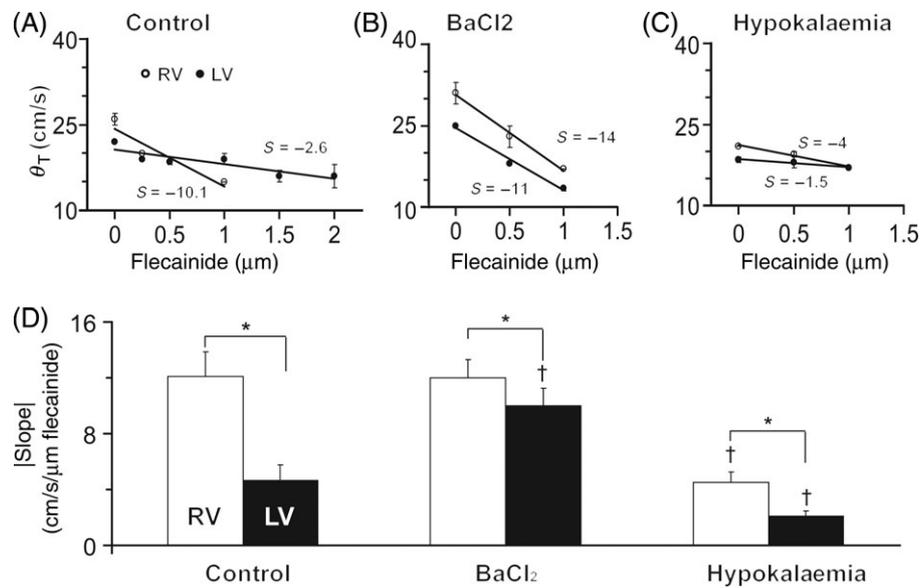
### 3.4 Effect of $I_{Na}$ availability on conduction

$I_{Na}$  was reduced by perfusing flecainide ( $0.5 \mu\text{M}$ ), a relatively specific blocker of the cardiac sodium channel ( $\text{Na}_v1.5$ ). Representative activation isochrone maps in the presence of flecainide (Figure 2D) demonstrate that the latest time of activation is increased and the spacing between isochrones decreased in both ventricles relative to control, indicating conduction slowing. Overall, flecainide reduced  $\theta_T$  from  $26.1 \pm 3.3$  to  $16.9 \pm 1.4$  cm/s in the RV and from  $21.9 \pm 2.2$  to  $15.5 \pm 1.9$  cm/s in the LV ( $n = 4$ , Figure 5). Although flecainide ( $0.5 \mu\text{M}$ ) caused conduction slowing in both ventricles, the fractional decrease in  $\theta_T$  was greater in the RV than in the LV.

In order to assess interventricular heterogeneities in conduction dependence on sodium channel availability, different concentrations of flecainide ( $0.25$ ,  $0.50$ ,  $1.00$ ,  $1.50$ , and  $2.00 \mu\text{M}$ ) were perfused. A representative plot of  $\theta_T$  vs. the concentrations of flecainide is presented in Figure 6A. In both ventricles,  $\theta_T$  decreased with increasing concentrations of flecainide until eventually pacing was no longer possible. The decrease of  $\theta_T$  with increasing flecainide concentrations was fit to a straight line ( $R^2 = 0.91 \pm 0.03$  for all experiments). Conduction dependence on sodium channel availability was approximated by the absolute slope of the linear fit of  $\theta_T$  vs. flecainide concentrations in each ventricle. Importantly, the dependence of  $\theta_T$  on sodium channel blockade was significantly greater in the RV than in the LV ( $n = 3$ , Figure 6D).

### 3.5 Effect of $I_{K1}$ blockade on conduction

Although  $I_{K1}$  blockade by  $\text{BaCl}_2$  caused a significant increase in  $\theta_T$  in the absence of flecainide, it did not significantly increase  $\theta_T$  in the presence of flecainide ( $n = 3$ , Figure 5). This suggests that  $I_{K1}$  heterogeneities may be a significant determinant of conduction heterogeneities under control conditions. However, the role of  $I_{K1}$  may be diminished when sodium channel availability is reduced. Therefore, we studied the role partial  $I_{K1}$  blockade plays in modulating conduction dependence on sodium channel availability.  $\theta_T$



**Figure 6** Representative plots of  $\theta_T$  vs. flecainide concentration are shown in (A) (control conditions), (B) ( $\text{BaCl}_2$ ), and (C) (hypokalaemia). In all cases,  $\theta_T$  decreased in both ventricles with increasing flecainide concentration. The slopes ( $S$ ) of linear fits are indicated in  $\text{cm/s}/\mu\text{M}$  flecainide. (D) Conduction dependence on sodium channel availability. Under control conditions, the slope was significantly greater in the RV compared with LV ( $*P < 0.05$ ). During  $I_{K1}$  blockade, the slope was significantly greater in the LV relative to LV control ( $^\dagger P < 0.05$ ) but remained unchanged in the RV ( $P = \text{ns}$ ). Although the difference in slope during  $\text{BaCl}_2$  between RV and LV was significantly reduced from control, the RV conduction dependence on sodium channel availability remained significantly different from LV ( $*P < 0.05$ ). During hypokalaemia, the slope was significantly reduced in both ventricles in comparison with control ( $^\dagger P < 0.05$ ) but the RV slope still remained significantly higher than the LV slope ( $*P < 0.05$ ).

was measured in the two ventricles while varying flecainide concentration in the presence of  $10 \mu\text{M}$   $\text{BaCl}_2$ . A representative plot of  $\theta_T$  vs. flecainide concentrations in the presence of  $\text{BaCl}_2$  is presented in Figure 6B. As demonstrated previously,  $\theta_T$  was greater in the RV relative to the LV before flecainide was perfused, and  $\theta_T$  decreased in both ventricles with increasing concentrations of flecainide. Interestingly, the dependence of  $\theta_T$  on flecainide increased significantly in the LV but did not change in the RV when compared with control. However, the dependence of  $\theta_T$  on flecainide remained significantly greater in the RV than in the LV, although the difference was reduced in magnitude when compared with control conditions. For all experiments,  $\text{BaCl}_2$  significantly increased the slope of  $\theta_T$  vs. flecainide concentration in the LV ( $n = 3$ ) but did not significantly change conduction dependence on sodium channel availability in the RV (Figure 6D). Again, the difference of conduction dependence on sodium channel availability between the ventricles was significantly reduced by  $\text{BaCl}_2$  when compared with control conditions.

These data suggest that  $I_{K1}$  heterogeneities underlie interventricular conduction heterogeneities under control conditions, but the interventricular heterogeneity in  $\text{Na}_v1.5$  expression becomes functionally relevant under challenging conditions where  $I_{\text{Na}}$  is functionally reduced.

### 3.6 Effect of hypokalaemia on conduction

Changes in extracellular potassium concentration ( $[\text{K}^+]_o$ ) affect  $I_{K1}$  as well as the resting membrane potential and thus have an important effect on conduction. Hypokalaemia ( $3 \text{ mM } [\text{K}^+]_o$ ) by itself significantly reduced  $\theta_T$  in both ventricles ( $n = 3$ ). When varying concentrations of flecainide were applied during hypokalaemia,  $\theta_T$  decreased in

both ventricles ( $n = 3$ ) as seen from the representative plot in Figure 6C. Interestingly, the dependence of  $\theta_T$  on flecainide decreased in both ventricles during hypokalaemia but RV dependence remained significantly greater than that of the LV ( $n = 3$ , Figure 6D).

## 4. Discussion

Conduction slowing secondary to decreased  $I_{\text{Na}}$  has been well established as a pro-arrhythmic factor<sup>1,2</sup>; however, the precise role of conduction heterogeneities in arrhythmogenesis remains incompletely understood. The aim of the study was to identify ion channel heterogeneities related to phase 0 depolarization and to determine their effects on conduction. Although the role of the transient-outward potassium current ( $I_{\text{to}}$ ) in arrhythmogenesis with reference to increased repolarization gradients has been well established,<sup>23</sup> the  $I_{\text{to}}$  peak occurs about 20 ms after initial depolarization.<sup>24,25</sup> It is therefore unlikely that  $I_{\text{to}}$  plays a significant role in determining heterogeneities related to phase 0 depolarization. Therefore, guinea pig, which does not functionally express  $I_{\text{to}}$ ,<sup>26</sup> was chosen as the animal model for this study.

We hypothesized that the mechanism of interventricular electrophysiological heterogeneity during loss of sodium channel function is based on heterogeneous distribution of cardiac sodium channels, leading to preferential conduction slowing and decreased depolarization reserve in the RV. Indeed,  $\text{Na}_v1.5$  expression in the RV is reduced relative to the LV, consistent with previous findings in sheep.<sup>14</sup> Although  $I_{\text{Na}}$  availability is a well-established determinant of conduction velocity,<sup>2</sup> the observed  $\text{Na}_v1.5$  expression heterogeneity does not correlate with conduction patterns.

#### 4.1 Heterogeneous conduction in the normal myocardium

Baseline values for  $\theta_L$  and  $\theta_T$  are consistent with previously reported results in guinea pig.<sup>27</sup> In particular, RV  $\theta_T$  is significantly greater than LV  $\theta_T$ , which is also in agreement with previous results.<sup>28</sup> The effects of ion channel heterogeneities on  $\theta$  are not expected to be directionally dependent, and  $\theta_L$  was not significantly different between the ventricles. One possible explanation is that the large variation of  $\theta_L$  (as measured by the standard deviation) may mask conduction heterogeneities.  $\theta_T$  on the other hand had a smaller variability, allowing relatively more precise measurement of changes in  $\theta$ . No intervention changed the anisotropic ratio, consistent with the idea that ion channel heterogeneities do not affect  $\theta$  in a directionally dependent manner.<sup>29</sup> Therefore, the significantly different RV and LV conduction anisotropy may be due to greater measurement sensitivity of  $\theta_T$  than  $\theta_L$ . Therefore,  $\theta_T$  was used as the more sensitive measure of conduction changes for this study.

#### 4.2 $I_{K1}$ heterogeneities underlie conduction heterogeneities in the normal heart

As mentioned previously, interventricular  $\theta_T$  heterogeneity does not agree with  $\text{Na}_v1.5$  expression patterns. We hypothesized that this apparently paradoxical behaviour may result from heterogeneities in  $I_{K1}$ . The magnitude of the  $I_{K1}$  current is small in comparison with that of the sodium current, but  $I_{K1}$  opposes depolarization particularly before sodium channels fully activate. Therefore, we hypothesized that reducing  $I_{K1}$  opposition to  $I_{\text{Na}}$ -mediated depolarization may increase  $\theta$ .

A smaller peak  $I_{K1}$  current and lower Kir2.1 RNA levels in the RV than in the LV have been previously reported in guinea pig,<sup>21</sup> which are consistent with reduced RV Kir2.1 protein expression levels reported in this study. However, this is inconsistent with other studies that have been unable to demonstrate difference in Kir2.1 RNA levels between ventricles.<sup>30</sup> The discrepancy may be due to difference between animals, experimental technique, or that RNA quantity does not necessarily correlate to protein expression.

Regions with reduced Kir2.1 demonstrated the greatest  $\theta_T$ . Furthermore, when  $I_{K1}$  was blocked with  $\text{BaCl}_2$ ,  $\theta_T$  significantly increased. Importantly, LV  $\theta_T$  during  $\text{BaCl}_2$  increased to similar values as RV  $\theta_T$  under control conditions. This suggests that the faster conduction in the RV under control conditions may in part be explained on the basis of the reduced  $I_{K1}$  in the RV relative to the LV.

#### 4.3 Effect of $I_{\text{Na}}$ availability on conduction: normal vs. disease states

Although  $I_{K1}$  heterogeneities may explain baseline  $\theta$  heterogeneities, they do not fully explain ventricular-specific responses to sodium channel blockade. Therefore, one would expect that interventricular differences in conduction dependence on sodium channel availability reflect regional  $\text{Na}_v1.5$  heterogeneities. Flecainide significantly decreased  $\theta_T$  in both ventricles,<sup>31</sup> but the fractional decrease in  $\theta_T$  was greater in the RV relative to the LV. In order to determine whether there was any difference between the ventricles in conduction dependence on sodium channel

availability, varying concentrations of flecainide were applied. Shaw and Rudy<sup>2</sup> previously demonstrated in a mathematical model that  $\theta$  and sodium conductance ( $g_{\text{Na}}$ ) were curvilinearly related. Our data were well fit by a line, and the absolute value of the slope of this line was used as a measure of conduction dependence on sodium channel availability. The absence of the curvilinear relationship in this study may be due to the use of flecainide concentration steps that were not sufficiently fine, particularly near the point of conduction failure.

RV  $\theta_T$  exhibited significantly greater dependence on flecainide concentration than LV  $\theta_T$ , and the results were independent of the order of flecainide doses. This is consistent with the reduced expression of  $\text{Na}_v1.5$  in the RV relative to the LV. These results suggest that interventricular  $I_{K1}$  heterogeneities mask  $\text{Na}_v1.5$  heterogeneities under control conditions but fail to do so during sodium channel blockade.

#### 4.4 $I_{K1}$ blockade unmasks effect of $\text{Na}_v1.5$ heterogeneities on conduction

Blockade of  $I_{K1}$  by  $10 \mu\text{M}$   $\text{BaCl}_2$ , which caused  $\theta_T$  to increase significantly under control conditions, did not cause a significant change in  $\theta_T$  in either ventricle in the presence of  $0.5 \mu\text{M}$  flecainide. This indicates that the dependence of interventricular conduction heterogeneities shifts from  $I_{K1}$  heterogeneities under control conditions to  $I_{\text{Na}}$  heterogeneities when  $I_{\text{Na}}$  is reduced. Therefore, the roles played by  $I_{\text{Na}}$  and  $I_{K1}$  in conduction are likely synergistic, not summative. In order to test this hypothesis, we applied different concentrations of flecainide to the heart in the presence of  $\text{BaCl}_2$ . Although flecainide decreased  $\theta_T$  in both ventricles,  $I_{K1}$  blockade attenuated the chamber-specific differences in conduction dependence on sodium channel availability. This further supports the hypothesis that  $I_{K1}$  heterogeneities can compensate for  $I_{\text{Na}}$  heterogeneities during impulse propagation under normal conditions.

Interestingly,  $I_{K1}$  blockade increased  $\theta_T$  dependence on flecainide in the LV but did not cause a significant change in the RV. This suggests that the mechanism underlying the interventricular heterogeneity in conduction dependence on sodium channel availability may be two-fold. The larger  $I_{K1}$  current in the LV depresses control LV  $\theta$ , whereas the greater  $\text{Na}_v1.5$  expression may act to lower the dependence of LV conduction on sodium channel blockade. These effects may act synergistically to attenuate the dependence of LV  $\theta_T$  on sodium channel blockade.

Since the LV expresses more  $I_{K1}$  and  $I_{\text{Na}}$  than the RV, further reducing  $I_{K1}$  unmasks LV conduction dependence on sodium channel availability, suggesting a greater 'depolarization reserve' in the LV. The RV on the other hand may function with a reduced depolarization reserve. Therefore, additional  $I_{K1}$  reductions do not significantly affect conduction dependence on sodium channel availability when  $I_{\text{Na}}$  is functionally reduced. In other words, conduction dependence on sodium channel availability under conditions of reduced  $I_{K1}$  most likely reflects  $\text{Na}_v1.5$  expression heterogeneities.

#### 4.5 Synergistic conduction slowing by hypokalaemia and sodium channel blockade

Apart from specific blockade by compounds such as  $\text{BaCl}_2$ ,  $I_{K1}$  is also affected by changes in extracellular potassium

concentration ( $[K^+]_o$ ). However, changes in  $[K^+]_o$  also affect the resting membrane potential and consequently the availability of sodium channels. Thus, changes in  $[K^+]_o$  have a significant bearing on conduction.<sup>32</sup> Further, some anecdotal reports have linked hypokalaemia to an increased risk of VT/VF in Brugada patients,<sup>33,34</sup> suggesting that hypokalaemia may exacerbate conduction abnormalities. Therefore, we studied the effect of hypokalaemia (3 mM  $[K^+]_o$ ) on the dependence of conduction on sodium availability. Hypokalaemia significantly decreased  $\theta_T$  in both ventricles and caused a significant decrease in the dependence of  $\theta_T$  on flecainide in both ventricles. Although the depression of conduction dependence on sodium channel availability may appear anti-arrhythmic, this depression may be insufficient to counteract the pro-arrhythmic effects of conduction slowing. Therefore, hypokalaemia in the RV may result in reduced safety of conduction at lower concentrations of flecainide when compared with control.

## 5. Conclusions

Interventricular  $I_{K1}$  heterogeneities underlie the conduction heterogeneities observed under control conditions. However, under conditions where the sodium current is reduced, the heterogeneity in sodium channel expression plays a greater role in determining conduction heterogeneities. Importantly,  $I_{Na}$  heterogeneities could exacerbate regional susceptibility to arrhythmias in disease states where  $I_{Na}$  may be functionally reduced.

### 5.1 Limitations

Although BaCl<sub>2</sub> is a potent  $I_{K1}$  blocker, it has some cross-reactivity with the L-type calcium channel.<sup>35</sup> However, the 10  $\mu$ mol/L used in this study is orders of magnitude smaller than concentrations reported to significantly affect L-type calcium channels.<sup>36</sup> Similarly, although flecainide is relatively specific in blocking  $I_{Na}$ , it has been shown to inhibit  $I_{to}$  and a maintained outward potassium current ( $I_K$ ) in rat.<sup>37</sup> Guinea pigs do not functionally express  $I_{to}$ , and  $I_K$  block is significant at higher flecainide concentrations than used in this study. BDM has been shown to cause no significant change in conduction velocity in the guinea pig heart.<sup>38</sup> In other animals, BDM is associated with a small depression of conduction velocity at doses significantly greater than the one used in this study.<sup>39,40</sup> Therefore, it is unlikely that BDM significantly affected the principal findings of this study.

This study does not address the effects of heterogeneities in cell size,<sup>41</sup> cell geometry, wall thickness, or connexin distribution on conduction. Although these effects may be significant, particularly with respect to differences in conduction anisotropy between the ventricles, they are unlikely to affect the principle finding of this study that interventricular conduction differences are in part dependent on  $I_{K1}$  and  $I_{Na}$  heterogeneities.

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