Heart block in mice overexpressing calcineurin but not NF-AT3

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Abstract

Objective: Overexpression of calcineurin causes cardiac hypertrophy and arrhythmic deaths. During disease development, sinus bradycardia followed by high degree atrioventricular (AV) block finally culminating in ventricular asystole has been observed over time in calcineurin hearts. AV block is associated with the development of pleomorphic ventricular tachycardia in mice and downregulation of potassium currents in ventricular myocytes. We tested the hypothesis that the abnormalities of AV block and propensity to ventricular tachycardia relate to overexpression of calcineurin independent of the development of hypertrophy.

Methods: Cardiac electrophysiologic properties were compared in isolated perfused hearts with ventricular hypertrophy due to overexpression of calcineurin or NF-AT3 and in their corresponding wild types at 15 or 30 days of age.

Results: Compared to wild-type hearts, significant prolongation of sinus node recovery times was noted in both NF-AT3 and calcineurin hearts. Compared to wild-type hearts, Wenckebach cycle length (WCL) and the left ventricular effective refractory period (LVERP) were significantly prolonged in the calcineurin hearts (\(p<0.05\)) but not NF-AT3 hearts. In calcineurin mice, left ventricular effective refractory period impinged on Wenckebach cycle length resulting in a significant correlation between left ventricular effective refractory period and Wenckebach cycle length (\(r^2=0.56\)). No such correlation was observed for wild type or NF-AT3 hearts. At 30 days of development, ventricular tachycardia developed in 70% of calcineurin hearts compared to 0% wild-type hearts (\(p=0.003\)), whereas ventricular tachycardia was observed in 33% of NF-AT3 hearts and 10% of corresponding wild-type hearts (\(p=NS\)).

Conclusions: The prolonged ventricular refractoriness, seen only in calcineurin hearts, impinges on Wenckebach cycle length resulting in heart block and is associated with propensity to ventricular tachycardia.

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Keywords: Hypertrophy; Sinus node; Transgenic animal models; Arrhythmias

1. Introduction

Not all forms of ventricular hypertrophy have equivalent cardiovascular phenotypes. Experimental data suggest that separate signaling pathways regulate pathological and physiological hypertrophy. The calcineurin–NF-AT3 signaling pathway is believed to contribute to pathological hypertrophy [1]. Calcineurin is a calcium/calmodulin-activated phosphatase that dephosphorylates nuclear factor of activated cells (NF-AT3). Dephosphorylated NF-AT3 translocates into the nucleus and interacts with transcription factors that activate a range of genes involved in hypertrophy. In contrast, insulin-like growth factor is believed to mediate physiological hypertrophy, independent of calcineurin [1].

While most forms of hypertrophy are associated with a downregulation of potassium currents [2–6], overexpression of L-type Ca(2+) channels results in the development of cardiac hypertrophy and myocardial dysfunction in the absence of downregulation of potassium currents [7]. Heart
block is another cardiovascular phenotype that is observed in some, but not all models of cardiac hypertrophy [6,8]. Some recombinant mouse models of hypertrophic cardiomyopathy, e.g., myosin binding protein C, manifest no abnormalities of ECG intervals [9]. In contrast, mice overexpressing calcineurin experience sudden cardiac death [10] which is secondary to heart block [6]. Mice overexpressing NF-AT3 also develop hypertrophy, but the risk of sudden death is reduced [4]. We hypothesized that abnormalities of atrioventricular (AV) conduction and propensity to ventricular tachycardia (VT) relate to overexpression of calcineurin independent of the development of hypertrophy. Since activated calcineurin results in down-regulation of potassium channels, we also assessed whether the smaller residual potassium currents would result in exaggerated responses to pharmacologic blockade of the potassium channels [2,11]. The approach used in this study was to compare the electrophysiologic properties of hearts manifesting similar extents of ventricular hypertrophy either due to overexpression of calcineurin or NF-AT3.

2. Methods
2.1. Study models

Transgenic mice with hypertrophy secondary to calcineurin or NF-AT3 overexpression and the corresponding wild-type mice were studied. Genotypes were determined by Southern blot PCR of DNA obtained from tail biopsy specimens [10]. For PCR analysis, oligonucleotides to detect the NF-AT3 transgene were 5'-TGTGACTTCTGCAGCA-CAA-3' upstream and 5'-TTGACCCAGCATCTCAGTCAC -3' downstream. The PCR conditions were as follows: denaturation at 95 °C for 1 min, followed by 30 cycles of 1 min at 94 °C, 1 min at 60 °C, and 1 min at 72 °C. All animals were housed in the animal care unit of the University of Calgary. All procedures were approved by our institutional Animal Care Committee. These guidelines comply with the Canadian Council on Animal Care and to the National Institutes of Health guidelines for care and use of animals.

The phenotype of only one line of each transgenic mouse model was studied. However, Molkentin et al. [10] reported that the phenotypes of the transgenic mice are consistent across different lines. Specifically, the phenotype of three transgenic lines of NF-AT3 and three transgenic lines of calcineurin has been evaluated. The phenotype of the NF-AT3 transgene line is the same in three different lines as is the phenotype of the calcineurin mice in the three different lines.

2.2. Experimental preparation

Prior to sacrifice, the ECG was recorded in conscious unsedated mice for measurement of ECG parameters. Measurements included sinus cycle length (R–R interval), P–R interval, and Q–T interval. The end of the T wave was measured as time when the repolarization process returns to the isoelectric point. The isoelectric point was defined as the position between the end of the P wave and the beginning of the QRS. These definitions have been used in our previous work [12]. Following anesthesia with methoxyfluorane, hearts were isolated for perfusion studies 15 or 30 days following birth. Hearts were perfused in the Langendorf mode with a modified Krebs–Henseleit buffer consisting of (mM) NaCl 118.5, NaHCO3 25, KCl 3.3, MgSO4 1.2, KH2SO4 1.2, CaCl2 2.5, EDTA 0.5, glucose 11 at a flow rate of 1.5 ml/min.

Hearts were atrially paced via a bipolar platinum electrode at a pacing cycle length of 170 ms (pulse width 2.0 ms; twice diastolic threshold intensity). A bipolar platinum electrode was also positioned on the left ventricle for ventricular pacing protocols. Electrograms were recorded on the epicardial surface of the ventricles via custom-made Ag–AgCl electrodes [11,13]. SNRT was measured following atrial pacing at 170 and 120 ms for 30 s. SNRT was defined as the interval between the last atrial depolarization of the pacing train and first spontaneous atrial depolarization. Wenckebach cycle length (WCL) was determined during decremental atrial pacing starting at a cycle length of 150 ms and decrementing by increments of 10 ms until AV block occurred. The left ventricular effective refractory period (LVERP) was measured at pacing cycle lengths of 170 and 120 ms by the introduction of ventricular premature stimuli following an 8-beat drive train delivered at the ventricular apex. The extrastimulus was decremented in 2 ms intervals until refractoriness was reached.

2.3. Experimental protocol

A total of 84 hearts were studied (Table 1). Following a stabilization period of 10 min, baseline electrophysiologic parameters were measured including SNRT, WCL, LVERP and monophasic action potentials signals. The development of ventricular tachycardia (VT) during each study was documented. Since the transient outward current (Ito) is diminished in most models of cardiac hypertrophy and the rapidly inactivating component of the delayed rectifying current (Ik1) is not [2,11], the effects of the Ito blocker, dofetilide (15 nM), and the Ik1 blocker, 4-aminopyridine (0.1 mM), on electrophysiologic parameters were measured after perfusion of each drug for 15 min. A washout period of 15 min was allowed prior to perfusion of the second drug.

Hearts were blotted on completion of the perfusions studies and weighed. In separate experiments, myocytes were isolated from hearts of mice at 14 and 28 days and their capacitance was determined [6].

2.4. Statistical analysis

Data are presented as median with 25th and 75th percentiles or mean±1 standard deviation (S.D.) where
Appropriate. Differences between groups were compared using the paired t-test, unpaired t-test, factorial analysis of variance (ANOVA) for repeated measures, Friedman’s test or chi-square analysis where appropriate [14]. Logistic regression analysis was performed to assess the impact of age and species on the development of ventricular tachycardia [14]. Differences were considered statistically significant at a p value < 0.05.

3. Results

3.1. Cardiac hypertrophy

The characteristics of the hearts studied are shown in Table 1. Significant cardiac hypertrophy with increased septal and left ventricular wall thickness was observed by 15 days post gestation in both NF-AT3 and calcineurin mice. Hypertrophy continued to develop over time in both groups (p < 0.001). To assess the extent of hypertrophy of the individual ventricular myocytes, their capacitance was measured at 15 and 30 days of age (Table 1). The capacitance of calcineurin and NF-AT3 cells were significantly greater than their wild-type myocytes respectively (p < 0.001). The capacitance of calcineurin myocytes was similar to NF-AT3 at 30 days (p < 0.05).

3.2. Sinus node recovery times

Examples of a SNRT recorded in one wild-type heart and one calcineurin heart following atrial pacing at a cycle length of 170 ms are shown in Fig. 1. SNRTs measured at baseline, during dofetilide and 4-aminopyridine perfusion are shown in Table 2. SNRTs were significantly longer in the hypertrophied hearts compared to the wild-type hearts (p < 0.01, Friedman’s test). Similar changes were observed following atrial pacing at a cycle length of 120 ms (data not shown). No significant difference was observed comparing calcineurin to NF-AT3 hearts, either at baseline or during treatment with the potassium channel blockers.

3.3. Wenckebach cycle length

The WCL, measured at baseline, during dofetilide and 4-aminopyridine perfusion are shown in Fig. 2. WCL was similar in NF-AT3 and wild-type hearts at baseline and remained unchanged during dofetilide and 4-aminopyridine perfusion. At 15 days, baseline WCL was significantly longer (p < 0.05, factorial ANOVA for repeated measures) in the calcineurin hearts compared to wild-type hearts. While dofetilide and 4-aminopyridine perfusion had no significant effect in wild-type and NF-AT3 hearts, both drugs significant prolonged WCL in the calcineurin hearts.

Table 1

Experiments over time

<table>
<thead>
<tr>
<th></th>
<th>Calcineurin (15 days)</th>
<th>Wild type (15 days)</th>
<th>Calcineurin (30 days)</th>
<th>Wild type (30 days)</th>
<th>NF-AT3 (15 days)</th>
<th>Wild type (15 days)</th>
<th>NF-AT3 (30 days)</th>
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<td>6</td>
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<td>4</td>
<td>5</td>
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<td>Days post gestation</td>
<td>15 ± 1</td>
<td>16 ± 2</td>
<td>29 ± 1</td>
<td>29 ± 1</td>
<td>14 ± 1</td>
<td>15 ± 1</td>
<td>29 ± 2</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>0.115 ± 0.015*</td>
<td>0.049 ± 0.007</td>
<td>0.256 ± 0.049*</td>
<td>0.108 ± 0.018</td>
<td>0.086 ± 0.020*</td>
<td>0.045 ± 0.006</td>
<td>0.181 ± 0.038*</td>
<td>0.113 ± 0.025</td>
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<tr>
<td>Cell capacitance (pf)</td>
<td>141.6 ± 9.99*</td>
<td>77.38 ± 1.78</td>
<td>159.2 ± 6.40*</td>
<td>116.7 ± 3.61</td>
<td>130.2 ± 4.18*</td>
<td>74.50 ± 2.60</td>
<td>155.6 ± 10.5*</td>
<td>129.9 ± 2.46</td>
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<td>ECG intervals</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td>PR (ms)</td>
<td>46 ± 7*</td>
<td>33 ± 4</td>
<td>51 ± 21*</td>
<td>34 ± 4</td>
<td>28 ± 6</td>
<td>29 ± 2</td>
<td>29 ± 6</td>
<td>32 ± 6</td>
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<tr>
<td>QT (ms)</td>
<td>87 ± 29*</td>
<td>52 ± 13</td>
<td>77 ± 23*</td>
<td>40 ± 10</td>
<td>63 ± 19*</td>
<td>41 ± 8</td>
<td>56 ± 20</td>
<td>47 ± 6</td>
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<tr>
<td>RR (ms)</td>
<td>130 ± 26*</td>
<td>100 ± 30</td>
<td>103 ± 17*</td>
<td>76 ± 4</td>
<td>129 ± 27*</td>
<td>92 ± 7</td>
<td>104 ± 15*</td>
<td>79 ± 5</td>
</tr>
</tbody>
</table>

Data are mean ± S.D.

* p < 0.001 compared to wild type at same age.

† p < 0.001 compared to NF-AT3 at same age.
Although WCL tended to be longer in the calcineurin hearts compared to the NF-AT3 hearts, these differences were only statistically significant during dofetilide perfusion ($p<0.05$, factorial ANOVA for repeated measures). Consistent with the differences in WCL noted in the calcineurin hearts compared to wild-type hearts, the PR interval measured prior to study were longer in the calcineurin hearts compared to age-matched wild-type and NF-AT3 hearts (Table 1, $p<0.001$, factorial ANOVA for repeated measures).

At baseline, spontaneous episodes of heart block were observed at 30 days of development in 7/10 hearts overexpressing calcineurin (Fig. 3) and 0/9 wild-type hearts ($p<0.01$, chi-square analysis). All episodes were transient complete heart block except one episode of 2:1 second-degree AV block. In NF-AT3 overexpressors, spontaneous episodes occurred in 0/9 hearts and in 0/9 corresponding wild-type hearts ($p=NS$).

### 3.4. Left ventricular effective refractory period (LVERP)

LVERP measured at baseline and during dofetilide and 4-aminopyridine perfusion are shown in Fig. 4. LVERP was similar in the NF-AT3 hearts and wild-type hearts at 15 and 30 days post gestation and remained unchanged during dofetilide and 4-aminopyridine perfusion. In contrast, base-

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Calcineurin (15 days)</th>
<th>Wild type (15 days)</th>
<th>Calcineurin (30 days)</th>
<th>Wild type (30 days)</th>
<th>NF-AT3 (15 days)</th>
<th>Wild type (15 days)</th>
<th>NF-AT3 (30 days)</th>
<th>Wild type (30 days)</th>
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</thead>
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<tr>
<td>Baseline (s)</td>
<td>1.65 (0.79–15.00)*</td>
<td>0.14 (0.11–0.34)</td>
<td>4.02 (2.5–6.9)*</td>
<td>0.14 (0.11–0.18)</td>
<td>5.00 (0.92–6.44) j</td>
<td>0.24 (0.15–0.53)</td>
<td>2.78 (0.59–10.50)</td>
<td>1.00 (0.12–3.5)</td>
</tr>
<tr>
<td>Dofetilide (s)</td>
<td>3.30 (2.48–15.00)*</td>
<td>0.30 (0.18–0.89)</td>
<td>10.50 (6.03–15.00) j</td>
<td>0.33 (0.23–0.45)</td>
<td>3.68 (1.71–9.44) j</td>
<td>0.64 (0.11–1.48)</td>
<td>7.00 (1.25–11.50)</td>
<td>0.60 (0.18–2.50)</td>
</tr>
<tr>
<td>4-AP (s)</td>
<td>5.57 (2.38–14.35) j</td>
<td>0.50 (0.35–1.26)</td>
<td>10.00 (2.88–15.00) j</td>
<td>0.40 (0.31–0.52)</td>
<td>9.50 (5.92–15.00)</td>
<td>1.00 (0.64–3.07)</td>
<td>2.67 (0.74–6.31)</td>
<td>1.12 (0.70–6.69)</td>
</tr>
</tbody>
</table>

Median data with 25th and 75th percentiles shown. Differences compared by the Friedman test.

* $p<0.001$ compared to wild type at same age.

† $p<0.05$ compared to wild type at same age.

‡ $p<0.01$ compared to wild type at same age.

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Fig. 2. WCL measured at baseline and following dofetilide and 4-aminopyridine perfusion in NF-AT3 hearts (■) (upper panel) or calcineurin hearts (■) (lower panel) and corresponding wild-type hearts (■). Hearts were studied at 15 or 30 days post gestation. WCL was significantly longer in calcineurin hearts compared to wild-type hearts at baseline and during dofetilide or 4-aminopyridine perfusion. Data are mean±S.D. Differences compared by factorial ANOVA for repeated measures. *$p<0.05$ compared to wild type at same age; **$p<0.01$ compared to wild type at same age; †$p<0.05$ compared to NF-AT3 at same age.
LVERP was significantly prolonged in the calcineurin hearts ($p<0.05$ compared to the wild-type hearts, factorial ANOVA for repeated measures). Both dofetilide and 4-aminopyridine significantly prolonged LVERP only in the calcineurin hearts ($p<0.01$). At 30 days, the LVERP measured at baseline was significantly greater in the calcineurin hearts compared to the NF-AT3 hearts ($p<0.05$). Similar differences were observed during dofetilide and 4-aminopyridine perfusion ($p<0.05$). Consistent with the differences in LVERP noted in the calcineurin hearts compared to wild-type and NF-AT3 hearts, the QT intervals measured prior to study were significantly longer in the calcineurin hearts compared to age matched wild-type and NF-AT3 hearts (Table 1, $p<0.001$ factorial ANOVA for repeated measures).

To assess whether prolongation of WCL was physiologically related to prolongation of refractoriness of ventricular tissue, we correlated WCL to LVERP (Fig. 5). In wild-type and NF-AT3 hearts, the duration of LVERP was substantially shorter than WCL, and thus there was no correlation between LVERP and WCL. In contrast, in calcineurin mice, the measured LVERP impinges on WCL, and there was a significant correlation between LVERP and WCL ($r^2=0.56$).

3.5. Spontaneous VT

An example of nonsustained VT elicited during ventricular pacing in a calcineurin heart is shown in Fig. 6. Nonsustained VT developed more frequently during atrial or ventricular pacing protocols in the calcineurin hearts compared to the NF-AT3 and wild-type hearts ($p<0.01$, Fig. 6). At 15 days gestation, nonsustained VT was observed in 1/9 NF-AT3 hearts and 2/14 wild-type hearts ($p=NS$) compared to 6/9 calcineurin hearts and 2/15 wild-type hearts ($p<0.05$, chi-square analysis). At 30 days gestation, nonsustained VT was observed in 3/9 NF-AT3 hearts and 1/11 wild-type hearts ($p=NS$) compared to 7/10 calcineurin hearts and 0/15 wild-type hearts ($p=0.003$, chi-square analysis). Independent of age, VT was more often observed.

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**Fig. 3.** Examples of spontaneous episodes of heart block observed in two calcineurin hearts at 30 days post gestation.

**Fig. 4.** Upper Panel: Left ventricular effective refractory period (LVERP) measured at baseline and during dofetilide perfusion and 4-aminopyridine perfusion in NF-AT3 hearts secondary to NF-AT3 (■, upper panel) or calcineurin (▲, lower panel) overexpression and corresponding wild-type hearts (□). Hearts were studied at 15 or 30 days post gestation. LVERP was significantly longer in the calcineurin hearts at baseline (30 days) and during dofetilide and 4-aminopyridine perfusion. Data are mean±S.D. Differences compared by factorial ANOVA for repeated measures. *$p<0.05$ compared to wild type at same age; **$p<0.01$ compared to wild type at same age; ***$p<0.001$ compared to wild type at same age; # $p<0.05$ compared to NF-AT3 at same age.
mice is associated with a significant increase in the propensity to ventricular tachycardia.

4.1. Abnormalities of sinus node function

Similar prolongation of SNRTs was observed in the calcineurin and NF-AT3 hearts. Prolongation of SNRT has been reported by Berul et al. in transgenic mice with hypertrophic cardiomyopathy secondary to an α-myosin heavy chain mutation, although the magnitude of prolongation of SNRT was much less than observed in the present study [15]. Abnormal sinus node function has also been reported in a rabbit model of pressure-volume overload heart failure [16]. The ionic current determinants of sinus node recovery include activity of $I_f$, $K_{ACh}$, $K_{Kr}$, $I_{Ca}$ and the sodium–calcium exchanger [17]. The possible mechanisms of abnormal SNRT include downregulation of one or more ionic channels in hypertrophy. Dofetilide and 4-AP did not cause exaggerated prolongation of SNRT in hypertrophy suggesting that other channels or transporters contribute to the abnormalities of sinus node function observed in this study. These data suggest a common mechanistic link between sinus node dysfunction and the

Fig. 5. LVERP impinges on WCL resulting in a correlation between LVERP and WCL, only in calcineurin hearts. The upper panel shows the significant correlation of LVERP and WCL in the calcineurin hearts at 30 days compared to its wild-type controls. The lower panel shows the absence of a correlation for NF-AT3 hearts and its wild-type controls at 30 days.

Fig. 6. Upper Panel: Example of nonsustained VT during atrial pacing in one calcineurin heart. Lower Panel: VT at baseline in the NF-AT3 (■) and calcineurin hearts (▲) and their corresponding wild-type hearts (▲). VT was significantly higher in the hypertrophied hearts secondary to calcineurin overexpression compared to the wild-type hearts at 15 days ($p<0.05$) and at 30 days post gestation ($p=0.003$). The probability of developing spontaneous VT was not significantly increased in the hypertrophied hearts secondary to NF-AT3 overexpression. Independent of age, VT was significantly more likely to occur in calcineurin hearts compared to wild type or NF-AT3 hearts ($p<0.01$ by logistic regression analysis).
development of cardiac hypertrophy. However, the cellular mechanisms require further study.

4.2. Heart block and ventricular refractoriness

When the cycle length of pacing impinges on the refractory period of either the ventricular or His Purkinje tissue, Wenckebach periodicity is to be expected. To assess whether prolongation of WCL was physiologically related to prolongation of refractoriness of ventricular tissue, we correlated WCL to LVERP. In wild-type and NF-AT3 hearts, the duration of LVERP was substantially shorter, did not impinge on WCL and thus there was no correlation between LVERP and WCL. In calcineurin mice, the measured LVERP impinged on WCL, resulting in a significant correlation between LVERP and WCL. These data are consistent with the notion that refractoriness of ventricular tissue is an important determinant of WCL in the calcineurin mice. Previous studies have reported that prolongation of the ventricular action potential duration of calcineurin myocytes relates to downregulation of potassium currents [6]. Pharmacologic blockade of the residual potassium currents also prolonged WCL and LVERP in calcineurin hearts again resulting in a significant correlation between LVERP and WCL. These data indicate that the downregulation of potassium currents likely contributes to prolongation of WCL in calcineurin mice. This observation does not mean that potassium current downregulation is necessarily the sole element responsible for prolongation of WCL noted in the present study. Other important regulators of atrioventricular and infra Hisian conduction include electrotonic interactions, sodium channel density, and cell-to-cell coupling (connexins). In a previous study, we have also observed downregulation of the sodium channel in calcineurin ventricular myocytes, which could also contribute to prolongation of the WCL (unpublished observations). The development of transient AV block in vitro parallels our previous observations of spontaneous heart block in conscious calcineurin mice [6].

4.3. Dofetilide and 4-aminopyridine pharmacodynamics

Dofetilide and 4-aminopyridine caused greater prolongation of WCL and LVERP in the calcineurin hearts compared to wild-type hearts and also had greater effects on these parameters in calcineurin hearts compared to NF-AT3 hearts. These effects may be explained in part by greater downregulation of the transient outward current in the calcineurin hearts compared to the NF-AT3 hearts and wild-type hearts [4,6]. Although $I_{Kr}$ is not a dominant repolarizing current in adult mice [12,18], the role of this current may become more prominent in left ventricular hypertrophy when other potassium repolarizing currents are downregulated [2,11]. In other experimental models of cardiac hypertrophy, $I_{Kr}$ blocking drugs have been shown to cause greater prolongation of ventricular repolarization compared to control [11,19]. Although $I_{to}$ is downregulated in calcineurin hearts, 50% of this current remains [4,6]. It is thus possible that 4-aminopyridine causes greater prolongation of ventricular refractoriness in the calcineurin hearts due to the compromised repolarization reserve.

4.4. Ventricular arrhythmias

Nonsustained VT developed significantly more frequently in the calcineurin hearts compared to the NF-AT3 hearts, although significant left ventricular hypertrophy was observed in both groups. This difference decreased over time perhaps reflecting the increase in hypertrophy with age in the NF-AT3 hearts. The prolonged ventricular refractoriness seen only in calcineurin mice could contribute to the propensity to ventricular arrhythmias. Prolonged refractoriness is a known substrate for reentry. The previously reported downregulation of either potassium currents in calcineurin mice could contribute to the prolongation of ventricular refractoriness. However, our data does not define the mechanism of the ventricular arrhythmias observed in the calcineurin mice.

4.5. Potential mechanisms of electrophysiologic abnormalities in hypertrophy

The calcineurin–NF-AT3 signalling pathway is believed to contribute to pathological hypertrophy [1]. We assessed different components of the calcineurin/NF-AT3 signal transduction pathway. Calcineurin dephosphorylates NF-AT3, which then translocates into the nucleus and interacts with transcription factors that activate a range of genes involved in hypertrophy. Both models of cardiac hypertrophy manifested abnormalities of sinus node dysfunction whereas atrioventricular block, prolongation of ventricular refractoriness and propensity to VT were observed only in calcineurin mice. This suggests that overexpression of calcineurin mediates part of its phenotype independent of the NF-AT3 portion of the signal transduction pathway. One fundamental difference between the calcineurin and the NF-AT3 mice is that downregulation of ion channels is not manifest in NF-AT3 at this time in development [4,6]. These data suggest that differences in ion channel expression associated with the calcineurin mice contribute to the phenotypic difference. The data in the present study does not address the molecular mechanism by which calcineurin overexpression leads to downregulation of these ion channels. It is possible that the phosphatase activity of calcineurin and/or crosstalk between calcineurin and other signal transduction cascades may contribute to this phenotype [20].

4.6. Limitations

It is possible that some differences in electrophysiologic parameters may be due to aging [19,20]. However, we
compared differences to age matched wild-type hearts. Furthermore, we have previously reported that while developmental changes in mice occur in the first 14 days of life, no further changes in electrocardiographic parameters are observed after 14 days of age [17].

5. Conclusions

Significant abnormalities of sinus node function are associated with hypertrophy secondary to both calcineurin and NF-AT3 overexpression. The prolonged ventricular refractoriness, seen only in calcineurin hearts, impinges on WCL resulting in heart block and is associated with a propensity to ventricular tachycardia.

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References