No Contribution of IP₃-R(2) to Disease Phenotype in Models of Dilated Cardiomyopathy or Pressure Overload Hypertrophy

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Background—We investigated the contribution of inositol(1,4,5)-trisphosphate (Ins(1,4,5)P₃ [IP₃]) receptors (IP₃-R) to disease progression in mouse models of dilated cardiomyopathy (DCM) and pressure overload hypertrophy. Mice expressing mammalian sterile 20–like kinase and dominant-negative phosphatidylinositol-3-kinase in heart (Mst1×dn-PI3K-2Tg; DCM-2Tg) develop severe DCM and conduction block, associated with increased expression of type 2 IP₃-R (IP₃-R(2)) and heightened generation of Ins(1,4,5)P₃. Similar increases in Ins(1,4,5)P₃ and IP₃-R(2) are caused by transverse aortic constriction.

Methods and Results—To evaluate the contribution of IP₃-R(2) to disease progression, the DCM-2Tg mice were further crossed with mice in which the type 2 IP₃-R (IP₃-R(2)−/−) had been deleted (DCM-2Tg×IP₃-R(2)−/−) and transverse aortic constriction was performed on IP₃-R(2)−/− mice. Hearts from DCM-2Tg mice and DCM-2Tg×IP₃-R(2)−/− were similar in terms of chamber dilatation, atrial enlargement, and ventricular wall thinning. Electrophysiological changes were also similar in the DCM-2Tg mice, with and without IP₃-R(2). Deletion of IP₃-R(2) did not alter the progression of heart failure, because DCM-2Tg mice with and without IP₃-R(2) had similarly reduced contractility, increased lung congestion, and atrial thrombus, and both strains died between 10 and 12 weeks of age. Loss of IP₃-R(2) did not alter the progression of hypertrophy after transverse aortic constriction.

Conclusions—We conclude that IP₃-R(2) do not contribute to the progression of DCM or pressure overload hypertrophy, despite increased expression and heightened generation of the ligand, Ins(1,4,5)P₃. (Circ Heart Fail. 2013;6:318-325.)

Key Words: atrium ■ dilated cardiomyopathy ■ echocardiography ■ experimental models of heart failure ■ pressure overload

The failing myocardium from humans and experimental animals commonly shows increased expression of the receptors for the Ca²⁺-regulating second messenger inositol(1,4,5)-trisphosphate (Ins(1,4,5)P₃ [IP₃]) receptors (IP₃-R), often along with a decrease in ryanodine receptors, the master regulators of Ca²⁺ within the myocardium. The importance, or otherwise, of this has been debated. The online-only Data Supplement is available at http://circheartfailure.ahajournals.org/lookup/suppl/doi:10.1161/CIRCHEARTFAILURE.112.972158/-/DC1.

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Under physiological conditions, cardiomyocytes from all species studied show little or no change in Ins(1,4,5)P₃ in response to the activation of appropriately coupled receptors, and, in addition, the expression of IP₃-R is low. These findings argue against a strong involvement of IP₃-R under physiological conditions. However, our earlier and recent studies show that generation of Ins(1,4,5)P₃ is markedly heightened under pathological conditions such as atrial dilatation in humans, mice with dilated cardiomyopathy (DCM), and in hypertrophied mouse ventricle after chronic pressure overload. Furthermore, we have observed heightened expression of phospholipase Cβ1b, the enzyme responsible for generation of Ins(1,4,5)P₃, in myocytes, from failing human, mouse, and sheep myocardium (sheep data not shown). All these findings suggest that Ins(1,4,5)P₃ may be of heightened importance in failing myocardium, especially given the likelihood of increased IP₃-R expression.© 2012 American Heart Association, Inc.
We undertook an investigation of the possible pathological roles of IP$_3$-R using a mouse model with severe DCM and clear evidence of conduction block (DCM-2Tg), as well as a well-characterized model of pressure overload hypertrophy, induced by transverse aortic constriction (TAC). Both of these murine model show increased generation of Ins(1,4,5)P$_3$ and increased expression of type 2 IP$_3$-R (IP$_3$-R2), the IP$_3$-R subtype expressed in working cardiomyocytes. To examine possible contributions of the heightedn Ins(1,4,5)P$_3$ and IP$_3$-R2 to hypertrophy and dilatation, we performed TAC on IP$_3$-R2$^{-/-}$ mice and crossed the DCM-2Tg with IP$_3$-R2+/− mice and examined the effect on progression of disease in both scenarios.

**Methods**

**Experimental Animals**

All experiments were approved by the Alfred Medical Research and Education Precinct Animal Ethics Committee.

**DCM-2Tg Model**

Cardiac-specific Mst1 (line no. 28; C57BL/6 background) and dn-PI3K (FVB/N) transgenic mice were generated and genotyped as described previously. Male heterozygous Mst1-Tg (Mst1+/−) were genetically crossed with dnPI3K−/− and IP$_3$-R2−/− to generate DCM-2Tg mice (Mst1+/−/dnPI3K−/−, Mst1×dnPI3K-2Tg, DCM-2Tg) and DCM-2Tg-knockout mice (Mst1+/−/dnPI3K+/−/IP$_3$-R2+/−). The crossed strain (Mst1×dnPI3K-2Tg) was chosen for these studies because, unlike the Mst1-Tg, they show severe conduction block and we were interested in possible atrial actions of IP$_3$-R2. All mice were bred on the same mixed genetic background (C57BL/6/FVB/N).

**Transverse Aortic Constriction**

Two-month-old male mice were anesthetized with ketamine/xylazine and underwent a sham operation or were subjected to pressure overload induced by TAC as previously described. Echocardiography measurements were performed before surgery and at 1 and 4 weeks after TAC. The pressure gradients generated by aortic banding were measured by introducing high-fidelity pressure transducers into the left and right common carotids. Pressure gradients in IP$_3$-R2−/− and wild-type (WT) littermates were similar at 4 weeks post-TAC.

**Transthoracic Echocardiography and Electrocardiography**

Anesthesia was maintained with 1.7% isoflurane. Echocardiography was performed using a Philips iE33 ultrasound machine and a 15-MHz linear transducer. Electrocardiography (ECG) recordings were measured using the Powerlab System and BioAmp (ADInstruments). Data were measured digitally using ADInstruments (Chart 5 Pro ECG analysis module). Details are provided in the online-only Data Supplement.

**Measurement of Ins(1,4,5)P$_3$ and Its Metabolites in Atria and Ventricles (Phospholipase C Activation)**

Tissues were labeled with [3H]inositol and subsequently stimulated with norepinephrine (50 µmol/L). [3H]Ins(1,4,5)P$_3$ and its metabolites were extracted and quantified by high-performance liquid chromatography, as described previously. Details are provided in the online-only Data Supplement.

**Results**

**Hearts From DCM-2Tg Mice or TAC Mice Have Heightened Expression of IP$_3$-R2 and Heightened Generation of Ins(1,4,5)P$_3$**

The IP$_3$-R2 is expressed in working cardiomyocytes, and we measured expression in hearts from DCM-2Tg mice that have DCM, as well as from mice that have undergone TAC to induce pressure overload hypertrophy. The DCM-2Tg mice had heightened IP$_3$-R2 expression in atria and left ventricles (Figure 1A). Loss of IP$_3$-R2 in IP$_3$-R2−/− mice did not result in altered expression of either of the other IP$_3$-R subtypes, IP$_3$-R1 or IP$_3$-R3 (Figure 1 in the online-only Data Supplement). IP$_3$-R2 expression also was substantially elevated 4 weeks after TAC (Figure 1B and 1C).

We have previously reported that TAC-induced hypertrophy leads to heightened InsP responses in mouse hearts, resulting in increased generation of Ins(1,4,5)P$_3$. In the current study, we assessed Ins(1,4,5)P$_3$ generation in atria and ventricles from DCM-2Tg and littermate WT mice. This was accomplished by labeling the inositol phospholipids with [3H]inositol and subsequently measuring the generation of [3H]-labeled Ins(1,4,5)P$_3$ and its metabolites as a measure of total [3H]Ins(1,4,5)P$_3$ generation. As described previously, atria and ventricles from WT mice have low levels of [3H]InsPs after 20 minutes of treatment with norepinephrine (50 µmol/L), indicating minimal generation of Ins(1,4,5)P$_3$. Responses were substantially heightened in atria and ventricles from DCM-2Tg mice, reflecting increased Ins(1,4,5)P$_3$ production (Figure 1D).

**Deletion of IP$_3$-R2 Does Not Influence Heart Size in DCM-2Tg Mice or TAC Mice**

DCM-2Tg mice develop DCM, indicated by chamber dilatation, left ventricular wall thinning, and decreased contractile function. We argued that heightened generation...
been deleted (DCM-2Tg×IP 3-R(2)−/−) and compared this phenotype of the DCM-2Tg mice in which IP3-R(2) had in these animals. To test this possibility, we examined the R(2), might contribute substantially to the DCM phenotype of IP3-R(2) (DCM-2Tg). As reported previously, 12 DCM mice with DCM mice expressing the endogenous complement of IP3-R(2) in atria and ventricles from DCM-2Tg mice and littermate controls (wild type [WT]). Values shown are IP3-R(2) mRNA relative to GAPDH) in left ventricle of mice 4 weeks after TAC. The experiment was performed 3× with similar results. D, Generation of [3H]Ins(1,4,5)P3 and its metabolites shown are [3H]inositol phosphates, counts per min/mg of tissue, mean±SEM (n=5–7); *P<0.05 relative to WT or sham (Sh)-operated control (Mann-Whitney rank-sum test). LA indicates left atrium; LV, left ventricle; RA, right atrium; and RV, right ventricle.

of Ins(1,4,5)P3, together with heightened expression of IP3-R(2), might contribute substantially to the DCM phenotype in these animals. To test this possibility, we examined the phenotype of the DCM-2Tg mice in which IP3-R(2) had been deleted (DCM-2Tg×IP3-R(2)−/−) and compared this with DCM mice expressing the endogenous complement of IP3-R(2) (DCM-2Tg). As reported previously,12 DCM mice exhibited severe left ventricular dilatation (Figure IIB in the online-only Data Supplement and Table 1), increased atrial size, and ventricular wall thinning compared with WT mice (Table 1). There was no hypertrophy observed in the DCM-2Tg hearts. DCM-2Tg mice with IP3-R(2) deleted showed a phenotype indistinguishable from that of DCM-2Tg mouse (Tables 1 and 2).

In contrast to the DCM-2Tg model, mice subjected to TAC develop ventricular hypertrophy indicated by increased left ventricle/body weight ratio and increased ventricular wall thickness. Deletion of IP3-R(2) did not alter the cardiac phenotype either in TAC mice or in sham-operated controls. Measurements were made both early (1 week) and late (4 weeks after TAC), and no effect of IP3-R(2) deletion was observed at either time point (Figure 2).

Deletion of IP3-R(2) Does Not Alter Gene Expression Profiles in DCM-2Tg Mice or TAC Mice

DCM-2Tg mice show alterations in ventricular gene expression, reflecting disease progression.13 We examined transcriptional responses in DCM mice with and without their complement of IP3-R(2). As shown in Figure 3A, DCM in the DCM-2Tg was associated with increased expression of atrial natriuretic peptide and α-skeletal actin. Deletion of IP3-R(2) did not alter these expression changes. As reported previously,14 TAC induced changes in hypertrophic gene expression profiles, atrial natriuretic peptide, α-myosin heavy chain, β-myosin heavy chain, and α-skeletal actin, and these were not altered by deletion of IP3-R(2) (Figure 3C and 3D).

Ins(1,4,5)P3/IP3-R(2) signaling has been suggested to contribute to hypertrophy by generating the Ca2+ required to activate calcineurin, which in turn dephosphorylates the nuclear factor of activated T cells family of transcription factors culminating in altered gene expression profiles.17 Calcineurin is known to be activated by TAC, and we examined whether deletion of IP3-R(2) altered calcineurin signaling after TAC. Modulatory calcineurin-interacting protein is a direct transcriptional target of calcineurin, and we measured modulatory calcineurin-interacting protein as an index of calcineurin signaling. As shown in Figure 3B, modulatory calcineurin-interacting protein expression was increased at 4

Table 1. Echocardiographic Parameters in DCM-2Tg mice and the Effect of Deletion of IP3-R(2)

<table>
<thead>
<tr>
<th></th>
<th>WT (7)</th>
<th>IP3-R(2)−/− (5)</th>
<th>DCM-2Tg (6)</th>
<th>DCM-2Tg×IP3-R(2)−/− (12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>5/2</td>
<td>4/1</td>
<td>2/4</td>
<td>5/7</td>
</tr>
<tr>
<td>Age, wk</td>
<td>9.1±1.4</td>
<td>7.2±0.5</td>
<td>8.0±0.1</td>
<td>8.8±0.7</td>
</tr>
<tr>
<td>HR, bpm (echo)</td>
<td>571±30</td>
<td>628±48</td>
<td>490±34</td>
<td>507±20</td>
</tr>
<tr>
<td>LVPWs, mm</td>
<td>1.3±0.10</td>
<td>1.2±0.05</td>
<td>0.75±0.05</td>
<td>0.8±0.05</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>0.92±0.09</td>
<td>0.94±0.06</td>
<td>0.66±0.04</td>
<td>0.68±0.05</td>
</tr>
<tr>
<td>LVIDs, mm</td>
<td>1.97±0.18</td>
<td>2.2±0.26</td>
<td>3.69±0.14</td>
<td>3.7±0.18</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>3.5±0.17</td>
<td>3.4±0.2</td>
<td>4.2±0.18</td>
<td>4.3±0.16</td>
</tr>
<tr>
<td>IVSs, mm</td>
<td>1.53±0.11</td>
<td>1.4±0.12</td>
<td>0.79±0.06</td>
<td>0.87±0.06</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>0.96±0.07</td>
<td>1.02±0.04</td>
<td>0.68±0.04</td>
<td>0.71±0.04</td>
</tr>
<tr>
<td>LA area, mm²</td>
<td>7.6±0.58</td>
<td>7.24±0.9</td>
<td>21.7±2.3</td>
<td>19.68±1.6</td>
</tr>
</tbody>
</table>

Values are mean±SEM. The number of animals is indicated in brackets. Data were analyzed by 2-way ANOVA, and P values shown are for the effect of DCM, effect of IP3-R(2)−/−, and the interaction (int.) between DCM and IP3-R(2)−/−. bpm indicates beats per minute; DCM, dilated cardiomyopathy; HR, heart rate; IP3-R (2), type 2 inositol(1,4,5)-trisphosphate (Ins(1,4,5)P3 [IP3]) receptors; LVIDs, intraventricular septum diastole; IVSs, intraventricular septum systole; LA, left atrium; LVIDd, LV internal diameter diastole; LVIDs, LV internal diameter systole; LVPWs, LV posterior wall diastole; LVPWd, LV posterior wall systole; NS, not significant; and WT, wild type.
weeks after TAC, but deletion of IP$_3$-R(2) did not influence expression either in TAC or in sham-operated mice.

**Deletion of IP$_3$-R(2) Does Not Alter ECG Profiles in DCM-2Tg Mice**

We next examined whether deletion of IP$_3$-R(2) influenced electrophysiological changes that we have previously found to be associated with heart failure in the DCM model. No arrhythmias were observed over the time periods studied. Relative to WT littermates, ECG profiles from DCM-2Tg mice showed prolonged P-R interval, prolonged Q-T interval, and reduced R amplitude. R-R interval, QRS interval, and ST height were not significantly altered in the DCM mice. Deletion of IP$_3$-R(2) did not influence these changes, and data obtained from DCM-2Tg and DCM-2Tg×IP$_3$-R(2)$^{-/-}$ were not different in terms of any of these parameters. Deletion of IP$_3$-R(2) also did not alter ECG profiles in the absence of DCM (Figure IIC in the online-only Data Supplement and Table 3).

**Deletion of IP$_3$-R(2) Does Not Alter Functional Parameters in DCM-2Tg Mice or in TAC Mice**

DCM-2Tg mice die prematurely by 10 to 12 weeks of age. Deletion of IP$_3$-R(2) did not alter the death rate in this strain (Figure 4A). DCM-2Tg mice showed a high incidence of pleural effusion, lung congestion, and atrial thrombus, consistent with severe heart failure. Deletion of IP$_3$-R(2) did not alter any of these parameters (Figure 4B). Contractility, measured as fractional shortening by echocardiography, was substantially reduced in the DCM-2Tg mice, but was not influenced by deletion of IP$_3$-R(2) (Figure 4C and Table 1). Lung congestion was similar in DCM-2Tg mice with and without their complement of IP$_3$-R(2) (Figure 4D). Thus, IP$_3$-R(2) do not contribute substantially to the heart failure phenotype in this model.

TAC resulted in a progressive decrease in fractional shortening that was not altered by IP$_3$-R(2) deletion (Figure 4E). Similarly, increases in left ventricular weight were similar in WT and IP$_3$-R(2)$^{-/-}$ mice after TAC (Figure 4F).

**Discussion**

Heart failure in humans and experimental animals has repeatedly been reported to be associated with increased ventricular expression of IP$_3$-R(2) in the left ventricle. Increased atrial expression has been reported in valvular heart disease and atrial fibrillation. Furthermore, increased IP$_3$-R(2) expression has been reported in neonatal rat cardiomyocytes undergoing hypertrophy. In some cases, the increased IP$_3$-R(2) expression is paralleled by a lowering of ryanodine receptor expression. These findings might suggest that IP$_3$-R(2) are of increased importance in hypertrophic and failing myocardium. It has been suggested that the increased IP$_3$-R activity

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**Table 2. Morphometric Parameters in DCM-2Tg Mice and the Effect of Deletion of IP$_3$-R(2)**

<table>
<thead>
<tr>
<th></th>
<th>WT (6)</th>
<th>IP$_3$-R(2)$^{-/-}$ (6)</th>
<th>DCM-2Tg (12)</th>
<th>DCM-2Tg×IP$_3$-R(2)$^{-/-}$ (18)</th>
<th>p</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, men/women</td>
<td>4/2</td>
<td>4/2</td>
<td>5/7</td>
<td>8/10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, wk</td>
<td>13±0.58</td>
<td>10±0.75</td>
<td>10.5±1.3</td>
<td>11.8±1.4</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ventricle/tibia, mg/mm</td>
<td>7.05±0.17</td>
<td>6.85±0.41</td>
<td>6.41±0.41</td>
<td>6.47±0.30</td>
<td>&lt;0.001</td>
<td>0.486</td>
<td>0.294</td>
</tr>
<tr>
<td>Atria/tibia, mg/mm</td>
<td>0.39±0.09</td>
<td>0.37±0.05</td>
<td>0.94±0.2</td>
<td>0.88±0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>28.2±2.4</td>
<td>31.4±3.8</td>
<td>23.8±1.6</td>
<td>26.8±1.3</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. The number of animals is indicated in brackets. Data were analyzed by 2-way ANOVA, and P values shown are for the effect of DCM, effect of IP$_3$-R(2)$^{-/-}$, and the interaction (int.) between DCM and IP$_3$-R(2)$^{-/-}$. DCM indicates dilated cardiomyopathy; IP$_3$-R(2), type 2 inositol(1,4,5)-trisphosphate (Ins(1,4,5)P$_3$ [IP3]) receptors; NS, not significant; and WT, wild type.

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**Figure 2. Deletion of type 2 inositol(1,4,5)-trisphosphate (Ins(1,4,5)P$_3$ [IP3]) receptors (IP$_3$-R(2)) does not alter hypertrophic responses at 1 or 4 weeks after transverse aortic constriction (TAC).**

Echocardiographic parameters measured in IP$_3$-R(2)$^{-/-}$ mice and littermate controls (WT, +/+). Values shown are mean±SEM (n=6 for sham groups, 13 for +/+ TAC, and 15 for −/− TAC). Analyses used a 2-way repeated measures ANOVA, as described in the Methods section. Significant effect of TAC, relative to sham; ††P<0.001, †P<0.01, and †P<0.05. There was no significant effect of IP$_3$-R(2)$^{-/-}$ on any parameter, irrespective of the TAC status. IVSD indicates intraventricular septum diastole; IVSs, intraventricular septum systole; LVIDd, LV internal diameter diastole; LVIDs, LV internal diameter systole; LVPWd, LV posterior wall diastole; and LVPWs, LV posterior wall systole.
contributes to pathology by promoting hypertrophic growth of the cardiomyocytes, or that the increased IP_3-R might contribute to arrhythmia by perturbing Ca^{2+} responses close to sarcoplasmic Ca^{2+} channels and the Na^+/Ca^{2+} exchanger.

Alternatively, the changes might reflect a loss of muscle phenotype because the cardiomyocytes undergo the transition to failure and have little functional consequence. We recently reported that dilated atrial tissues from patients with valvular heart disease have substantially heightened generation of Ins(1,4,5)P_3 and, furthermore, that generation correlated with atrial weight.

We reasoned that the heightened Ins(1,4,5)P_3/IP3-R axis contributes to hypertrophy, we used a well-established pressure overload model.22 When we compared the DCM-2Tg mice with DCM-2Tg mice lacking IP3-R(2), we found no difference in the extent of dilatation, chamber size, or contractile dysfunction. Indicators of heart failure, lung congestion, atrial thrombus, and pleural effusion also were similar between the 2 experimental groups.

Transgenic mice expressing Mst1 in heart have been previously reported to have DCM, and the phenotype is exacerbated by coexpression of dominant-negative PI3 kinase. The crossed strain (Mst1×dn-PI3K-2Tg; DCM-2Tg) was chosen for these studies because we were interested in a possible contribution of the DCM phenotype. The crossed strain (Mst1×dn-PI3K-2Tg; DCM-2Tg) has a worsened heart failure phenotype compared with Mst1-Tg and, in addition, shows clear evidence of conduction block, as well as to chamber dilatation and heart failure. The DCM model involves chamber dilatation, possibly related to enhanced cardiomyocyte apoptosis, without cardiomyocyte hypertrophy.20 In addition to a contribution to heart failure, IP3-R have been suggested to contribute to hypertrophic signaling pathways by supplying Ca^{2+} to activate the calcineurin/nuclear factor of activated T cells (NFAT) pathway or by activating calcium calmodulin-dependent protein kinase II (CaMKII) localized close to the nuclear membrane.

To evaluate possible contributions of the Ins(1,4,5)P_3/IP3-R(2) axis to hypertrophy, we used a well-established pressure overload model.

When we compared the DCM-2Tg mice with DCM-2Tg mice lacking IP3-R(2), we found no difference in the extent of dilatation, chamber size, or contractile dysfunction. Indicators of heart failure, lung congestion, atrial thrombus, and pleural effusion also were similar between the 2 experimental groups.

### Table 3. ECG Parameters in DCM-2Tg Mice and the Effect of Deletion of IP3-R(2)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT (8)</th>
<th>IP3-R(2)−/− (5)</th>
<th>DCM-2Tg (8)</th>
<th>DCM-2Tg×IP3-R(2)−/− (13)</th>
<th>P</th>
<th>P</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, males/females</td>
<td>5/3</td>
<td>3/2</td>
<td>4/4</td>
<td>5/8</td>
<td>DCM</td>
<td>IP3-R(2)−/−</td>
<td>Int.</td>
</tr>
<tr>
<td>Age, wk</td>
<td>9.3±1</td>
<td>10±3</td>
<td>9.2±0.8</td>
<td>11.4±1.6</td>
<td>&lt;0.001</td>
<td>0.252</td>
<td>0.950</td>
</tr>
<tr>
<td>R amplitude, mV</td>
<td>1.26±0.2</td>
<td>1.15±0.16</td>
<td>0.66±0.09</td>
<td>0.86±0.11</td>
<td>&lt;0.001</td>
<td>0.348</td>
<td>0.500</td>
</tr>
<tr>
<td>P amplitude, mV</td>
<td>0.13±0.01</td>
<td>0.16±0.01</td>
<td>0.057±.0.01</td>
<td>0.048±0.01</td>
<td>&lt;0.001</td>
<td>0.348</td>
<td>0.500</td>
</tr>
<tr>
<td>P-R interval, ms</td>
<td>37±1</td>
<td>35±2</td>
<td>54±4</td>
<td>56±3</td>
<td>&lt;0.001</td>
<td>0.825</td>
<td>0.485</td>
</tr>
<tr>
<td>Q-T interval, ms</td>
<td>17±1.5</td>
<td>14±1</td>
<td>42±5</td>
<td>39±3</td>
<td>&lt;0.001</td>
<td>0.482</td>
<td>0.239</td>
</tr>
<tr>
<td>QRS interval, ms</td>
<td>8±1</td>
<td>8±1</td>
<td>10±1</td>
<td>10±1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>R-R interval, ms</td>
<td>112±7</td>
<td>111±4</td>
<td>119±5</td>
<td>124±2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. The number of animals is indicated in brackets. Data were analyzed by 2-way ANOVA, and P values shown are for the effect of DCM, effect of IP3-R(2)−/− and the interaction (Int.) between DCM and IP3-R(2)−/−. DCM indicates dilated cardiomyopathy; ECG, electrocardiography; IP3-R(2), type 2 inositol(1,4,5)-trisphosphate (Ins(1,4,5)P3) receptors; NS, not significant; and WT, wild type.
Life span also was similar in the 2 groups (Figure 4A), showing that elimination of IP₃-R(2) did not slow disease progression. ECG studies on DCM mice revealed increased P-R interval indicative of conduction block and long Q-T interval, reflecting prolonged action potential duration. Neither of these perturbations was altered by deletion of IP₃-R(2). Number of positive animals relative to the total animal number is shown on the bars. The DCM-2Tg +/+ group contained 7 of 13 females and the −/− group contained 10 of 19 females. None of the values was significantly different between the 2 groups. C, Fractional shortening, measured by echocardiography, in DCM-2Tg mice and DCM-2Tg×IP₃-R(2)−/− mice. Values shown are means±SEM (n=6 for the DCM-2Tg group and 12 for the DCM-2Tg×IP₃-R(2)−/− group). ††P<0.001 relative to wild-type (WT) or IP₃-R(2)−/− mice. D, Lung weight relative to tibia length (mg/mm) in DCM-2Tg and DCM-2Tg×IP₃-R(2)−/− mice. Values shown are means±SEM (n=6 for the sham-operated groups, 13 for +/+ TAC, and 15 for −/− TAC). F, LV weight relative to body weight in sham-operated and TAC mice 4 weeks after surgery. Values shown are means±SEM (n=6 for sham groups, 13 for +/+ TAC, and 15 for −/− TAC). ††P<0.001 relative to sham. There was no significant effect of IP₃-R(2)−/− on any parameter, irrespective of the DCM-2Tg or TAC status, and there was no interaction detected between IP₃-R(2)−/− and DCM-2Tg or TAC. Except for the survival study (A), all measurements on DCM-2Tg mice were made between 6 and 8 weeks of age.

**Figure 4.** Deletion of type 2 inositol(1,4,5)-trisphosphate (Ins(1,4,5)P₃ [IP₃]) receptors (IP₃-R(2)) does not alter the phenotype of the dilated cardiomyopathy (DCM)-2Tg mice or transverse aortic constriction (TAC) mice. A, Kaplan-Meier survival curves of DCM-2Tg mice, 10 total (black symbols), and DCM-2Tg×IP₃-R(2)−/− mice, 10 total (red symbols). There was no detectable difference between death rates. B, Incidence of pleural effusion, left atrium (LA) and right atrium (RA) thrombus in DCM-2Tg mice with (+/+ ) and without (−/−) their complement of IP₃-R(2). Number of positive animals relative to the total animal number is shown on the bars. The DCM-2Tg +/+ group contained 7 of 13 females and the −/− group contained 10 of 19 females. None of the values was significantly different between the 2 groups. C, Fractional shortening, measured by echocardiography in sham-operated and TAC mice before and 1 and 4 weeks after surgery. Values shown are means±SEM (n=6 for the sham-operated groups, 13 for +/+ TAC, and 15 for −/− TAC). F, LV weight relative to body weight in sham-operated and TAC mice 4 weeks after surgery. Values shown are means±SEM (n=6 for sham groups, 13 for +/+ TAC, and 15 for −/− TAC). ††P<0.001 relative to sham. There was no significant effect of IP₃-R(2)−/− on any parameter, irrespective of the DCM-2Tg or TAC status, and there was no interaction detected between IP₃-R(2)−/− and DCM-2Tg or TAC. Except for the survival study (A), all measurements on DCM-2Tg mice were made between 6 and 8 weeks of age.
activate subtypes of protein kinase C and protein kinase D, as well as some sarcolemmal canonical transient receptor potential channels. Any of these sn-1,2-diacylglycerol-induced responses could have major influences on disease progression that are independent of IP$_3$-R. Changes in the precursor phosphatidylinositol(4,5)-bisphosphate also may be of critical significance, because this lipid is a direct regulator of the activity of a range of sarcolemmal ion channels critical in the maintenance of cardiac rhythm. It is also possible that Ins(1,4,5)P$_3$ has functions other than those that require IP$_3$-R activation. Ins(1,4,5)P$_3$ is the precursor of the highly phosphorylated inositol derivatives (InsP$_4$-InsP$_8$), some of which are known to be functionally important, but currently such functions have not been described in heart.

These studies show that IP$_3$-R(2) do not make a significant contribution to disease in the DCM-2Tg or TAC models, despite the increased activity of the Ins(1,4,5)P$_3$/IP$_3$-R(2) axis. We have recently reported that IP$_3$-R(2) contribute to the regulation of pacemaker function in mice by demonstrating a lowering of the Ca$^{2+}$ responses (by $\approx 12\%$) in isolated sinoatrial node tissue in IP$_3$-R(-/-) mice. In the current study, we did not detect any effect of deletion of IP$_3$-R(2) on heart rate in DCM-2Tg mice, TAC mice, or in WT or sham-operated littermates. However, this contribution of IP$_3$-R(2) to pacemaker activity would be expected to be buffered by the autonomic nervous system in vivo.

In conclusion, we have demonstrated that Ins(1,4,5)P$_3$ and its receptors do not contribute to disease in murine models of DCM and hypertrophy, both of which have heightened Ins(1,4,5)P$_3$ generation and increased IP$_3$-R(2) expression. This finding shows that heightened expression of IP$_3$-R in heart disease cannot be assumed to have functional significance.

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Disclosures

None.

References


CLINICAL PERSPECTIVE

The content of inositol(1,4,5)-trisphosphate (Ins(1,4,5)P3 [IP3]) receptors in the heart, IP3-R(2), has repeatedly been shown to be elevated under pathological conditions, particularly heart failure, valvular heart disease, and atrial fibrillation in human and animal models. Because IP3-R(2) are regulators of intracellular Ca2+, the increased IP3-R(2) activity has been suggested to contribute to the perturbed Ca2+ signaling central to heart diseases. However, there are many factors that change with disease progression, and the challenge is to identify those that contribute to disease, either positively or negatively. In the current study, we provide evidence that IP3-R(2) do not contribute to heart disease in two mouse models, one of dilated cardiomyopathy and the other of pressure overload hypertrophy. This is despite clear elevation in IP3-R(2) expression, together with increased generation of the ligand, IP3. Although our data do not rule out a contribution to human disease, it is clear that demonstrating heightened expression or activity of IP3-R(2) cannot be assumed to influence disease progression. It is likely that factors other than IP3-R(2) are the source of altered Ca2+ signaling in human heart diseases.
No Contribution of IP₃-R(2) to Disease Phenotype in Models of Dilated Cardiomyopathy or Pressure Overload Hypertrophy
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SUPPLEMENTAL MATERIAL

Supplementary Methods

Echo cardiography

After a short-axis 2-D image of the LV at the level of the papillary muscles of the LV was obtained, 2-D guided M-mode images were acquired digitally at a sweep speed of 100 mm/s. Images were blindly analyzed as described previously \(^1\). Left ventricular (LV) wall thicknesses [LV posterior wall (LVPW) and interventricular septum (IVS)], LV internal diameter (LVID) at end-diastole and end-systole (LVIDd and LVIDs), and fractional shortening \([FS=(LVDd-LVDs)/LVDd]\) were determined from M-mode images. Left atrial size was determined from long-axis 2-D images at end-systole.

Surface ECG recordings and analyses

ECG recordings were measured in anesthetized mice (isofluorane, 1.7%) using the Powerlab System and BioAmp (ADInstruments). Animals were kept warm using a heating pad (37° C). Two pairs of 27G needle electrodes were placed subcutaneously and recordings were made from a chest lead (equivalent to V5). All signals were sampled at 1 kHz for a period of 5-10 min. Averaged HRs, P-R intervals, R-R intervals, QRS intervals and amplitudes of positive R- and P-waves were measured digitally using ADInstruments (Chart 5 Pro ECG analysis module).

Measurement of mRNA expression.

The following primer sets for mouse were used; IP3-R(2) (5’ AAGGTGTGCCTATGAATCG, 3’ ACCTCTTGTTCACCGTCAGG), IP3-R(1) (5’ GCAGAGGCAGAAAGAGGA, 3’ GTTTCCGGAGGATGTTTCTG), IP3-R(3) (5’ TGATGGACACCAAGCTGAAG, 3’ TGATGGTAGCAGTGTCTCTGG) atrial natriuretic peptide (ANP) (5’ ATCTGCCCTATTGAAAAAGCA, 3’ ACACACACAAAAGGGCTTAGG), \(\alpha\)-skeletal actin (5’ CCGACCACCGTCACCAGGTGTG, 3’ ATCCAACACGATGCCGGTG), MCIP, (5’ TCCAGCTTGGGGCTTGAATGAG, 3’ ACTG GAAGGTGGTGTCCTTGTC) \(\alpha\)-MHC (5’ CTGCTGGAGAGGTTATCCCTCG, 3’ GGAAGAGTGAGCCGCGCATCAAGG), \(\beta\)-MHC (5’
TGCAAAGGCTCCAGGTCTGAGGGC 3’ GCAACACCAACCTGTCCAAGTTC) Mouse GAPDH (5’ ATGACTCCACTCAGGCAAAT, 3’ TCCCATTCTCGGCCTTGAC).

**Western blot analysis**

Total protein was extracted from left ventricles of IP₃-R(2)+/+ and IP₃-R(2)-/- mice. A polyclonal antibody to IP₃R(2) was produced in rabbits immunized with a synthetic peptide for mouse IP₃,R(2) (amino acid sequence, RKNKQLGLGLGNSPH; Open Biosystems, 1:500), and was used for immunodetection as previously described ².

**Measurement of Ins(1,4,5)P₃ generation**

Atrial and ventricular tissues were labeled with [³H]inositol and subsequently stimulated with norepinephrine (50 µmol/L), in the presence of 1 µM propranolol and 10 mM LiCl for 20 min. Atria were labelled with [³H]inositol and subsequently stimulated in 96 well tissue culture dishes. Ventricles were perfused with medium containing [³H]inositol by the Langendorf method, and data include both left and right ventricle. [³H]-Labeled Ins(1,4,5)P₃ and its metabolites were extracted and subsequently quantified by HPLC, as described previously ³ ⁴.

**Supplementary data**

**IP₃-R(1) and IP₃-R(3) expression in LV for WT and DCM-2Tg mice with and without IP₃-R(2).**

IP₃-R(2) are thought to be exclusively expressed in working cardiomyocytes, IP₃-R(1) are regarded the sole subtype expressed in the conducting tissue, but expression on IP₃-R(3) has not been reported in cardiomyocytes. The possible effect of deletion of IP₃-R(2) on the expression levels of IP₃-R(1) and IP₃-R(3) in heart was assessed. There was no significant effect of deletion of IP₃-R(2) on the expression of IP₃-R(1) or IP₃-R(3) in any chambers of the hearts (Supplemental Figure 1). As expected for a subtype not expressed in cardiomyocytes, expression of IP₃-R(3) was extremely low, approximately 1/10 of the other two subtypes. There was no alteration in the expression of IP₃-R(3) caused by deletion of IP₃-R(2).
Deletion of IP₃-R(2) did not alter the pressure gradient induced by TAC.

As shown in Supplemental Figure 2A, there was no significant difference in trans-stenotic pressure gradients between IP₃-R(2)+/+ (WT) and IP₃-R(2)-/- mice.

Deletion of IP₃-R(2) did not alter echocardiographic parameters or ECG data in DCM-2Tg mice or in littermate controls (WT).

As shown in Supplemental Figure 2B echo cardiographic profiles in WT or DCM-2Tg mice were not altered by deletion of IP₃-R(2). Similarly, ECG recordings were similar either in WT or DCM-2Tg with or without IP₃-R(2) (Supplemental Figure 2C).

References


Supplemental Figure Legends

Supplemental Figure 1. Lack of effect of deletion of IP3-R(2).  A. Transstenotic pressure gradient measured in IP3-R(2) +/+ and IP3-R(2) +/- mice.  B. Representative echocardiographic profiles in WT and DCM-2Tg mice with and without IP3-R(2).  C. Representative ECG profiles in WT and DCM mice with and without IP3-R(2).

Supplemental Figure 2. Expression of IP3-R(1) and IP3-R(3) in left ventricle of mice with and without their complement of IP3-R(2). Values shown are mRNA expression relative to GAPDH, mean ± sem, n=4-8. No values differed between the relevant IP3-R(2)+/+ and IP3-R(2)-/− groups (1 way ANOVA).
Suppl. Fig. 1
Suppl. Fig. 2

Gene expression levels of IP$_3$-R(1) and IP$_3$-R(3) in different cardiac chambers (LA, RA, LV, RV) with two genotypes: DCM-2Tg and DCM-2Tg x IP$_3$-R(2)-/-.