Calmodulin Kinase Activity Links Green Fluorescent Protein Overexpression to a Cardiomyopathic Phenotype

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Background: Transgenic (TG) expression of green fluorescent protein (GFP) causes dilated cardiomyopathy in mice by unknown mechanisms. Calmodulin kinase (CaMK) activity has been implicated in cardiomyopathic responses to GFP.

Methods: We developed TG mice with cardiac expression of GFP fused to a CaMK-specific inhibitory peptide (IP) or a scrambled inactive control peptide (CP) to test if CaMK activity determined cardiomyopathic responses to GFP.

Results: Cardiac dysfunction followed increased GFP expression and CaMK activity in the GFP-CF TG mice (Table; *P < 0.05). In contrast, GFP-IP TG mice with significantly higher GFP expression were protected from developing severe cardiomyopathy compared to GFP-CF TG mice. CaMK inhibition in GFP-IP TG mice disrupted the relationship between GFP expression and cardiac dysfunction seen in GFP-CF TG mice. Atrial natriuretic peptide (ANP) expression is a marker of cardiac dysfunction. ANP increased with GFP expression in GFP-CF TG mice, suggesting that GFP over-expression activated a CaMK signaling pathway linked to cardiomyopathy. GFP IP mice had lower ANP message levels than GFP-CF TG mice, showing that reduced CaMK activity translated into reduced activity of this CaMK-dependent cardiomyopathic signaling pathway.

Conclusion: These findings implicate CaMK as a specific signal activated in GFP TG cardiomyopathy and suggest the hypothesis that other types of cardiomyopathy with abnormal protein expression may also be linked to increased CaMK activity.

<table>
<thead>
<tr>
<th>TG (Line)</th>
<th>GFP Activity (ng/µl)</th>
<th>CaMK Activity (nmol/mg/min)</th>
<th>Left Ventricular Fractional Shortening (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP (L1)</td>
<td>(n = 10)</td>
<td>89.6 ± 4.8</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>IP (L2)</td>
<td>(n = 9)</td>
<td>125.7 ± 13.7</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>CP (L1)</td>
<td>(n = 10)</td>
<td>85.1 ± 5.9</td>
<td>4.3 ± 0.5</td>
</tr>
<tr>
<td>CP (L2)</td>
<td>(n = 9)</td>
<td>52.9 ± 1.6</td>
<td>3.4 ± 0.3</td>
</tr>
</tbody>
</table>

Optimal Preload Adjustment of Right Ventricular Power Allows for Single Beat Estimation of Contractility

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Background: Right ventricular (RV) maximal power (PWRmax) is closely related to RV stroke work, and is a sensitive, yet preload dependent parameter of contractility. We hypothesized that the relationship of PWR vs end-diastolic volume (EDV), analogous to the left ventricle, is nonlinear, and that PWRmax appears to be a preload-independent estimate of RV contractility.

Methods: Nine dogs were instrumented with pulmonary flow probe, RV micromanometer, and conductance catheter. RV volume signals were calibrated by flow probe and echocardiography. Data were obtained during bicaval occlusions (n=78) under various hemodynamic conditions (baseline, atrial pacing at 90, 120, and 160 bpm, and dobutamine infusion) and fitted to the equation PWR = a·(EDV- V0PWR)b.

Results: The PWR vs EDV relationship did not deviate from linearity (b=1.1 ± 0.6, p = NS vs 1.0), and PWRmax was defined and used for evaluation of PWRmax. Dividing PWRmax by the difference of EDV and V0PWR (PAMPV0-EST) eliminated preload dependency (0.30) down to 50% of baseline EDV. Preload independency of PWRmax was confirmed by numerical modeling of actual data testing various V0PWR, EDV, PSRW, and PWR. Similarly, PWRmax adjustment using V0PWR (PAMPV0-EST) showed preload independency down to 75% of baseline EDV. Enhancing contractility by dobutamine increased PAMPV0-PWR and PAMPV0-EST from 173 ± 87 to 417 ± 225 Wm² (p < 0.001) and 178 ± 48 to 264 ± 284 Wm² (p < 0.001), respectively (both p < 0.01), accompanied by increase of PSRW from 13.6 ± 4.3 to 30.1 ± 17.4 mm Hg (p < 0.05). Both PAMPV0-PWR and PAMPV0-EST correlated closely with PSRW (r=0.86, p<0.001; r=0.93, p<0.001).

Conclusion: Optimal preload adjustment of RF PWRmax can be achieved by considering linear vs EDV relationship and V0PWR presence. Adjusting PWRmax by steady state EDV and V0PWR (PAMPV0,EST) may be a useful, preload independent estimate of RV contractility that can potentially be determined non-invasively without the need for bicaval occlusion.

Calpain Activation and Inhibition in Acute Right Heart Failure

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Right ventricular (RV) failure from acute pressure overload (RVPO) is an important cause of morbidity and mortality in patients. We previously showed that RV contractile dysfunction develops during experimental RVPO in pigs in the absence of RV ischemia, and persists after normal loading conditions are restored. We hypothesized that RV dysfunction from RVPO is due in part to activation of the calcium sensitive proteasome calpain, and that calpain inhibition would attenuate RV dysfunction during RVPO.

Methods: Anesthetized open chest pigs were randomized to treatment with a calpain inhibitor (MDL-28170, INH) or inactive vehicle (VEH) infused into the right coronary artery, then subjected to RVPO by a constant degree of pulmonary artery constriction for 4 hrs. Contractile function was assessed by RV stroke work in a second series of experiments, calpain activation after RVPO was assessed by Western blotting of spectrin breakdown products.

Results: At the beginning of RVPO, RV systolic pressure was 60±5 mmHg in both groups. Compared with VEH pigs, INH pigs maintained significantly higher RV stroke work during 4 hrs RVPO (Figure Left). RV myocardium from INH pigs showed less calpain activation after RVPO than VEH pigs (Figure Right). Four VEH pigs, but no INH pigs, failed to complete the protocol due to RV failure and hypotension and were not included in this analysis.

Conclusions: RV dysfunction during RVPO is attenuated by calpain inhibition, offering a potential new therapeutic strategy in this condition.