Chronic infusion of bradykinin preserves vascular function and delays the progression of heart failure. The study examined the effects of chronic BK infusion on cardiac and vascular nitric oxide (NO)-mediated endothelial functions during pacing-induced heart failure in conscious dogs. It has been shown that bradykinin (BK) is involved in the beneficial effects of angiotensin-converting enzyme inhibitors on vascular endothelial and left ventricular (LV) functions in heart failure (HF). However, it is not known whether BK per se exerts a protective action on vascular and cardiac functions during the development of HF. This study examined the effects of chronic BK infusion on cardiac and vascular nitric oxide (NO)-mediated endothelial functions during pacing-induced HF in conscious dogs. Sixteen beagle dogs were chronically instrumented to measure cardiac output, LV pressure, LV wall thickness, and left and right atrial and aortic pressures. After baseline recording and examining the vasodilator responses to acetylcholine (ACh) and nitroglycerin (NTG), the study was performed to randomize the use of left atrial either water (vehicle group) or BK (1 µg/min, which does not change systemic hemodynamics: BK group) during right ventricular pacing (250 beats/min, 5 weeks). The vasodilator responses to ACh and NTG were again examined after 3 and 5 weeks of pacing. The expression of endothelial NO synthase (eNOS) in femoral, carotid, and renal arteries was determined by Western blot analysis. After 3 weeks of pacing, the BK group had a lesser degree of HF than the vehicle group as indicated by less alterations in LV end-diastolic pressure (+10±3 vs +19±2 mmHg, p<0.05), LV wall thickening (-33±18 vs -22±11 mm, p<0.05) and cardiac output (-16±6 vs -32±6%, p<0.05). Total peripheral resistance (TPR) increased in the vehicle group (+18±10%, p<0.05) but remained unchanged in the BK group (+1±8%). In both groups during HF, TPR response to NTG was not altered. In contrast, TPR response to ACh was blunted in the vehicle group (p<0.01) but was unchanged in the BK group. Similar trends were also observed after 5 weeks of pacing. In all examined arterial beds, eNOS protein expression decreased significantly in the vehicle group but was preserved in the BK group. Thus, in conscious dogs, chronic BK infusion limits alterations in LV function and preserves vascular endothelial NO-mediated vasodilation and thereby delays the progression of HF.

**Conclusions.** These data suggest that anemia is associated with reduced QoL in patients with HF. Additional studies will be required to establish if this is a cause and effect relationship.
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 is increased in cardiomyopathy and GFP expression in GFP-CP TG mice, suggesting that GFP over-expression activated a CaMK signaling pathway linked to cardiomyopathy. GFP IP mice had lower ANP with GFP expression in GFP-CP TG mice, suggesting that GFP over-expression activated a CaMK signaling pathway linked to cardiomyopathy. GFP IP mice with significantly higher GFP expression were protected from developing severe cardiomyopathy compared to GFP-CP TG mice. CaMK inhibition in GFP-IP TG mice disrupted the relationship between CaMK expression and cardiomyopathy seen in GFP-CP TG mice. Atrial natriuretic peptide (ANP) expression is a marker of cardiac dysfunction. ANP increased with GFP expression in GFP-CP 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-CP 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.

TG (Line) | [GFP] ng/µl | CaMK Activity (nmol/min/mg) | Left Ventricular Fractional Shortening (%) |
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<tr>
<td>IP (L1) (n = 10)</td>
<td>89.6 ± 4.8</td>
<td>2.4 ± 0.2</td>
<td>58.9 ± 1.9</td>
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<tr>
<td>IP (L2) (n = 9)</td>
<td>125.7 ± 13.7</td>
<td>3.2 ± 0.2</td>
<td>46.2 ± 5.7</td>
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<tr>
<td>CP (L1) (n = 9)</td>
<td>85.1 ± 5.9</td>
<td>4.3 ± 0.5</td>
<td>27.7 ± 2.9</td>
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<tr>
<td>CP (L2) (n = 9)</td>
<td>52.9 ± 1.6</td>
<td>3.4 ± 0.3</td>
<td>54.7 ± 1.7</td>
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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 RV stroke work vs EDV relationship (PWR-EDV), may be linearly modeled with an x-axis intercept corresponding to PRSW intercept (V0EDV).

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 bivacual 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 - V0EDV) b.

Results: The PWR vs EDV relationship did not deviate from linearity (R² = 0.90, p < 0.001). V0EDV was related to steady state EDV by equation V0EDV = 4.63 EDV 0.65 (R² = 0.83, p < 0.001), allowing for single beat estimation of V0EDV. V0EDV was closely related to PWRmax (R² = 0.83, p < 0.001), and for preload adjustment of PWRmax, dividing PWRmax by the difference of EDV and V0EDV (PAMPV0-EDV) eliminated preload dependency (R² = 0.50) to 50% of baseline EDV. Preload independency of PAMPV0-EDV was confirmed by numerical modeling of actual data testing various V0EDV, EDV, PRSW, and PWR. Similarly, PAMPV0-EDV adjusted using PAMPV0-EST showed preload independency down to 75% of baseline EDV. Enhancing contractility by dobutamine increased PAMPV0-EDV and PAMPV0-EST from 173 ± 87 to 417 ± 225 W/ml·10 4 and 178 ± 48 to 284 W/ml·10 4, respectively (both p < 0.03), accompanied by increase of PRSW from 13.6 ± 4.3 to 30.1 ± 17.4 mm Hg (p < 0.05). Both PAMPV0-EDV and PAMPV0-EST correlated closely with PRSW (R² = 0.86, p < 0.01) and V0EDV (R² = 0.93, p < 0.001). Conclusion: Optimal preload adjustment of RV PWRmax can be achieved by considering linear PRSW vs EDV relationship and V0EDV presence. Adjusting PWRmax by steady state EDV and V0EST may be a useful, preload independent estimate of RV contractility that can potentially be determined non-invasively without the need for bivacual 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 protease 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.

AKT Signaling in Pacing-Induced Heart Failure

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Background: Marked changes in energy substrate utilization occur during the progression of congestive heart failure (CHF) where free fatty acid utilization, as the primary source of cardiac energy is severely diminished, oxidative phosphorylation is down-regulated and glucose uptake and utilization increase. While the molecular basis for the shift in substrate utilization has not yet been elucidated, we sought to examine in the canine model of paced-induced heart failure the potential role of the AKT pathway (previously implicated in the events of cardiac hypertrophy) in signaling the metabolic transition of CHF.

Methods: Left ventricular samples from early (1-2 weeks) and late (3-4 weeks) stages of pacing-induced CHF animals (n=6 and 6 respectively) were evaluated for the levels of both phosphorylated and non-phosphorylated AKT, as well as for AKT kinase activity and were compared to control un paced animals (n=6). In addition, markers of cardiac hypertrophy (e.g. actin, ANP, BNP, beta-MHC and SERCA 2) were assessed by immunoblot analysis as were myocardial levels of free fatty acids and glucose.

Results: Significant increases in the phosphorylated AKT form (1.7 fold) and in AKT kinase activity (3 fold) were found in early paced animals; these levels declined in the late stages of pacing. Significant increases were also found in the myocardial free fatty acid content (mean 1.0 ± 0.2% vs. controls 0.1±0.1% and only a mild decrease in glucose level (10%) in late but not in early pacing. Markers of cardiac hypertrophy showed no differences in the paced animals compared to controls.

Conclusion: Our data demonstrate that AKT signaling pathway is a contributory element in the early signaling events leading to the progression of pacing-induced heart failure, accompanying the shift in substrate utilization and, contrary to the transgenic mice model, is independent of cardiac hypertrophy. The decline in AKT phosphorylation and kinase activation in the later stages of the paced-induced events is also consistent with the observed onset of myocardial apoptosis previously described in the pacing model, a process known to be reversed by AKT pathway activation.