Multi-Scale Model of Phospholamban Mutations in the Mouse Heart

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1. INTRODUCTION

Phospholamban (PLB) is a key regulator of cardiac excitation-contraction coupling and PLB polymorphisms can induce congenital cardiac pathologies. Phospholamban knockout (PLB-KO) produces greatly enhanced in vivo contractility while phospholamban mutation (PLB-N27A) reduces contractility. While genetic mutations clearly alter organ function, there is a large gap in mechanistic understanding between molecular perturbations and in vivo phenotypes as >100 proteins exhibit altered expression in these mice. Moreover, cardiac function is well described at individual spatial scales spanning from intracellular domains to the entire circulatory system. However, it is unclear how independent actions at these scales cohesively contribute to normal and pathological heart activity. Though many feedback mechanisms are hypothesized to constrain these independent actions, the specific mechanisms which constrain many aspects of global cardiac behavior remain unknown.

Because cells are complex, nonlinear systems, it is difficult to experimentally isolate the molecular mechanisms responsible for specifying in vivo phenotypes. Data from transgenic mouse experiments can be difficult to interpret due to the onset of compensatory biological mechanisms. Moreover, the cost and time associated with these methods limit the throughput of these studies. Consequently, computational models have recently gained significant exposure as tools for systematically distilling complexity in biological systems. Carefully constrained multi-scale models functionally integrate across physiological subsystems and can provide unique comprehensive and quantitative insight into how molecular events affect organ system behaviors. Here, we develop a simple multi-scale model relating mouse β₁-adrenergic signaling to cardiac myocyte electrophysiology, trabecular force generation and circulatory system hemodynamics in vivo. This model is used to mechanistically link molecular events to in vivo phenotypes with examples of PLB knockout (PLB-KO) and mutation (PLB-N27A).

2. METHODS

We adapted our previous model of rat β₁-adrenergic signaling [1] for mouse and integrated this model with previously published models of myocyte excitation-contraction coupling [2], myofilament activation [3], simplified ventricular mechanics [4], and a lumped-parameter circulatory model [5]. This model accounts for a variety of feedbacks across scales, including the effect of myofilament activation on calcium, the effect of tissue strain and strain rate on myofilament activation, and the effect of the circulation on preload/afterload, modulating ventricular mechanics.

We validated this model at three functional scales against in vitro and in vivo experiments. Application of isoproterenol in our model stimulates the β₁-adrenergic signaling network and induces a rise in the calcium (Ca) transients which quantitatively agree with studies in primary cardiac myocytes. These behaviors are also evident on the tissue level with increases in force generation in quantitative agreement with measurements from trabecular papillary strips. Lastly, effects on left ventricular strain, pressure and volume are in good quantitative agreement with in vivo cardiac MRI and pressure measurements in mice.
3. RESULTS

Model analysis suggests that such integration filters global cardiovascular responses to cardiomyocyte stimulation; while β-adrenergic signals are greatly amplified at the biochemical level, β-adrenergic responses gradually diminish at increasing spatial scales due to negative feedback among functional modules of the heart. Moreover, our simulations suggest a possible mechanism for a key difference observed between mouse vs. human heart contractility. Mice have significantly faster physiologic heart rates than humans and exhibit limited contractile responsiveness. Our model suggests that in mice, cross-bridge cycling may significantly rate-limit ventricular strain development and adrenergic contractile responsiveness.

We use this model to test whether PLB’s actions on excitation-contraction coupling alone adequately explain the in vivo phenotypes of PLB knockout and gain-of-function transgenic mice or if these in vivo phenotypes require other compensatory changes. Endogenous PLB sequesters and therefore inhibits sarcoplasmic reticulum Ca pumps (SERCA) on the sarcoplasmic reticulum (SR). The result of this inhibition is that while free SERCA are able to pump Ca into the SR, a large fraction gets inactivated by phosphorylation. Phosphorylation of phospholamban by PKA and other kinases induces a conformational change which releases SERCA and relieves this inhibition, resulting in greater SR loads, larger calcium release events and faster diastolic calcium uptake. This is a critical step in β-adrenergic stimulated control of heart contractility and results in faster and stronger heart contractions.

Singularly removing PLB from the model, we observe stronger and faster Ca transients and pressure-volume relationships quantitatively consistent with reported values from PLB-KO mice. Singularly amplifying PLB function in this model yields depressed contractility quantitatively consistent with PLB-N27A gain-of-function transgenic mice. These findings suggest that perturbations to PLB expression or function are sufficient to explain the observed functional phenotypes in these transgenic mice.

4. CONCLUSIONS

In summary, we present a multi-scale model mechanistically linking molecular events to in vivo phenotypes. We validate this model at three functional scales from both in vitro and in vivo experiments. Our model predictions suggest PLB regulation of ventricular Ca transients sufficiently account for both amplitude and rate changes across all scales of heart function in these transgenic mice. These findings demonstrate how systems approaches can be useful for testing experimentally intractable hypotheses and for providing mechanistic understanding of gene-to-organ relationships. Moreover, such approaches are useful for prompting new experimental questions and can guide exploration of the global in vivo consequences of controlled molecular perturbations.

5. REFERENCES