A Model Based Analysis of Steady-State versus Dynamic Elements in the Relationship between Calcium and Force

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BACKGROUND

Cardiac contraction and calcium. Intracellular calcium is a key regulator of cardiac contraction. When an action potential stimulates a cardiac myocyte, extracellular calcium enters the cell and stimulates a huge secondary release of calcium from the sarcoplasmic reticulum (SR), rising the intracellular free [Ca^{2+}]. Calcium then binds to cardiac troponin C (cTnC) and triggers a cascade that allows actin and myosin to form a crossbridge, the force generating unit of muscle. For muscle relaxation to occur, [Ca^{2+}] is shuttled back to the SR or outside the cell, leading to the dissociation of calcium and cTnC. Physiologically, the intracellular free calcium concentration rises and falls transiently, causing the repetitive contraction of the cardiac muscle.

Static versus Dynamic Calcium-Force Relationship. Often, myofilament response to calcium is studied in skinned fibers. The technique involves "skinning" the fiber by removing the membrane. Force (generated by the myofilaments) is recorded for a range of free calcium concentrations in the solution surrounding the skinned muscle. Without the membrane, the concentration of calcium in the bath is the same as the intracellular calcium concentration. This data is presented as force-pCa curves. The dotted line in Figure 1 shows a typical force-pCa curve. As stated above, however, calcium and force rise and fall transiently, and so there exist characteristics of the calcium-force relationship that may not be described well

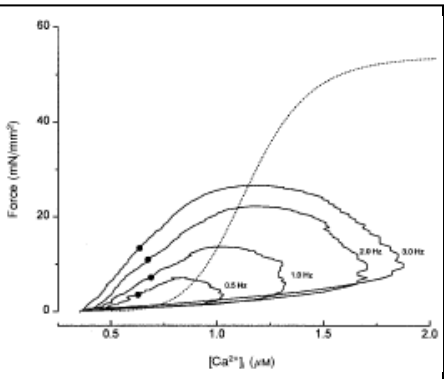

Figure 1. The static and dynamic calcium-force relationships [5]

by only analyzing steady-state activation. Represented by solid lines in Figure 1 are dynamic force-calcium loops, generated from beating isolated heart studies [5]. With the dynamic data overlaid on the traditional force-pCa data, it is easy to see that one particular intracellular calcium concentration does not produce one unique force.

We are interested in studying how alterations in processes such as the calcium-troponin interaction, crossbridge cycling and the cooperativity mechanism affect both dynamic and static characteristics. Parameters describing these processes may have a larger effect on the dynamic aspects of the calcium-force relationships or have a greater effect on the steady state one. Although it is possible to study the dynamic and static characteristics of calcium and force separately, there is not yet a way to study both at the same time. Moreover, it is impossible to experimentally control these specific parameters (crossbridge cycling, cooperativity, etc.). Therefore, we will use a model-based approach where we can vary parameters systematically in a way that allows us to simultaneously study both dynamic and steady state aspects of the calcium-force relationship.

METHODS

The Four-State Model. The model that we use must be able to recreate both steady state and dynamic calcium force pairs (one as the input, one as the output). The Four-State model represents actual elements in the physiological pathway ([2], Fig. 2) and has been used in several studies to simulate calcium-force pairs. In Dr. Shroff's laboratory, this

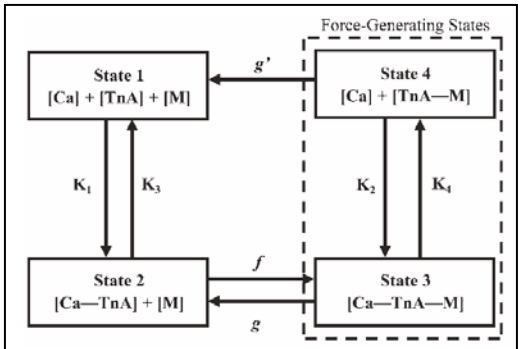

Figure 2. Block diagram of the four-state model

model of myofilament interaction was applied to calcium and pressure (force) data obtained in intact mouse hearts to explain the dynamic relationship between the two [6]. The input to the model is intracellular calcium concentration, [Ca](t), and the output is force, F(t), schematically shown in Figure 3.

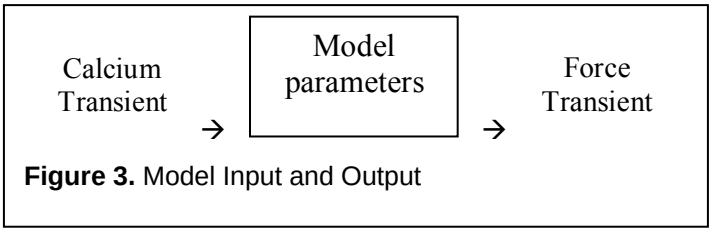

Figure 3. Model Input and Output

State 1 represents the condition when calcium, troponin, actin and myosin are all in their unbound state. In *State 2* calcium is bound to troponin, but actin and myosin have not formed a crossbridge. The rate constants of calcium binding and unbinding, K_1 and K_3, regulate the movement between these states. *State 3* is a force-generating state, and has calcium bound, and the strong crossbridge formed. The classical *f* and *g* rate constants represent the formation and deformation of the strongly bound crossbridge. *State 4* is a second force-generating state, where

$$\frac{d[\mathrm{TnCA}]}{dt} = -K_1[Ca][TnCa] + K_3[Ca \bullet TnCA] + g'[TnCA \bullet M] \quad (1)$$

$$\frac{d[\mathrm{M}]}{dt} = g'[TnCA \bullet M] - f[Ca \bullet TnCA][M] + g[Ca \bullet TnCA \bullet M] \quad (2)$$

$$\frac{d[\mathrm{Ca \bullet TnCA}]}{dt} = K_1[Ca][TnCa] - K_3[Ca \bullet TnCA] - f[Ca \bullet TnCA][M] + g[Ca \bullet TnCA \bullet M] \quad (3)$$

$$\frac{d[\mathrm{Ca \bullet TnCA \bullet M}]}{dt} = f[Ca \bullet TnCa][M] + K_2[Ca][TnCA \bullet M] - (g + K_4)[Ca \bullet TnCA \bullet M] \quad (4)$$

$$\frac{d[\mathrm{TnCA \bullet M}]}{dt} = K_4[Ca \bullet TnCa \bullet M] - g'[TnCA \bullet M] - K_2[Ca][TnCA \bullet M] \quad (5)$$

$$K_1(t) = \alpha_1 \{[Ca \bullet TnCa \bullet M](t) + [TnCa \bullet M](t)\}^{0.5} + \beta_1 \quad (6)$$

$$f(t) = \alpha_f \{[Ca \bullet TnCa \bullet M](t) + [TnCa \bullet M](t)\}^2 + \beta_f \quad (7)$$

$$F(t) = \alpha \{[Ca \bullet TnCa \bullet M](t) + [TnCa \bullet M](t)\} \quad (8)$$

calcium unbinds from troponin but the crossbridge is still formed. This state is controlled by rate constants K_2 and K_4.

This model is described by equations 1-8, and is solved through simultaneous numerical integration of equations 1-5. The equations represent a relationship between cardiac troponon C-actin complex (TnCA), myosin (M), and calcium (Ca). Equations 6 and 7 are calculations for K_1 and *f*, which can increase based on the number of strong crossbridges that have been formed, a

feedback known as cooperativity. The total force is found by adding the two force generating states together and multiplying by a gain parameter (Eq. 8). We will apply time-varying calcium and constant calcium inputs in order to observe the dynamic and steady state responses, respectively. A double exponential function will be used to generate the input $[Ca^{2+}]$ transient

Determination of Baseline Variables. Simultaneous calcium and force measurements from stimulated isolated papillary muscle will be used to optimize our model. This was accomplished in a previous study with calcium and pressure data collected in intact mouse hearts [6]. We will apply the observed intracellular calcium concentration transient to the model then alter the model parameters until the estimated waveform matches the experimentally obtained force waveform as best as possible. This optimization procedure will be done using the Levenberg-Marquadt algorithm. This will provide a set of baseline parameters.

Systematic Varying of Parameters. Subsets of the parameters in the four-state model can be lumped into three processes:

Process	Parameters
1. Calcium-Troponin interaction	K_1, K_2, K_3, K_4
2. Crossbridge cycling	f, g
3. Cooperativity feedback mechanism	α_1, α_f, β_1, β_f

We are interested in how changes in each of these parameter sets affect the static and dynamic force responses. As mention previously, we will apply time-varying calcium and constant calcium inputs in order to observe the dynamic and steady state responses. For both forms of calcium input, we will make the same adjustments to the parameters. Specifically, we will alter parameters from 50% to 200% of the baseline parameter values.

Evaluation of Force Response Waveforms. In order to quantitatively describe the effects of parameter changes on steady-state and dynamic calcium force relationships a number of indices of both relationships will be calculated.

For the steady-state data, force will be plotted as a function of calcium concentration in pCa units ($-\log[Ca^{2+}]$). The steady-state waveform characteristic values we will consider include: F_{max}, pCa_{50} and Hill coefficient. The relation between free calcium and isometric force resembles a sigmoidal function and should fit the modified Hill equation (Eq. 9, [4]). F is the isometric force, F_{max} is the force at maximum activation, pCa_{50} is the $[Ca^{2+}]$ at which F is 50% of F_{max} and represents a compound affinity constant (i.e., the calcium sensitivity index) and $[Ca^{2+}]_i^{nH}$ represents the slope of the F-$[Ca^{2+}]$ relation (the Hill coefficient). The Hill coefficient is the maximal slope of pCa_{50} and a quantitative measure of cooperativity.

$$F = (F_{max} \times [Ca^{2+}]_i^{nH}) / ([Ca^{2+}]_i^{nH} + pCa_{50}) \quad (9)$$

To evaluate dynamic characteristics of the calcium-force relationship we will plot developed force (F_{dev}) and developed calcium ($[Ca^{2+}]_{dev}$) with respect to time. F_{dev} refers to the difference between force generated during systole and force generated during diastole ($F_{sys} - F_{dia}$). Similarly, $[Ca^{2+}]_{dev}$ refers to $[Ca^{2+}]_{sys} - [Ca^{2+}]_{dia}$. The dynamic waveform characteristics we will consider include: T_{rise}, T_{relax}, F_{max}, dF/dt_{max} and dF/dt_{min}. T_{rise} is the rise time of F_{dev} or $[Ca^{2+}]_{dev}$, T_{relax} is the relax time of F_{dev} or $[Ca^{2+}]_{dev}$, F_{max} is the maximum force for the full range of calcium concentrations, dF/dt_{max} is the maximal rate of rising force (during contraction) and dF/dt_{min} is the maximal rate of falling force (during relaxation).

ANTICIPATED RESULTS

Our results will show how changes to the baseline parameter set affect the indices of the

steady state force-pCa relationship (F_{max}, pCa_{50},nH) and the dynamic calcium-force relationship (T_{rise}, T_{relax}, F_{max}, dF/dt_{max} and dF/dt_{min}). It is difficult to assess whether these alterations will affect the steady-state or dynamic relationships more, but it is unlikely that it will be an absolute behavior, i.e. parameter alterations will most likely affect both. For each of the processes (sets of parameters), however, they may have a larger impact on one versus the other.

REFERENCES

1. Bers D: Calcium Fluxes Involved in Control of Cardiac Myocyte Contraction *Circ Res.* (2000) 87:275-281.

2. Burkhoff D: Explaining load dependence of ventricular contractile properties with a model of excitation-contraction coupling. *Journal of molecular and cellular cardiology* (1994) 26: 959-978.

3. Dobrunz L E, Backx P H, Yue D T: Steady-State $[Ca^{2+}]$-Force Relationship in Intact Twitching Cardiac Muscle: Direct Evidence for Modulation by Isoproterenol and EMD 53998. *Biophysical Journal*. (1995) Vol 69 189-201.

4. Fuchs F, Martyn D A: Length-dependent Ca^{2+} activation in cardiac muscle: some remaining questions. *Journal of Muscle Research and Cell Motility* (2005) 26:199-212.

5. Gao W D, Perez N G, Marban E: Calcium cycling and contractile activation in intact mouse cardiac muscle. *Journal of Physiology* (1998), 507.1, pp. 175-184.

6. MacGowan G A, Kirk J A, Evans C, Shroff S G: Pressure-calcium relationships in perfused mouse hearts. *Am J Physiol Heart Circ Physiol*. (2006) H2614-H2624.

7. Research Training Plan: Kirk, Jonathan Alder 04/05/2007

8. Zipes: Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine, 7th ed. (2005)

9. Silverthorn: Human Physiology: An Integrated Approach, 3rd ed.

10. Solaro RJ, Rarick HM: Troponin and tropomyosin: Proteins that switch on and tune in the activity of cardiac myofilaments. *Circ Res* (1998) 83:471.