POSITIVE INOTROPY IN HUMAN MYOCARDIUM

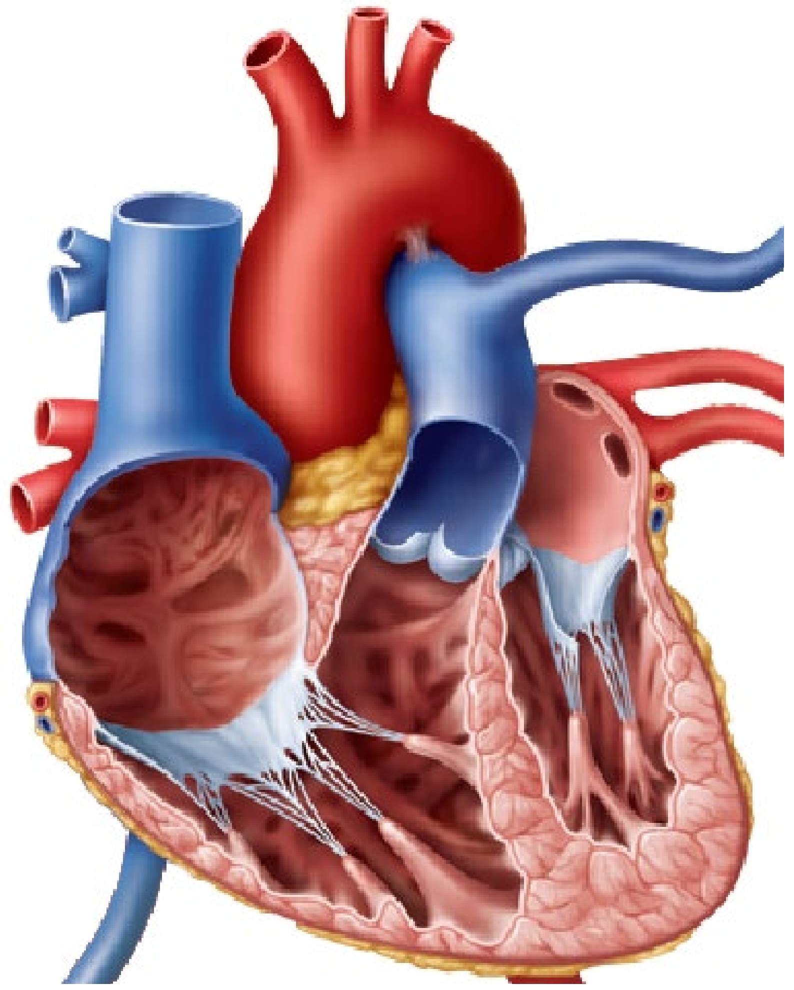
EXPLORING THE ROLE OF MyBP-C

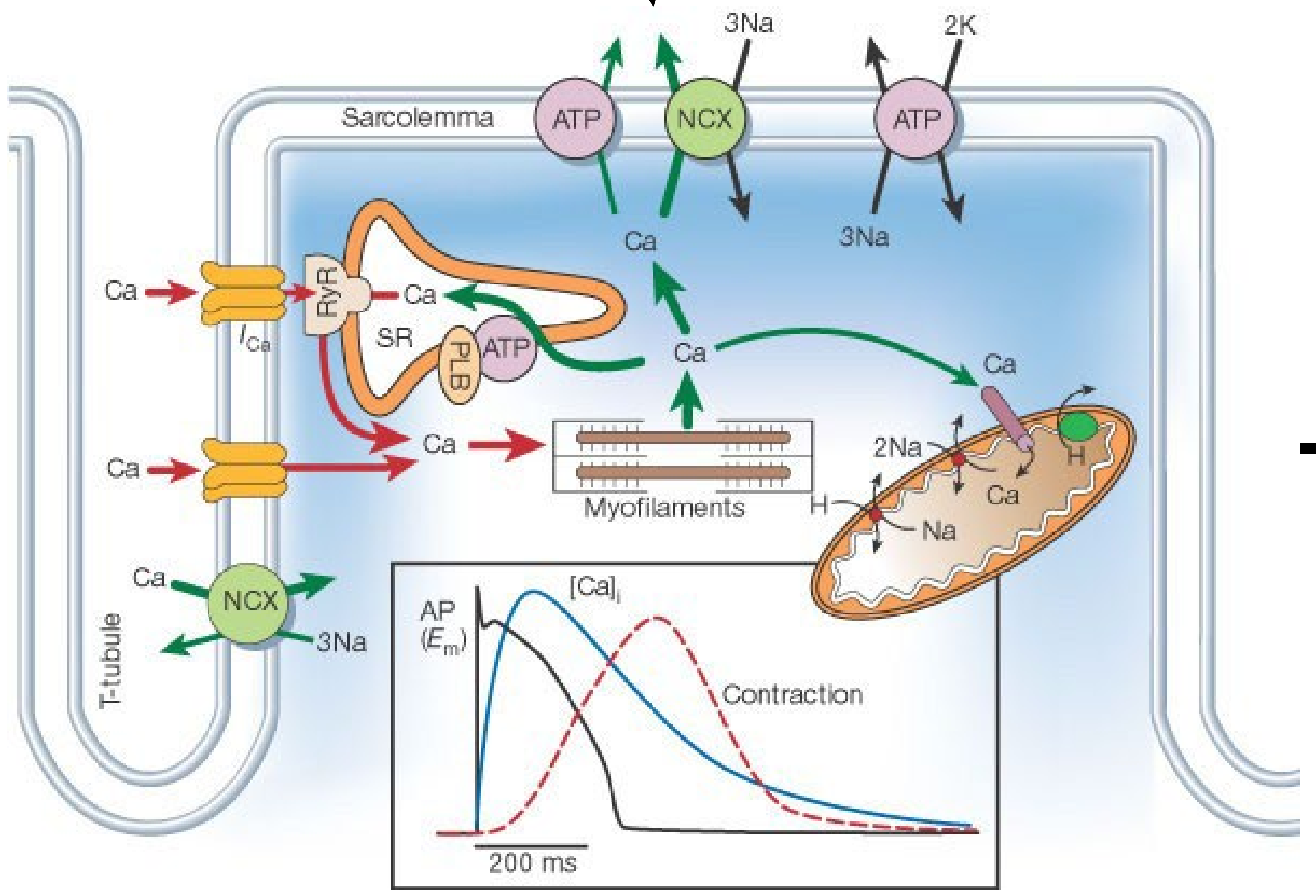
DR. DANIEL FITZSIMONS

ANIMAL, VETERINARY AND FOOD SCIENCES

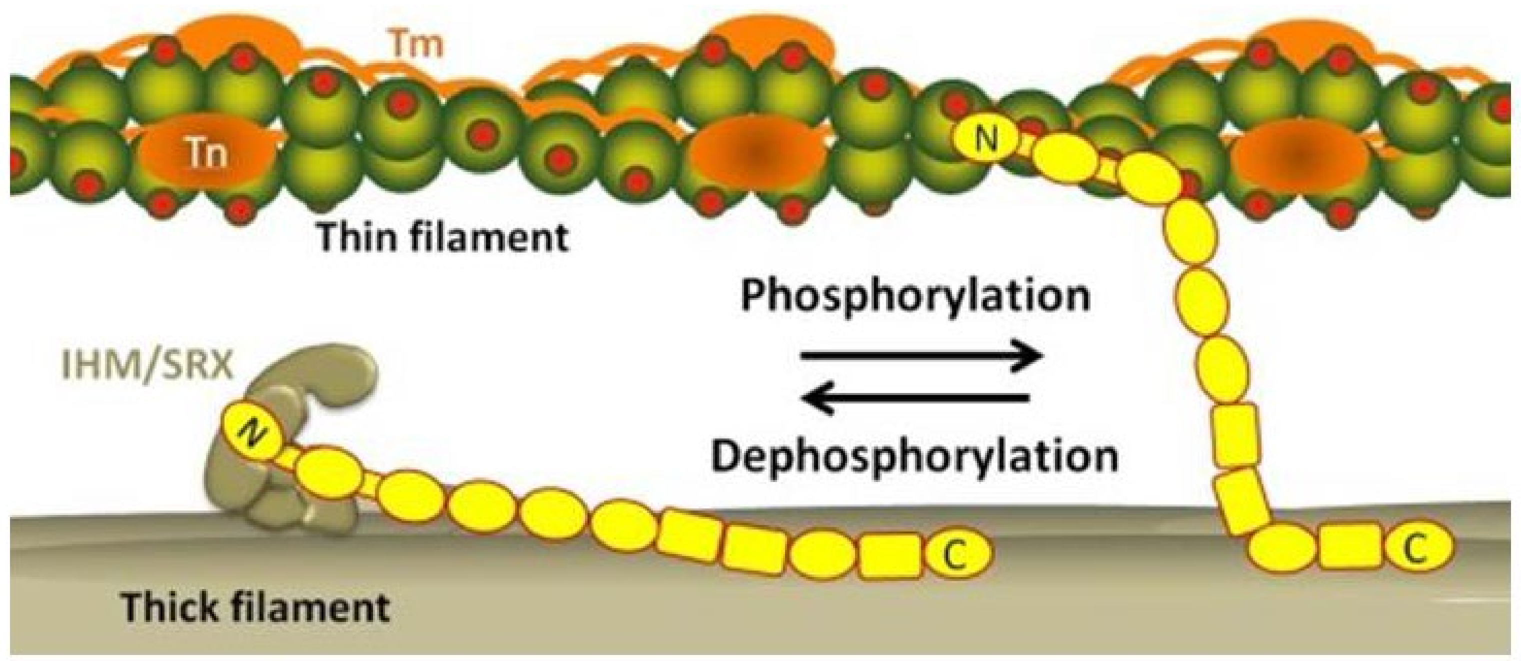
UNIVERSITY OF IDAHO

Ventricular Contractility – A Multidimensional Property of the Heart

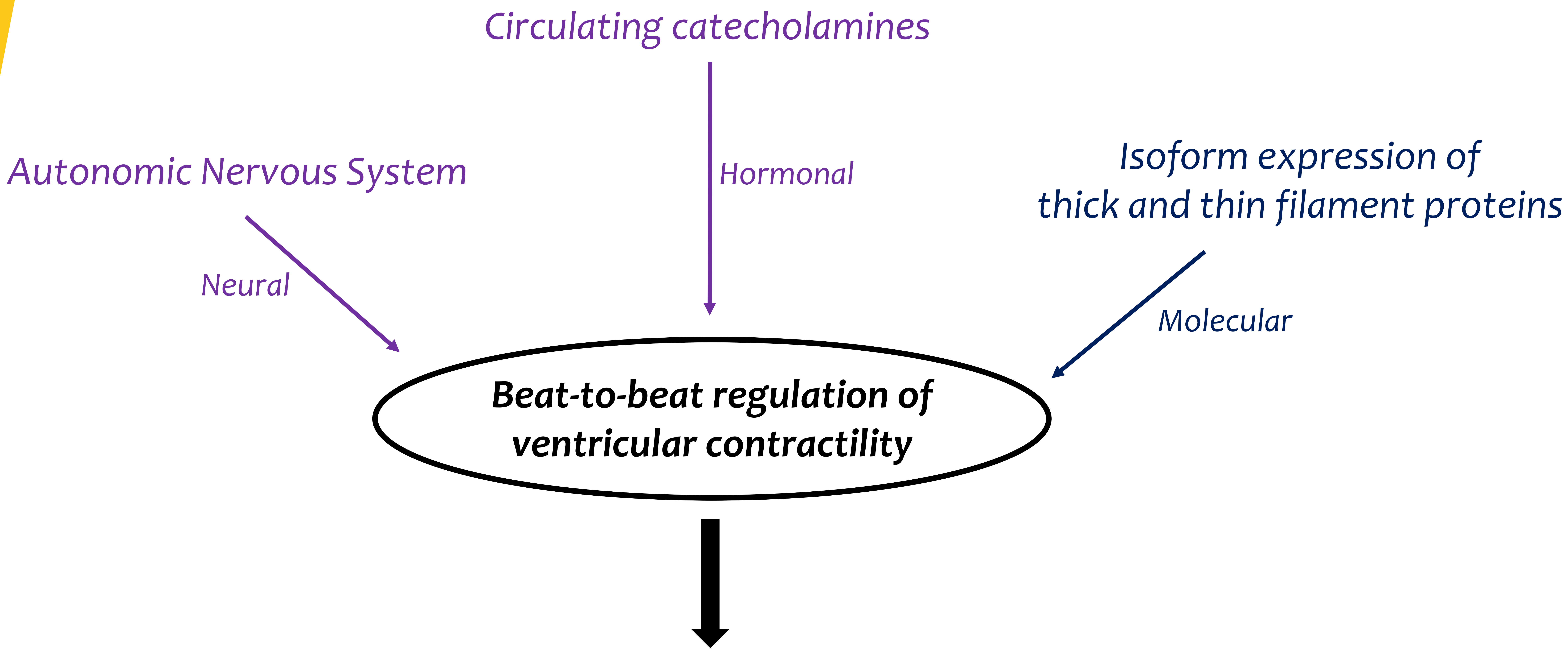




Bers, DM. Nature 415:198-205, 2002

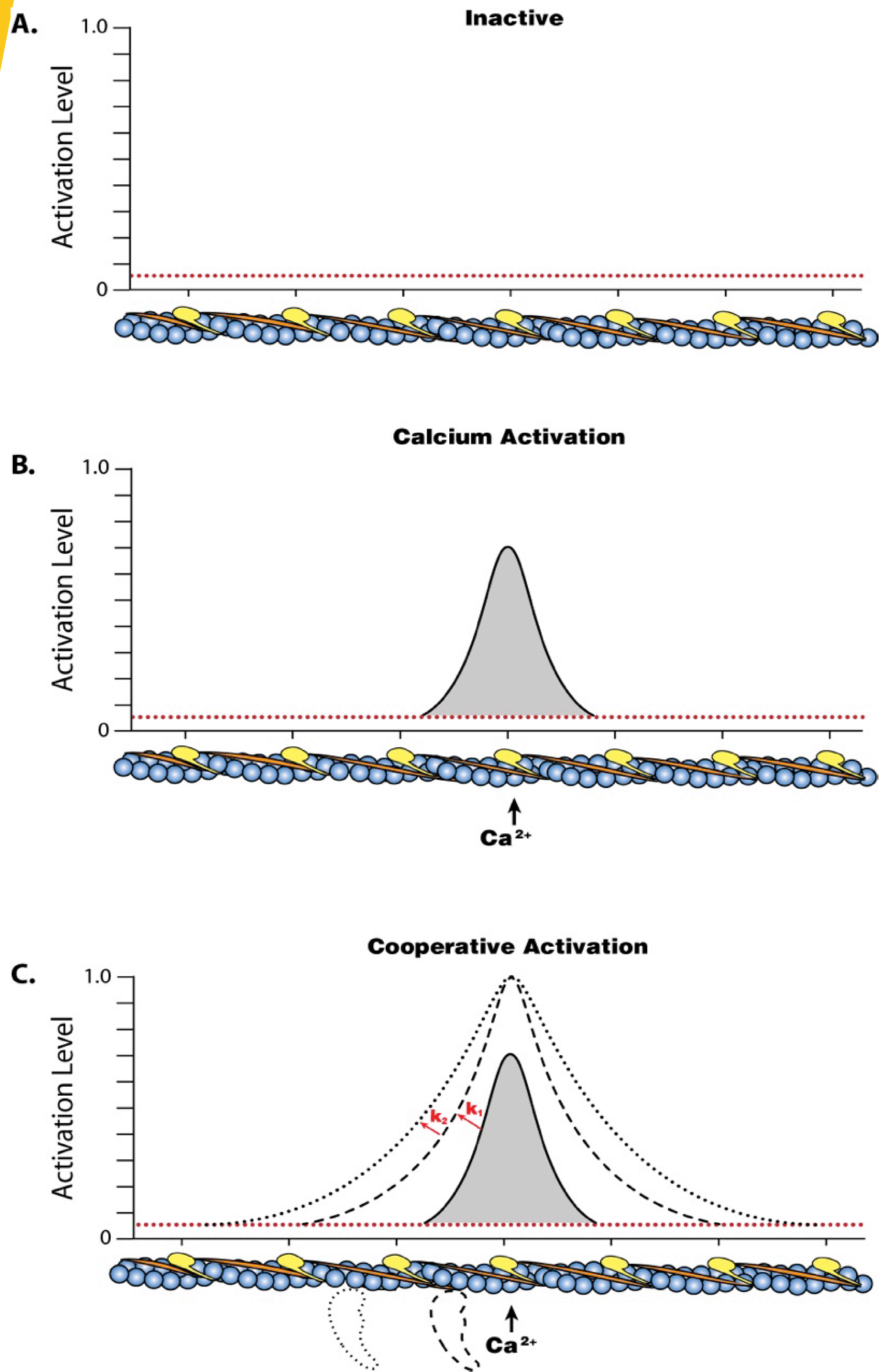


Ventricular Contractility – A Multidimensional Property of the Heart



Significant variations in the strength of contraction and systolic pressure to ensure that peripheral tissue demands are met.

Diagrammatic Representation of Thin Filament Activation



Initiation of contraction appears to involve the cooperative activation of a short segment of thin filament by Ca^{2+} and / or strong binding myosin cross-bridges, which then activate adjacent regions of thin filament via near-neighbor interactions.

Moss, RL. Circ Res 116:183-192, 2015

Idea:

Given the nearly ten-fold difference in resting heart rates and myocardial twitch kinetics between rodents and humans, it is highly unlikely that the relative contributions of contractile and regulatory proteins of the thick and thin filament to the cooperative activation of force are the same in these species.

Study:

To test this idea, we examined the Ca^{2+}-dependencies of steady-state force and the rate constant of force redevelopment (k_{tr}) in murine and human ventricular myocardium prior to and following treatment with protein kinase A.

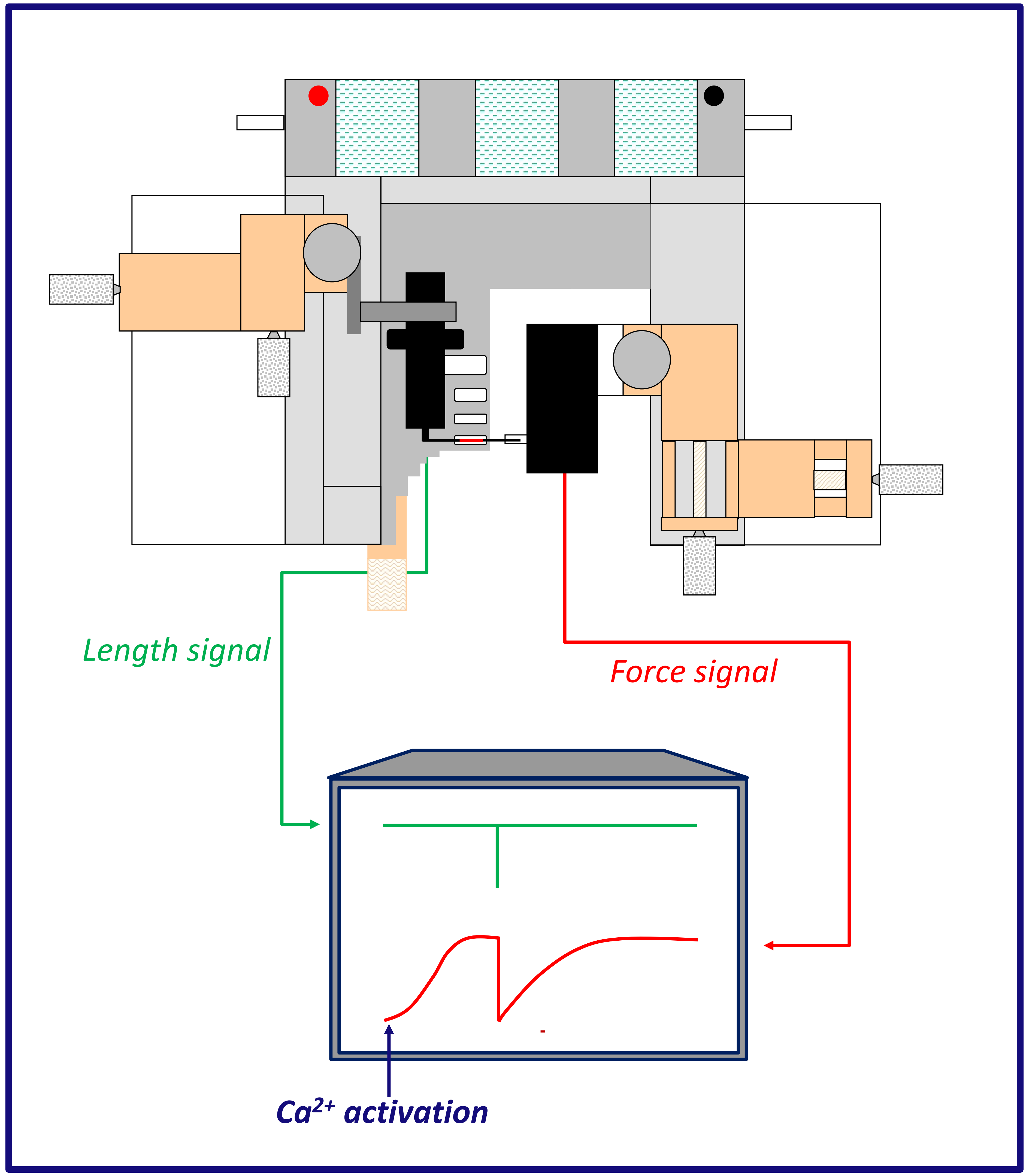
Experimental Workflow

1. Homogenize

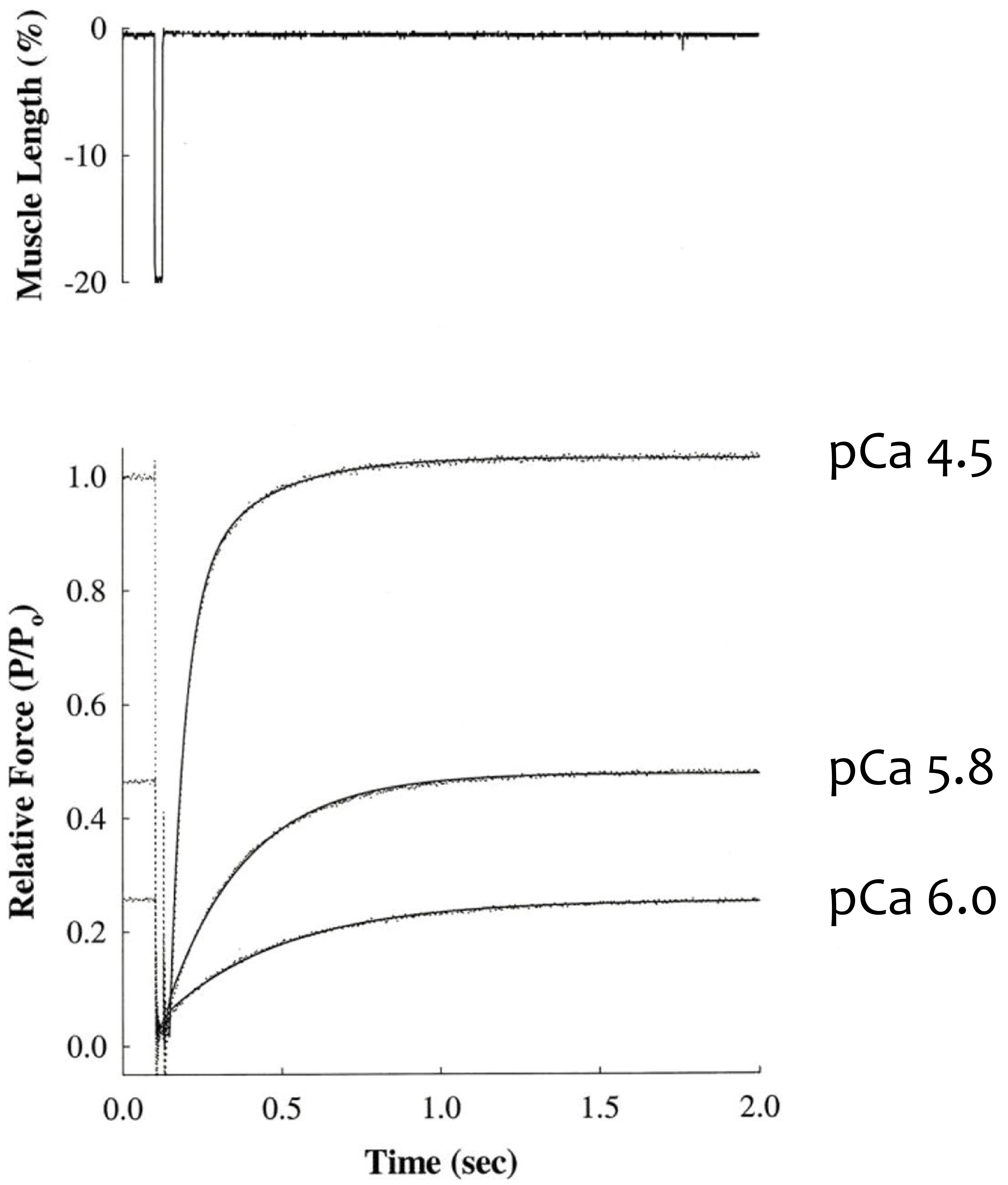
2. Chemically skin

3. Mount

Permeabilized multicellular myocardial preparations
(dimensions: 800-1200 μm x 150-250 μm)



Force Redevelopment in Permeabilized Myocardium

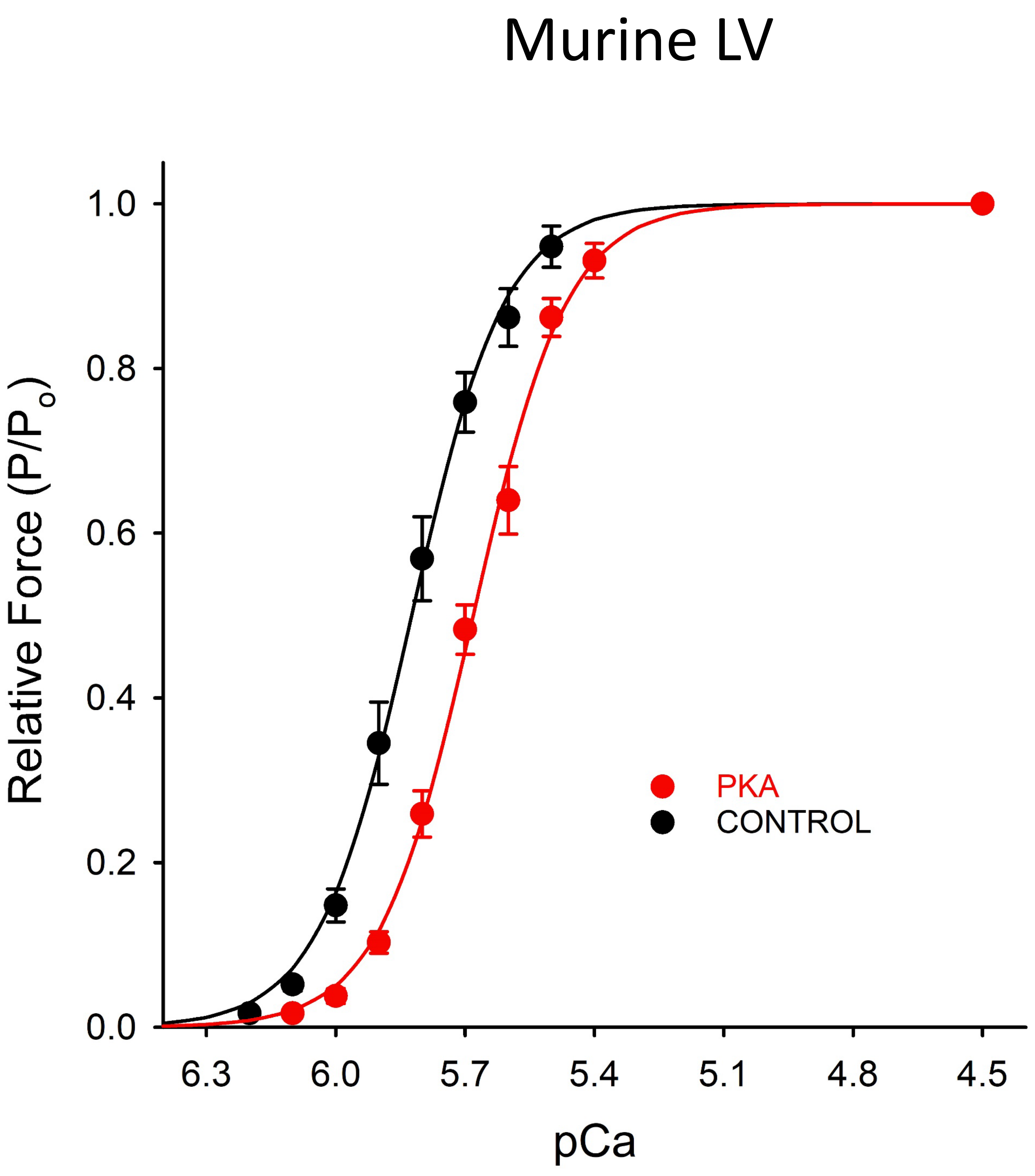


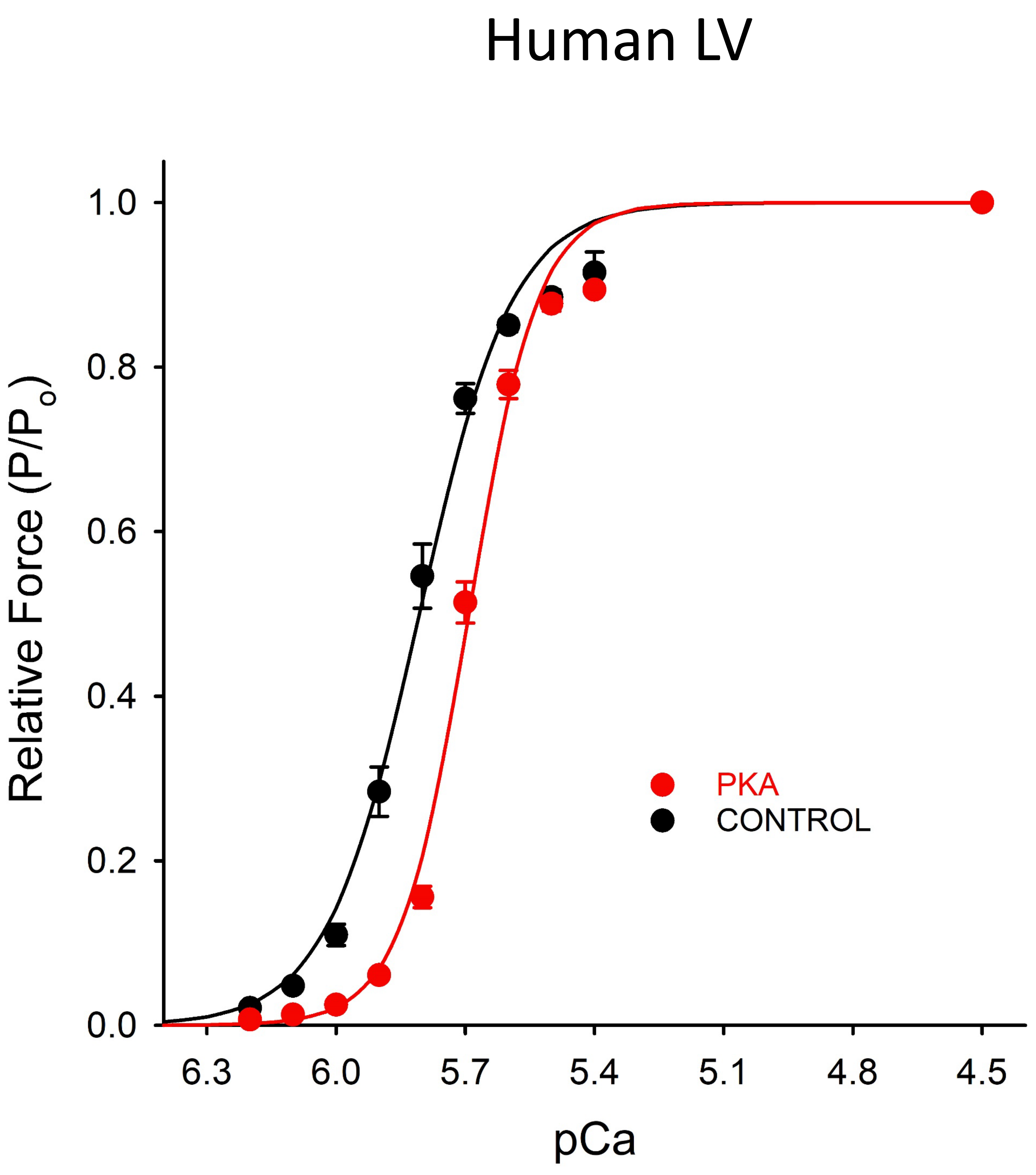
Mechanical release-restretch maneuver to determine the Ca^{2+}-sensitivity of force and the rate constant of force redevelopment at varying levels of Ca^{2+} activation.

Steady-State Mechanical Measurements

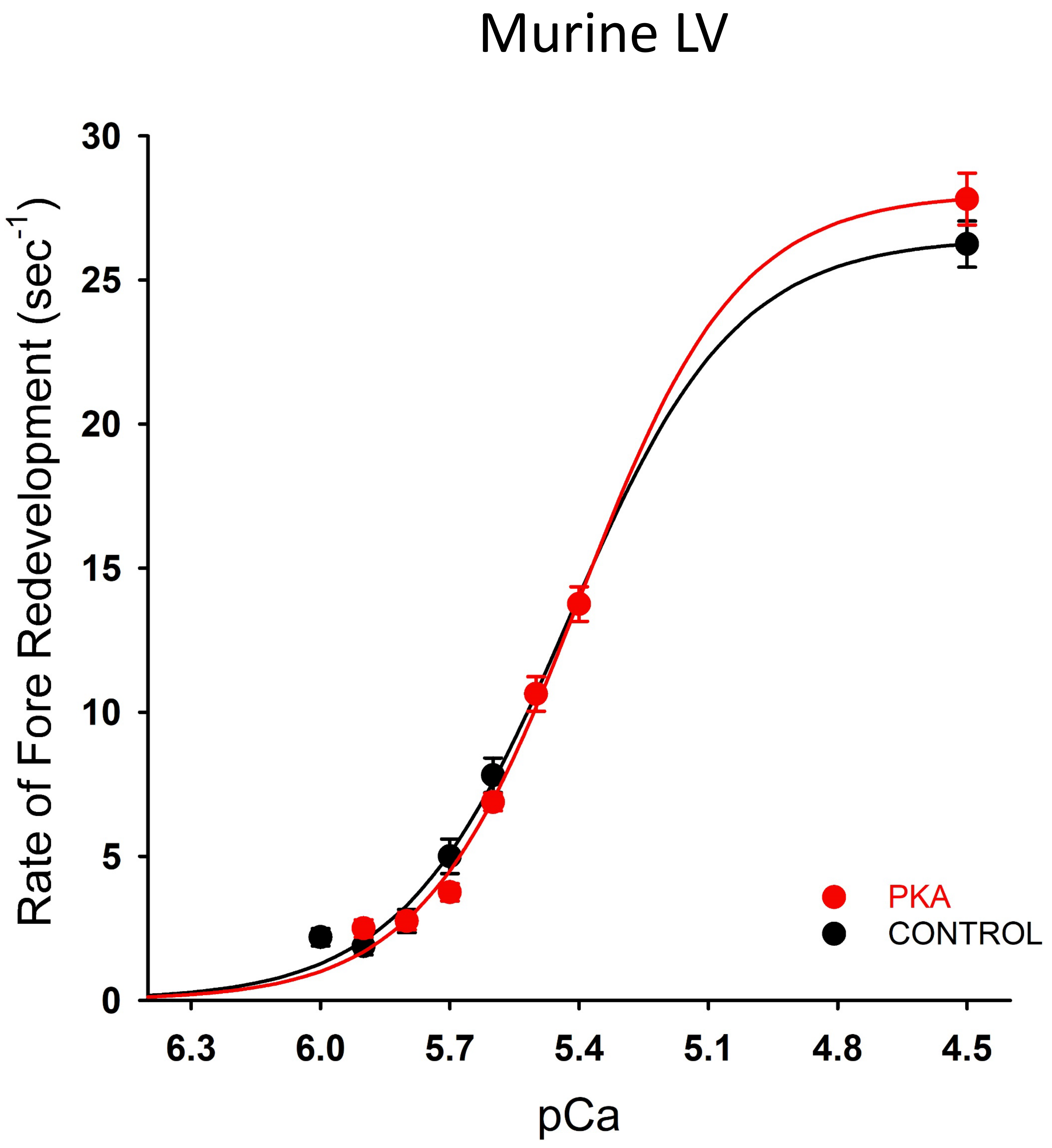
Measurement	Murine LV (Control)	Murine LV (PKA)	Human LV (Control)	Human LV (PKA)
P_o (mN mm^{-2})	14.4 ± 1.4	12.6 ± 1.4	36.9 ± 2.9	30.5 ± 3.0
P_{rest} (mN mm^{-2})	0.7 ± 0.1	0.5 ± 0.1	1.8 ± 0.2	1.0 ± 0.2
n_H	4.2 ± 0.2	4.2 ± 0.2	4.3 ± 0.4	5.5 ± 0.1
pCa_{50}	5.82 ± 0.01	5.69 ± 0.01	5.81 ± 0.01	5.69 ± 0.01
*k*tr (sec^{-1})	26.6 ± 0.9	28.0 ± 1.3	2.7 ± 0.1	2.9 ± 0.2
	95% α-MyHC / 5% β-MyHC		90% β-MyHC / 10% α-MyHC	

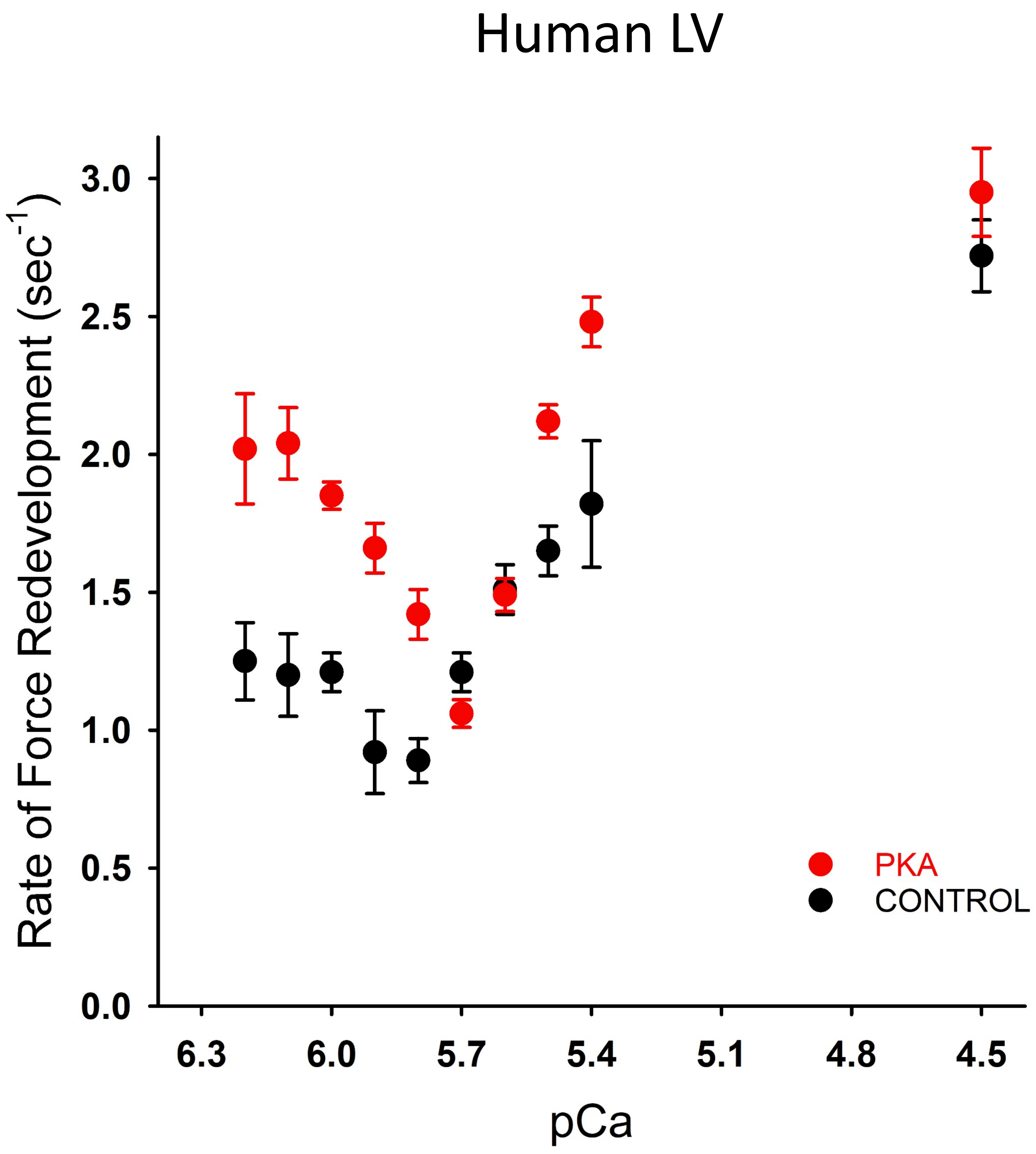
Force-pCa Relationships in Mammalian Myocardium





Rate Constant of Force Redevelopment in Mammalian Myocardium



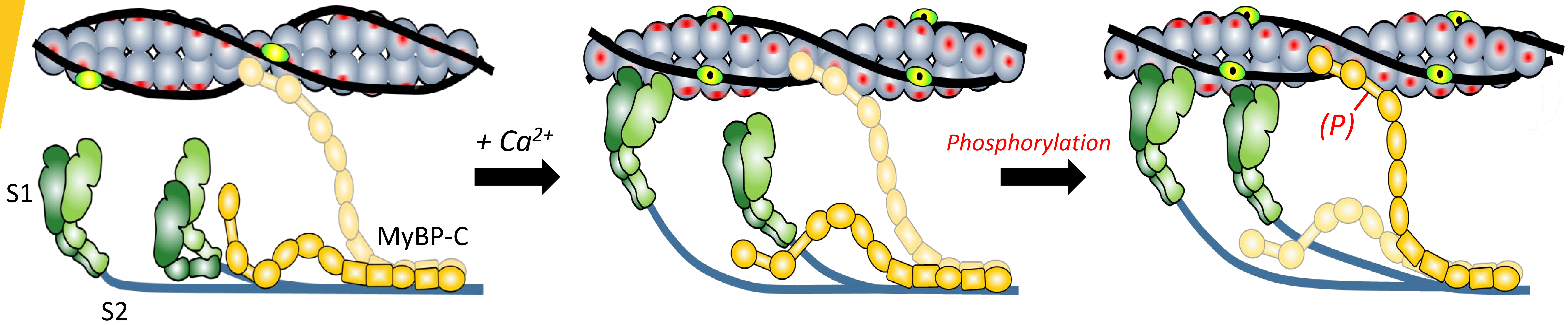


Model for Accelerated Contraction due to MyBP-C Phosphorylation

Relaxed
Dephosphorylated

Ca^{2+}-activated
Dephosphorylated
Inhibition of some XBs

Ca^{2+}-activated
Phosphorylated
Release of XB inhibition



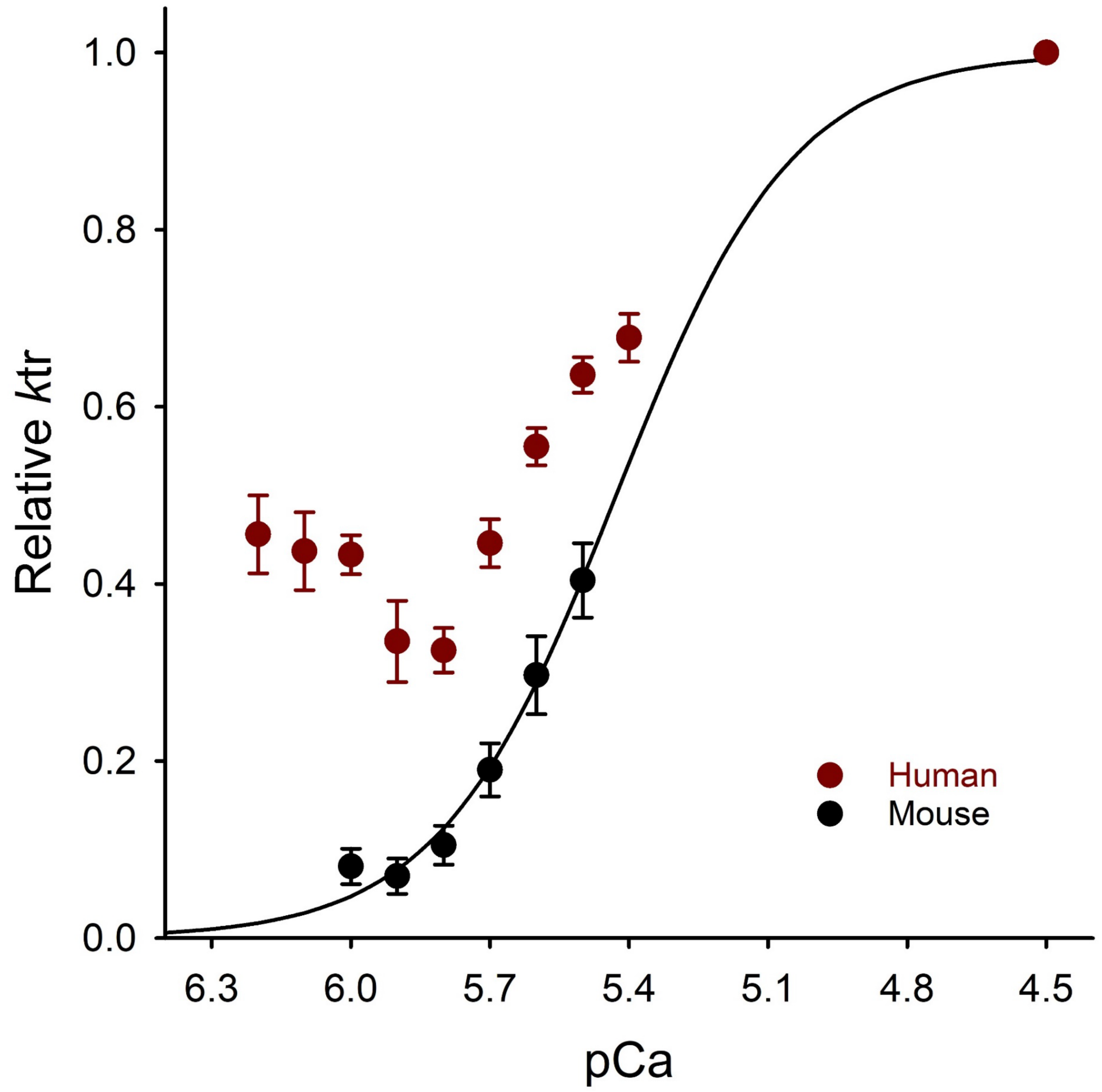
Myosin binding

Myosin binding

Actin binding

Acute modulation of myocardial work capacity

Conclusions

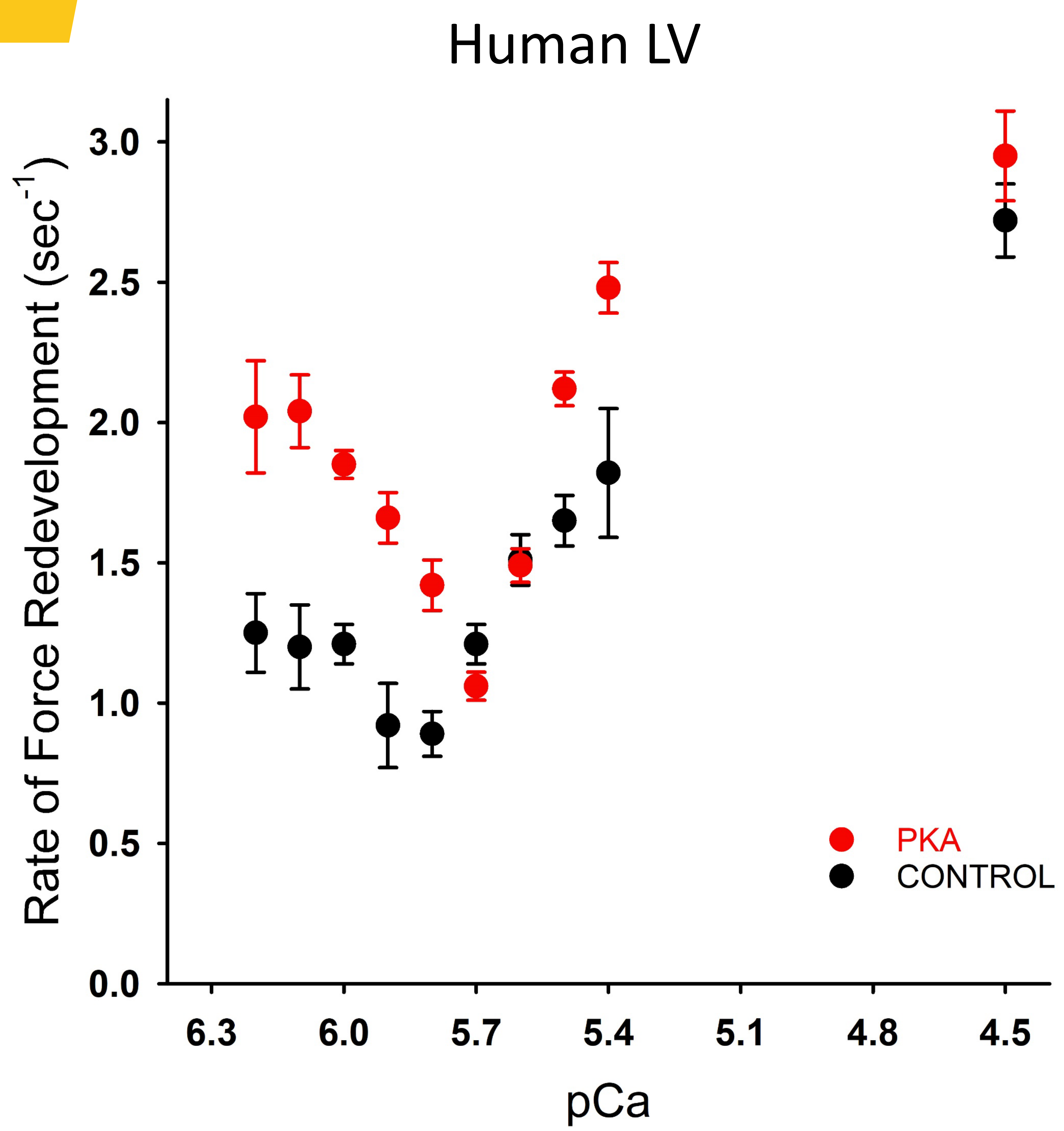


Under control conditions, the faster normalized *k*tr values at low and intermediate levels of Ca^{2+} activation in human myocardium may reflect an altered level of thin filament activation due to the synergistic expression of α-MyHC and β-MyHC.

When Ca^{2+} is released during EC coupling, α-MyHC is the earliest/first to bind to the thin filament.

Due to its greater activating effect on the thin filament, the binding of α-MyHC opens the thin filament for initial β-MyHC binding and the subsequent cooperative spread of β-MyHC binding.

Conclusions



The effect of MyBP-C phosphorylation on contractile kinetics was amplified in human myocardium.

This may be due, at least in part, to an enhanced level of thin filament activation by the initial binding of fast α-MyHC and phosphorylated MyBP-C to the thin filament.

Thereby, resulting in a greater rate and spread of cooperative β-MyHC binding along the human thin filament.

Acknowledgments

<u>University of Wisconsin School of Medicine and Public Health</u>

Jitandrakumar Patel, PhD (Dept. of Cell and Regenerative Biology)

Eric Schmuck, PhD (Dept. of Medicine)

Amish Raval, MD (Dept. of Medicine)

<u>University of Idaho</u>

Tuan Phan, PhD (Institute for Modeling Collaboration and Innovation)

Kayla Park

Aidan Bradshaw

<u>Mountain West Clinical & Translational Research Infrastructure Network</u>

Pilot Grant Funding 2021-2022

Thin Filament Regulatory Unit – Myosin Cross-bridge Model (Four-State Model)