Supplement

PKA catalytic subunit compartmentation regulates contractile and hypertrophic responses to β-adrenergic signaling

Jason H. Yang, Ph.D., Renata K. Polanowska-Grabowska, Ph.D., Jeffrey S. Smith, B.S., Charles W. Shields, IV, B.S., Jeffrey J. Saucerman, Ph.D.

Methods

Cardiomyocyte Isolation and Culture

Neonatal rat ventricular myocytes were isolated from 1-2 day old Sprague-Dawley rats using the Neomyt Cardiomyocyte Isolation Kit (Cellutron Life Technologies, Baltimore, MD) and cultured on Surecoat-treated 35 mm glass-bottom dishes (MatTek, Ashland, MA), Surecoat-treated 6-well plates or CellBIND-coated 96-well plates (Corning, Corning, NY) as described previously [1]. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and approved by the University of Virginia Institutional Animal Care and Use Committee. Myocytes were cultured in media containing 65% Dulbecco's Modified Eagle Medium, 17% Medium 199, 10% horse serum, 5% fetal bovine serum, 2% penicillin / streptomycin and 1% L-glutamine on either 35 mmol/L glass-bottom dishes (MatTek, Ashland, MA) or CellBIND coated 96-well plates (Corning, Corning, NY).

Spatially Targeted PKA Over-Expression

mCherry-labeled PKA catalytic subunits containing a C-terminal nuclear export sequence (-NES) or nuclear localization sequence (-NLS) were constructed by ligating the respective PKA-NES or PKA-NLS segments from CMV-EGFP-PKA-NES [2] or CMV-EGFP-PKA-NLS [2] into the mCherry-C1 expression vector (Clontech, Mountain View, CA) at the BSPEI/BamHI restriction sites. Targeted PKA over-expression was achieved using Lipofectamine 2000 (Invitrogen, Carlsbad, CA), following the manufacturer's instructions.

Ca^{2+} Imaging

Two days after isolation, myocytes cultured in 35 mm glass-bottom dishes were transferred to serum-free media (76.8% Dulbecco's Modified Eagle Medium, 19.2% Medium 199, 2% penicillin / streptomycin and 1% insulin-transferrin-sodium selenite) for 24 hours. Three days after isolation, cultured myocytes were loaded by incubating in Fluo-4 AM (Invitrogen, Carlsbad, CA) dissolved in Tyrode's Solution for 30 minutes. Loaded myocytes were then de-esterified by washing out the Fluo-4/Tyrode's mixture and incubating myocytes for an additional 30 minutes in Tyrode's Solution. De-esterified myocytes were then field stimulated at 1 Hz pacing using the C-Pace EP Culture Pacer (IonOptix, Milton, MA) and stimulated using isoproterenol (ISO; Tocris, Minneapolis, MN) dissolved in Tyrode's Solution. Paced myocytes were imaged on an IX-81 inverted microscope (Olympus, Center Valley, PA) with a Digital CCD C9300-221 camera (Hamamatsu, Bridgewater, NJ) at 10 Hz using MetaMorph (Molecular Devices, Sunnyvale, CA). Cells were segmented in ImageJ (National Institutes of Health, Bethesda, MA) and analyzed in MATLAB (Mathworks, Natick, MA).

Hypertrophy Measurements

Two days after isolation, myocytes cultured in 96-well plates were transfected with cTnT-EGFP plasmid [3] using Lipofectamine 2000. Two days following transfection, myocytes were cultured in serum-free media containing various concentrations of ISO for 24 hours. Following incubation, myocytes were imaged on an Olympus IX-81 inverted microscope with an automated stage (Prior Scientific, Rockland, MA) and an Orca-AG CCD camera (Hamamatsu, Bridgewater, NJ) using IPLab (Scanalytics, Fairfax,

VA), as described previously[1]. Images were segmented and analyzed in MATLAB using custom image processing algorithms.

FRET Imaging
Two days after isolation, myocytes cultured in 35 mmol/L glass-bottom dishes were transfected with CMV-AKAR-NES [4], CMV-AKAR-NLS [4], CMV-ICUE-NES [5] or CMV-ICUE-NLS [5] plasmid using Lipofectamine 2000. Following transfection, myocytes were cultured in serum-free media for 24 hours. Following incubation, myocytes were washed and incubated in Tyrode's Solution. Imaging was performed on an Olympus IX-81 inverted microscope with an Orca-AG CCD camera using IPLab. Cells were segmented in ImageJ and analyzed in MATLAB. One minute after the beginning of each experiment, Tyrode's Solution was added to each dish as a negative control. Cells were then treated with ISO dissolved in Tyrode's Solution at different concentrations. Finally, cells were treated with 50 μmol/L forskolin (FSK; Tocris, Minneapolis, MN) and 100 μmol/L 3-isobytl-1-methylxanthine (IBMX; Sigma-Aldrich, St. Louis, MO) as a positive control. FRET computations were performed in MATLAB using the PFRET algorithm [6]. Cells were then segmented in ImageJ and FRET responses were normalized to positive and negative controls in MATLAB. For wheat germ agglutinin (WGA, Sigma-Aldrich, St. Louis, MO) experiments, myocytes were pre-incubated in Tyrode's Solution with 20 μg/mL WGA before each experiment.

Computational Modeling
Nuclear PKA activity was modeled by modifying our previously published ordinary differential equation implementation of cardiac β-adrenergic signaling [7-9] to include nuclear PKA transport, PKI transport and AKAR expression/phosphorylation (*Supplement*). The expanded model was implemented in MATLAB and constrained to parameters estimated from published literature. The final model contained 34 state variables and 104 parameters. Before each simulation, fresh initial conditions were generated by running the model to steady-state with no ISO stimulation.

Immunofluorescence
Two days after isolation, myocytes cultured in 35 mmol/L glass-bottom dishes were fixed in 4% paraformaldehyde (Fisher Scientific, Pittsburgh, PA) for 20 minutes. Myocytes were then permeabilized with 0.2% Triton X-100 (MP Biomedicals, Solon, OH) for 2 minutes. Myocytes were then blocked with 1% bovine serum albumin (Sigma-Aldrich, St. Louis, MO) for 45 minutes. After blocking, myocytes were incubated with 1:200 rabbit polyclonal anti-PKIA primary antibodies (Lifespan Biosciences, Seattle WA) for 1 hour. Myocytes were then blocked with 2% normal goat serum (Sigma-Aldrich, St. Louis, MO) for 30 minutes. After blocking, myocytes were incubated with 1:200 goat anti-rabbit secondary antibodies conjugated with Alexa fluorophors (Invitrogen, Carlsbad, CA). Following washout, cells were imaged on an Olympus IX-81 inverted microscope with an Orca-AG CCD camera using IPLab. Cells were analyzed using ImageJ.

Western Blot Measurements
Two days after isolation, myocytes cultured in 6-well plates were treated with ISO for 30 minutes and then rinsed with ice cold phosphate buffered solution. Cellular lysates were prepared in Pierce RIPA Buffer (Thermo Scientific, Rockford, IL) supplemented with Complete Protease Inhibitor Cocktail Tablets (Roche, Indianapolis, IN) and Halt Phosphatase Inhibitor Cocktail (Thermo Scientific, Rockford, IL). To quantify nuclear PKA catalytic subunit accumulation, cells were fractionated into nuclear and cytosolic fractions using NE-PER Nuclear Protein Extraction Kit (Thermo Scientific) supplemented with Protease Inhibitor Cocktail. Protein concentrations from cell lysates were quantified using the Micro BCA Protein Assay Kit (Thermo Scientific, Rockford, IL) and all samples were normalized for protein concentration. Lysed cells were then mixed with 4x SDS-sample buffer. Samples for pCREB detection were boiled for 5 min at 95°C. Equal amounts of total protein from each sample were electrophoresed on 10% SDS-PAGE gels and then electrotransferred onto Immobilon-FL PVDF membranes (Millipore,

Billerica, MA) at 100 V for 1 hour. These membranes were rinsed with phosphate buffer solution and then incubated with Odyssey Blocking Buffer (LI-COR Biosciences, Lincoln, NE). The membranes were probed with affinity-purified rabbit polyclonal antibodies specific for PKA Cα (Cell Signaling Technology, Danvers, MA). Mouse anti-fibrillarin antibodies (Abcam, Cambridge, England) were used as nuclear loading controls, while mouse anti-α-tubulin antibodies (LI-COR Biosciences, Lincoln, NE) were used as cytosolic loading controls. To quantify CREB and phospholamban (PLB) phosphorylation, samples were resolved on 15% SDS-polyacrylamide gels by electrophoresis and then transferred to Immobilon-FL PVDF membranes (Millipore, Billerica, MA). Blots were probed using rabbit anti-phospho-PLB (Ser16/Thr17) or rabbit anti-phospho-CREB (Ser133) antibodies (Cell Signaling Technology). Mouse anti-α-tubulin antibodies (LI-COR Biosciences) were used as protein loading controls. Phosphorylated PLB and CREB were visualized using goat anti-rabbit IRDye800 CW secondary antibodies, while α-tubulin was detected using goat anti-mouse IRDye680 CW secondary antibodies (LI-COR Biosciences). All membranes were scanned on a LI-COR Odyssey scanner. Signal intensities for each experiment were quantified using Image Studio Lite (LI-COR Biosciences). All bands were normalized to their respective loading controls.

Statistics

All statistical analyses were performed using Prism 5.0 (GraphPad, La Jolla, CA). EC_{50}s for ISO-stimulated Ca^{2+} enhancements, hypertrophy, FRET responses and PLB and CREB phosphorylation were fitted to a variable slope dose-response curve. Unpaired *t*-tests were performed on ICUE and AKAR t_{50}s for statistical significance. A one-way ANOVA was performed on Western blot measurments of nuclear PKA catalytic subunit enrichment. Non-parametric Mann-Whitney tests were performed on hypertrophy measurements. Hypertrophy measurements are reported as median±standard error of median. All other statistics are reported as mean±standard error of mean.

Supplementary Figures

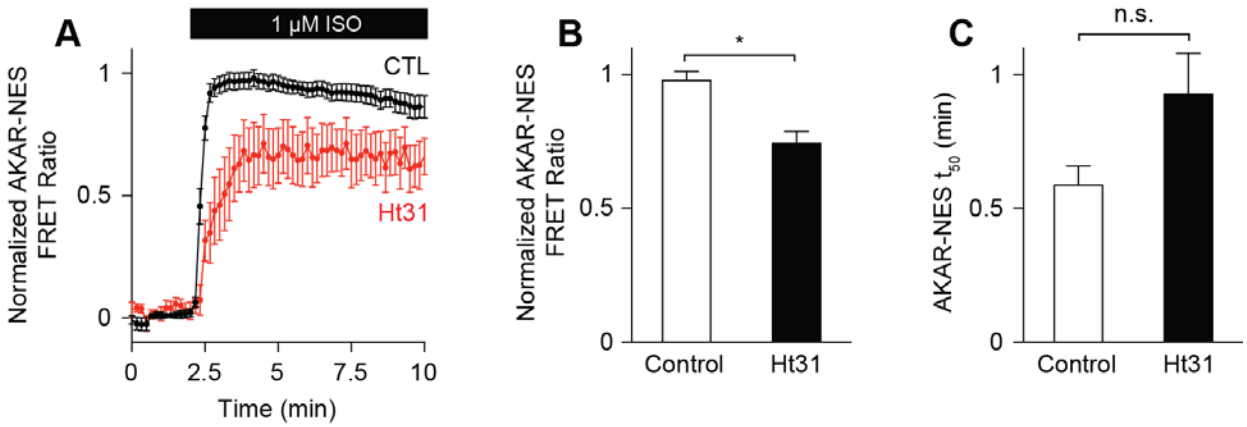

Fig. S1. AKAP disruption reduces cytosolic PKA responses to 1 μmol/L ISO. A, Mean cytosolic AKAR-NES responses to 1 μmol/L ISO following pre-treatment with 50 μmol/L Ht31 (n = 8 cells). Ht31 pre-treatment significantly reduces the peak amplitude (B), but does not significantly alter the t_{50} (C).

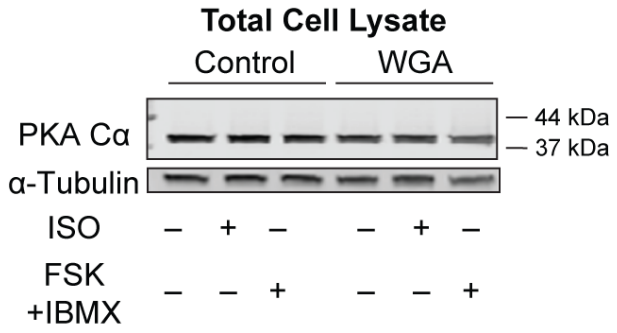

Fig. S2. Representative Western blots for PKA catalytic subunits from whole cell lysates of control myocytes and cells pre-treated with 20 μg/mL WGA. WGA pre-treatment does not alter whole cell catalytic subunit expression.

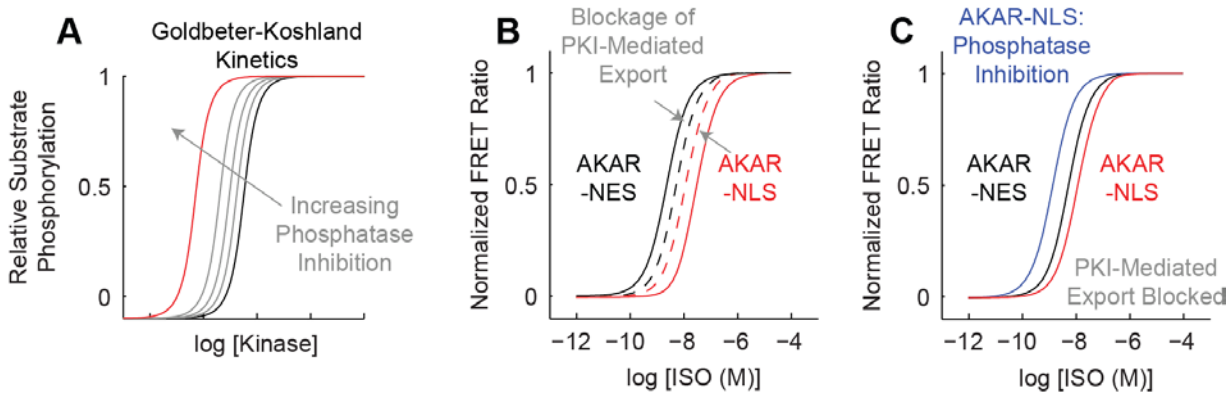

Fig. S3. PKI-mediated export of nuclear PKA catalytic subunit may contribute to differences in ISO sensitivity between cytosolic and nuclear PKA activity. A, Goldbeter-Koshland kinetics can be used to model steady-state substrate phosphorylation in a simplified system consisting of only a kinase, substrate and phosphatase [10]. Intuitively, in this system phosphatase inhibition increases the sensitivity of substrate phosphorylation to a kinase. In contrast, the computational model for nuclear PKA activity in cardiac myocytes makes a counter-intuitive prediction that phosphatase inhibition does not increase the sensitivity of nuclear PKA activity to ISO stimulation (Fig. 5B). B, The computational model predicts that PKI-mediated export of nuclear PKA catalytic subunits contributes to the lower ISO sensitivity differences between cytosolic and nuclear PKA activity. C, Under conditions of blocked PKI-mediated PKA-C export, phosphatase inhibition does increase the ISO sensitivity of AKAR-NLS (similar to that seen with Goldbeter-Koshland kinetics). Thus in normal cells it appears that PKI-mediated export acts as an alternative brake on nuclear PKA activity, reducing the effect of phosphatase inhibition on ISO sensitiivty. Blockage of PKI-mediated export increases total nuclear PKA and restores the intuitive sensitivity of nuclear PKA activity to phosphatase inhibition.

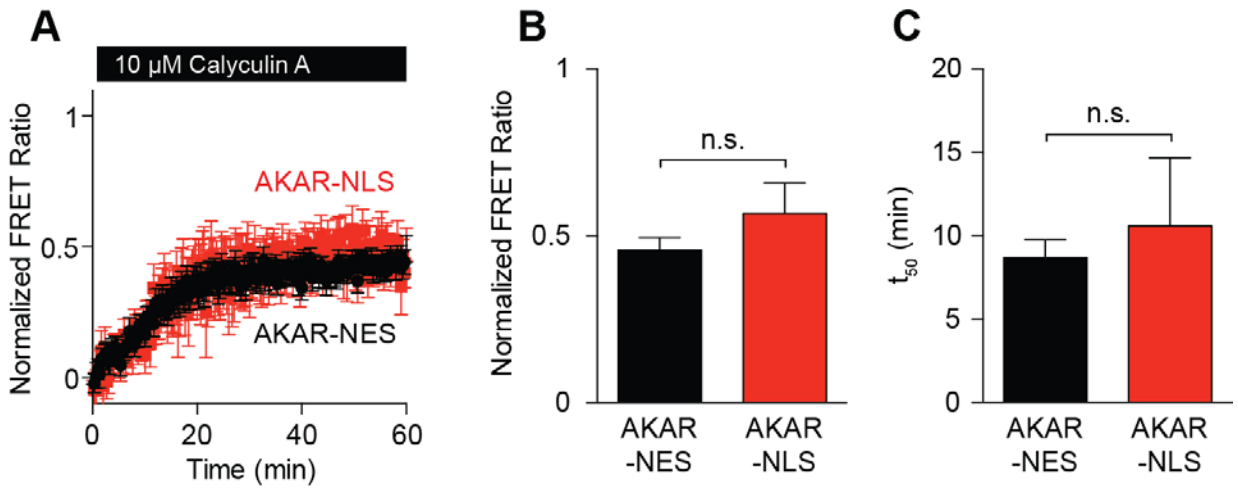

Fig. S4. Phosphatase inhibition reveals basal cytosolic and nuclear phosphatase activity. A, Mean AKAR-NES and AKAR-NLS responses to 10 μmol/L Calyculin A ($n \geq 9$ cells each). AKAR-NES and AKAR-NLS responses shared similar peak amplitudes (B) and kinetics (C).

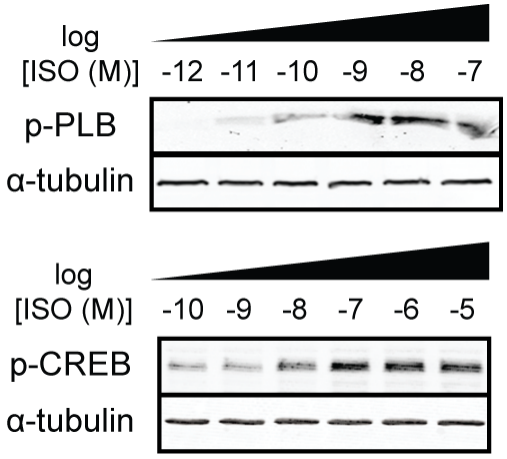

Fig. S5. Representative Western blots for differential ISO sensitivity of endogenous PKA substrates. PLB phosphorylation has significantly higher ISO sensitivity than CREB phosphorylation.

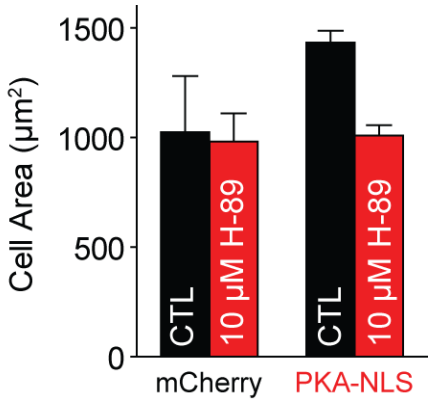

Fig. S6. Cell hypertrophy responses to 10 µmol/L H-89. 10 µmol/L H-89 blocked PKA-NLS induced cell hypertrophy, indicating a specific action by the PKA catalytic subunit (n > 100 cells each).

Model Equations and Parameters

β-Adrenergic Receptor / Gsα

Parameter	Description	Value	Units	Source
ISO	isoproterenol concentration (when used)	1	μmol/L	-
b1ARtot	total β1-adrenergic receptors	0.0132	μmol/L	[7-8]
Gstot	total Gs protein	3.83	μmol/L	[7]
kf_LR	β1-AR binding to ligand	1	1/[μmol/L ms]	[7-8]
kr_LR	β1-AR binding to ligand	0.285	1/ms	[7-8]
kf_LRG	ligand bound β1-AR associating with G-protein	1	1/[μmol/L ms]	[7-8]
kr_LRG	ligand bound β1-AR associating with G-protein	0.062	1/ms	[7-8]
kf_RG	unbound β1-AR associating with G-protein	1	1/[μmol/L ms]	[7-8]
kr_RG	unbound β1-AR associating with G-protein	33.0	1/ms	[7-8]
k_G_act	Gs-alpha activation	16.0e-3	1/ms	[7-8]
k_G_hyd	Gs-alpha hydrolysis	0.8e-6	1/ms	[7-8]
k_G_reassoc	Gs-alpha reassociation	1.21	1/[μmol/L ms]	[7-8]
kf_bark	β1-AR desensitization by β-arrestin	1.1e-6	1/ms	[7-8]
kr_bark	β1-AR resensitization	2.2e-6	1/ms	[7-8]
kf_pka	β1-AR desensitization by PKA	3.6e-6	1/[μmol/L ms]	[7-8]
kr_pka	β1-AR resensitization	2.2e-6	1/ms	[8]

$$b1ARact = b1ARtot - b1AR\_S464 - b1AR\_S301$$

$$b1AR = b1ARact - LR - LRG - RG$$

$$Gs = Gstot - LRG - RG - Gsby$$

$$\frac{dLR}{dt} = kf\_LR \cdot ISO \cdot b1AR - kr\_LR \cdot LR$$

$$\frac{dLRG}{dt} = kf\_LRG \cdot LR \cdot Gs - kr\_LRG \cdot LRG - k\_G\_act \cdot LRG$$

$$\frac{dRG}{dt} = kf\_RG \cdot b1AR \cdot Gs - kr\_RG \cdot Gs - k\_G\_act \cdot RG$$

$$\frac{db1AR\_S464}{dt} = kf\_bARK \cdot (LR + LRG) - kr\_bARK \cdot b1AR\_S464$$

$$\frac{db1AR\_S301}{dt} = kf\_PKA \cdot PKACI \cdot b1ARact - kr\_PKA \cdot b1AR\_S301$$

$$\frac{dGsaGTPtot}{dt} = k\_G\_act \cdot (RG + LRG) - k\_G\_hyd \cdot GsaGTPtot$$

$$\frac{dGsaGDP}{dt} = k\_G\_hyd \cdot GsaGTPtot - k\_G\_reassoc \cdot GsaGDP \cdot Gsby$$

$$\frac{dGsby}{dt} = k\_G\_act \cdot (RG + LRG) - k\_G\_reassoc \cdot GsaGDP \cdot Gsby$$

cAMP

Parameter	Description	Value	Units	Source
ACtot	total adenylyl cyclase	49.7e-3	µmol/L	[7-8]
ATP	total ATP	5.0e3	µmol/L	[7-8]
PDEtot	total phosphodiesterases	22.85e-3	µmol/L	[7]
IBMX	isobutylmethylxanthine concentration	0.0	µmol/L	[8]
FSK	forskolin concentration	0.0	µmol/L	[8]
k_AC_basal	basal AC activity	0.2e-3	1/ms	[7-8]
Km_AC_basal	basal AC affinity for ATP	1.03e3	µmol/L	[7-8]
k_AC_Gsa	AC activity with Gs-alpha activation	8.5e-3	1/ms	[8]
Km_AC_Gsa	AC:Gs-alpha affinity for ATP	315.0	µmol/L	[7-8]
kf_AC_Gsa	AC activation by Gs-alpha	1	1/[µmol/L ms]	[8]
kr_AC_Gsa	AC activation by Gs-alpha	0.4	1/ms	[8]
k_AC_FSK	AC activation by forskolin	7.3e-3	1/ms	[8]
Km_AC_FSK	AC:FSK affinity for ATP	860.0	µmol/L	[8]
kf_AC_FSK	AC activation by forskolin	1	1/[µmol/L ms]	[8]
kr_AC_FSK	AC activation by forskolin	44	1/ms	[8]
k_cAMP_PDE	cAMP degradation by PDEs	5.0e-3	1/ms	[8]
k_cAMP_PDEp	cAMP degradation by phosphorylated PDEs	10.0e-3	1/ms	[11]
Km_PDE_cAMP	PDE affinity for cAMP	1.3	µmol/L	[7-8]
Kd_PDE_IBMX	PDE inhibition by IBMX	30.0	µmol/L	[7-8]
k_PKA_PDE	PDE phosphorylation by PKA	7.5e-3	1/ms	[11]
k_PP_PDE	PDE inhibition by IBMX	1.5e-3	1/ms	[11]

$$cAMP = cAMPtot - (RCcAMP\_I + 2 \cdot RCcAMPcAMP\_I + 2 \cdot RcAMPcAMP\_I) - (RCcAMP\_II + 2 \cdot RCcAMPcAMP\_II + 2 \cdot RcAMPcAMP\_II)$$

$$AC = ACtot - AC\_GsaGTP$$

$$GsaGTP = GsaGTPtot - AC\_GsaGTP$$

$$\frac{dAC\_GsaGTP}{dt} = kf\_AC\_Gsa \cdot GsaGTP \cdot AC - kr\_AC\_Gsa \cdot AC\_GsaGTP$$

$$AC\_FSK = \frac{FSK \cdot AC}{Kd\_AC\_FSK}$$

$$PDE\_IBMX = \frac{PDEtot \cdot IBMX}{Kd\_PDE\_IBMX}$$

$$PDE = PDEtot - PDE\_IBMX - PDEp$$

$$\frac{dPDEp}{dt} = k\_PKA\_PDE \cdot PKACII \cdot PDE - k\_PP\_PDE \cdot PDEp$$

$$PDE_{ACT} = \frac{k\_cAMP\_PDE \cdot PDE \cdot cAMP}{Km\_PDE\_cAMP + cAMP} + \frac{k\_cAMP\_PDEp \cdot PDEp \cdot cAMP}{Km\_PDE\_cAMP + cAMP}$$

$$\frac{dcAMPtot}{dt} = \frac{k\_AC\_basal \cdot AC \cdot ATP}{Km\_AC\_basal + ATP} + \frac{k\_AC\_Gsa \cdot AC\_GsaGTP \cdot ATP}{Km\_AC\_Gsa + ATP} + \frac{k\_AC\_FSK \cdot AC\_FSK \cdot ATP}{Km\_AC\_FSK + ATP} - \frac{k\_cAMP\_PDE \cdot PDE \cdot cAMP}{Km\_PDE\_cAMP + cAMP} - \frac{k\_cAMP\_PDEp \cdot PDEp \cdot cAMP}{Km\_PDE\_cAMP + cAMP}$$

PKA / PKI Transport

Parameter	Description	Value	Units	Source
VnucF	nuclear fractional volume	0.02	-	[12]
DPKIcn	PKI cytosol to nucleus diffusion rate	19.8e-6	1/ms	-
DPKACIIcn	PKA cytosol to nucleus diffusion rate	1.190476e-7	1/ms	-
DPKACII_PKInc	PKA-PKI active transport rate	3.2e-6	1/ms	-
PKIbias	PKI nuclear expression bias	50	-	-

$$PKI = PKItot - PKACI\_PKI - PKACII\_PKI - nPKI \cdot VnucF - nPKACII\_PKI \cdot VnucF$$

$$JPKACIIcn = DPKACIIcn \cdot (PKACII - nPKACII)$$

$$JPKIcn = DPKIcn \cdot \left(PKI - \frac{nPKI}{PKIbias}\right)$$

$$JPKACII\_PKIcn = -DPKACII\_PKInc \cdot nPKACII\_PKI \cdot VnucF$$

PKA

Parameter	Description	Value	Units	Source
PKAItot	total type 1 protein kinase A	0.59	µmol/L	[7]
PKAIItot	total type 2 protein kinase A	0.059	µmol/L	adapted
PKItot	total protein kinase inhibitor	0.18	µmol/L	[7-8]
kf_RC_cAMP	cAMP association with PKA	1	1/[µmol/L ms]	[7-8]
kr_RC_cAMP	cAMP association with PKA	1.64	1/ms	[7-8]
kf_RCcAMP_cAMP	cAMP association with PKA	1	1/[µmol/L ms]	[7-8]
kr_RCcAMP_cAMP	cAMP association with PKA	9.14	1/ms	[7-8]
kf_RcAMPcAMP_C	catalytic subunit dissociation	4.375	1/[µmol/L ms]	[7-8]
kr_RcAMPcAMP_C	catalytic subunit dissociation	1	1/ms	[7-8]
kf_PKA_PKI	PKA inhibition by PKI	1	1/[µmol/L ms]	[7-8]
kr_PKA_PKI	PKA inhibition by PKI	0.2e-3	1/ms	[7-8]

$$\frac{dRC\_I}{dt} = -kf\_RC\_cAMP \cdot RC\_I \cdot cAMP + kr\_RC\_cAMP \cdot RCcAMP\_I$$

$$\frac{dRCcAMP\_I}{dt} = -kr\_RC\_cAMP \cdot RCcAMP\_I + kf\_RC\_cAMP \cdot RC\_I \cdot cAMP - kf\_RCcAMP\_cAMP \cdot RCcAMP\_I \cdot cAMP + kr\_RCcAMP\_cAMP \cdot RCcAMPcAMP\_I$$

$$\frac{dRCcAMPcAMP\_I}{dt} = -kr\_RCcAMP\_cAMP \cdot RCcAMPcAMP\_I + kf\_RCcAMP\_cAMP \cdot RCcAMP\_I \cdot cAMP - kf\_RcAMPcAMP\_C \cdot RCcAMPcAMP\_I + kr\_RcAMPcAMP\_C \cdot RcAMPcAMP\_I \cdot PKACI$$

$$\frac{dRcAMPcAMP\_I}{dt} = -kr\_RcAMPcAMP\_C \cdot RcAMPcAMP\_I \cdot PKACI + kf\_RcAMPcAMP\_C \cdot RCcAMPcAMP\_I$$

$$\frac{dPKACI}{dt} = -kr\_RcAMPcAMP\_C \cdot RcAMPcAMP\_I \cdot PKACI + kf\_RcAMPcAMP\_C \cdot RCcAMPcAMP\_I - kf\_PKA\_PKI \cdot PKACI \cdot PKI + kr\_PKA\_PKI \cdot PKACI\_PKI$$

$$\frac{dPKA\_CI\_PKI}{dt} = -kr\_PKA\_PKI \cdot PKACI\_PKI + kf\_PKA\_PKI \cdot PKACI \cdot PKI$$

$$\frac{dRC\_II}{dt} = -kf\_RC\_cAMP \cdot RC\_II \cdot cAMP + kr\_RC\_cAMP \cdot RCcAMP\_II$$

$$\frac{dRCcAMP\_II}{dt} = -kr\_RC\_cAMP \cdot RCcAMP\_II + kf\_RC\_cAMP \cdot RC\_II \cdot cAMP - kf\_RCcAMP\_cAMP \cdot RCcAMP\_II \cdot cAMP + kr\_RCcAMP\_cAMP \cdot RCcAMPcAMP\_II$$

$$\frac{dRCcAMPcAMP\_II}{dt} = -kr\_RCcAMP\_cAMP \cdot RCcAMPcAMP\_II + kf\_RCcAMP\_cAMP \cdot RCcAMP\_II \cdot cAMP - kf\_RcAMPcAMP\_C \cdot RCcAMPcAMP\_II + kr\_RcAMPcAMP\_C \cdot RcAMPcAMP\_II \cdot PKACII$$

$$\frac{dRcAMPcAMP\_II}{dt} = -kr\_RcAMPcAMP\_C \cdot RcAMPcAMP\_II \cdot PKACII + kf\_RcAMPcAMP\_C \cdot RCcAMPcAMP\_II$$

$$\frac{dPKACII}{dt} = -kr\_RcAMPcAMP\_C \cdot RcAMPcAMP\_II \cdot PKACII + kf\_RcAMPcAMP\_C \cdot RCcAMPcAMP\_II - kf\_PKA\_PKI \cdot PKACII \cdot PKI + kr\_PKA\_PKI \cdot PKACII\_PKI - JPKACIIcn$$

$$\frac{dPKA\_CII\_PKI}{dt} = -kr\_PKA\_PKI \cdot PKACII\_PKI + kf\_PKA\_PKI \cdot PKACII \cdot PKI - JPKACII\_PKIcn$$

$$\frac{dnPKI}{dt} = kr\_PKA\_PKI \cdot nPKACII\_PKI - kf\_PKA\_PKI \cdot nPKACII \cdot nPKI + \frac{JPKIcn}{VnucF}$$

$$\frac{dnPKACII}{dt} = kr\_PKA\_PKI \cdot nPKACII\_PKI - kf\_PKA\_PKI \cdot nPKACII \cdot nPKI + \frac{JPKACIIcn}{VnucF}$$

$$\frac{dnPKACII\_PKI}{dt} = -kr\_PKA\_PKI \cdot nPKACII\_PKI + kf\_PKA\_PKI \cdot nPKACII \cdot nPKI + \frac{JPKACII\_PKIcn}{VnucF}$$

I-1/PP1

Parameter	Description	Value	Units	Source
PP1tot	total phosphatase 1	0.89	µmol/L	[7-8]
I1tot	total inhibitor 1	0.3	µmol/L	[7-8]
k_PKA_I1	PKA phosphorylation of inhibitor 1	60e-3	1/ms	[7-8]
Km_PKA_I1	PKA phosphorylation of inhibitor 1	1.0	µmol/L	[7-8]
Vmax_PP2A_I1	PP2A dephosphorylation of phospholamban	14.0e-3	µmol/L /ms	[7-8]
Km_PP2A_I1	PP2A dephosphorylation of phospholamban	1.0	µmol/L	[7-8]
kf_PP1_I1	PP1 inhibition by inhibitor 1	1.0	1/[µmol/L ms]	[7-8]
kr_PP1_I1	PP1 inhibition by inhibitor 1	1.0e-3	1/ms	[7-8]

$$I1 = I1tot - I1ptot$$

$$PP1 = PP1tot - I1p\_PP1$$

$$I1p = I1ptot - I1p\_PP1$$

$$\frac{dI1p\_PP1}{dt} = kf\_PP1\_I1 \cdot PP1 \cdot I1p - kr\_PP1\_I1 \cdot I1p\_PP1$$

$$\frac{dI1ptot}{dt} = \frac{k\_PKA\_I1 \cdot PKACI \cdot I1}{Km\_PKA\_I1 + I1} - \frac{Vmax\_PP2A\_I1 \cdot I1ptot}{Km\_PP2A\_I1 + I1ptot}$$

LCC

Parameter	Description	Value	Units	Source
LCCtot	total L-type Ca channel	0.025	µmol/L	[7-8]
PKACII_LCCtot	total PKA local to L-type Ca channel	0.025	µmol/L	[7-8]
PP1_LCC	total PP1 local to L-type Ca channel	0.025	µmol/L	[7-8]
PP2A_LCC	total PP2A local to L-type Ca channel	0.025	µmol/L	[7-8]
epsilon	AKAP-mediated scaling factor	10	-	[7-8]
k_PKA_LCC	PKA phosphorylation of LCC	54e-3	1/ms	[7-8]
Km_PKA_LCC	PKA phosphorylation of LCC	21	µmol/L	[7-8]
k_PP1_LCC	PP1 dephosphorylation of LCC	8.52e-3	1/ms	[7-8]
Km_PP1_LCC	PP1 dephosphorylation of LCC	3	µmol/L	[7-8]
k_PP2A_LCC	PP2A dephosphorylation of LCC	10.1e-3	1/ms	[7-8]
Km_PP2A_LCC	PP2A dephosphorylation of LCC	3	µmol/L	[7-8]

$$PKACII\_LCC = \frac{PKACII\_LCCtot}{PKAIItot} \cdot PKACII$$

$$LCCa = LCCtot - LCCap$$

$$\frac{dLCCap}{dt} = \frac{epsilon \cdot k\_PKA\_LCC \cdot PKACII\_LCC \cdot LCCa}{Km\_PKA\_LCC + epsilon \cdot LCCa} - \frac{epsilon \cdot k\_PP2A\_LCC \cdot PP2A\_LCC \cdot LCCap}{Km\_PP2A\_LCC + epsilon \cdot LCCap}$$

$$LCCb = LCCtot - LCCbp$$

$$\frac{dLCCbp}{dt} = \frac{epsilon \cdot k\_PKA\_LCC \cdot PKACII\_LCC \cdot LCCb}{Km\_PKA\_LCC + epsilon \cdot LCCb} - \frac{epsilon \cdot k\_PP1\_LCC \cdot PP1\_LCC \cdot LCCbp}{Km\_PP1\_LCC + epsilon \cdot LCCbp}$$

PLB

Parameter	Description	Value	Units	Source
PLBtot	total phospholamban	106	µmol/L	[8]
k_PKA_PLB	PKA phosphorylation of phospholamban	54e-3	1/ms	[7-8]
Km_PKA_PLB	PKA phosphorylation of phospholamban	21	µmol/L	[7-8]
k_PP1_PLB	PP1 dephosphorylation of phospholamban	8.5e-3	1/ms	[7-8]
Km_PP1_PLB	PP1 dephosphorylation of phospholamban	7.0	µmol/L	[7-8]

$$PLB = PLBtot - PLBp$$

$$\frac{dPLBp}{dt} = \frac{k\_PKA\_PLB \cdot PKACI \cdot PLB}{Km\_PKA\_PLB + PLB} - \frac{k\_PP1\_PLB \cdot PP1 \cdot PLBp}{Km\_PP1\_PLB + PLBp}$$

PLM

Parameter	Description	Value	Units	Source
PLMtot	total phospholemman	48	µmol/L	[9]
k_PKA_PLM	PKA phosphorylation of phospholemman	54e-3	1/ms	[9]
Km_PKA_PLM	PKA phosphorylation of phospholemman	21	µmol/L	[9]
k_PP1_PLM	PP1 dephosphorylation of phospholemman	8.5e-3	1/ms	[9]
Km_PP1_PLM	PP1 dephosphorylation of phospholemman	7.0	µmol/L	[9]

$$PLM = PLMtot - PLMp$$

$$\frac{dPLMp}{dt} = \frac{k\_PKA\_PLM \cdot PKACI \cdot PLM}{Km\_PKA\_PLM + PLM} - \frac{k\_PP1\_PLM \cdot PP1 \cdot PLMp}{Km\_PP1\_PLM + PLMp}$$

TnI

Parameter	Description	Value	Units	Source
TnItot	total troponin I	70	µmol/L	[8]
PP2A_TnI	total PP2A local to troponin I	0.67	µmol/L	[8]
k_PKA_TnI	PKA phosphorylation of troponin I	54e-3	1/ms	[8]
Km_PKA_TnI	PKA phosphorylation of troponin I	21	µmol/L	[8]
k_PP2A_TnI	PP2A dephosphorylation of troponin I	10.1e-3	1/ms	[8]
Km_PP2A_TnI	PP2A dephosphorylation of troponin I	4.1	µmol/L	[8]

$$TnI = TnItot - TnIp$$

$$\frac{dTnIp}{dt} = \frac{k\_PKA\_TnI \cdot PKACI \cdot TnI}{Km\_PKA\_TnI + TnI} - \frac{k\_PP2A\_TnI \cdot PP2A\_TnI \cdot TnIp}{Km\_PP2A\_TnI + TnIp}$$

CREB

Parameter	Description	Value	Units	Source
CREBtot	total CREB	0.9	µmol/L	adapted
PP2A_CREB	total PP2A local to CREB	0.12	µmol/L	adapted
k_PKA_CREB	PKA phosphorylation of CREB	54e-3	1/ms	[7-8]
Km_PKA_CREB	PKA phosphorylation of CREB	10	µmol/L	adapted
k_PP2A_CREB	PP2A dephosphorylation of CREB	8.5e-3	1/ms	[7-8]
Km_PP2A_CREB	PP2A dephosphorylation of CREB	2.46	µmol/L	adapted

$$CREB = CREBtot - CREBp$$

$$\frac{dCREBp}{dt} = \frac{k\_PKA\_CREB \cdot nPKACII \cdot CREB}{Km\_PKA\_CREB + CREB} - \frac{k\_PP2A\_CREB \cdot PP2A \cdot CREBp}{Km\_PP2A\_CREB + CREBp}$$

AKAR FRET Reporters

Parameter	Description	Value	Units	Source
AKARnestot	total AKAR-NES	1.25	µmol/L	[13]
PP2A_AKARnes	total PP2A local to AKAR-NES	0.5	µmol/L	[14]
k_PKA_AKARnes	PKA phosphorylation of AKAR-NES	152e-3	1/ms	[14]
Km_PKA_AKARnes	PKA phosphorylation of AKAR-NES	16	µmol/L	[14]
k_PP2A_AKARnes	PP2A dephosphorylation of AKAR-NES	8.5e-3	1/ms	[7-8]
Km_PP2A_AKARnes	PP2A dephosphorylation of AKAR-NES	7	µmol/L	[7-8]
AKARnlstot	total AKAR-NLS	3.48	µmol/L	[13]
PP2A_AKARnls	total PP2A local to AKAR-NLS	0.5	µmol/L	[14]
k_PKA_AKARnls	PKA phosphorylation of AKAR-NLS	152e-3	1/ms	[14]
Km_PKA_AKARnls	PKA phosphorylation of AKAR-NLS	16	µmol/L	[14]
k_PP2A_AKARnls	PP2A dephosphorylation of AKAR-NLS	8.5e-3	1/ms	[7-8]
Km_PP2A_AKARnls	PP2A dephosphorylation of AKAR-NLS	7	µmol/L	[7-8]

$$AKARnes = AKARnestot - AKARnesp$$

$$\frac{dAKARnesp}{dt} = \frac{k\_PKA\_AKARnes \cdot PKACII \cdot AKARnes}{Km\_PKA\_AKARnes + AKARnes} - \frac{k\_PP2A\_AKARnes \cdot PP2A \cdot AKARnesp}{Km\_PP2A\_AKARnes + AKARnesp}$$

$$AKARnls = AKARnlstot - AKARnlsp$$

$$\frac{dAKARnlsp}{dt} = \frac{k\_PKA\_AKARnls \cdot nPKACII \cdot AKARnls}{Km\_PKA\_AKARnls + AKARnls} - \frac{k\_PP2A\_AKARnls \cdot PP2A \cdot AKARnlsp}{Km\_PP2A\_AKARnls + AKARnlsp}$$

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