Rich et al., http://www.jgp.org/cgi/content/full/jgp.201311095/DC1

Compartmental model used to describe the distribution of cyclic nucleotides

Compartmental models have been previously used to describe the localization of cyclic nucleotide signals (Saucerman et al., 2014, and references therein). Here, we present a compartmental model that incorporates recent estimates of near-membrane PDE activity. In this model, cAMP is produced by AC in compartment 1. The flux of cAMP from compartment 1 to the bulk cytosol (compartment 2) is markedly slower than the flux of cAMP within either compartment 1 or 2. In contrast to our previous models, PDE activity is markedly lower in compartment 1 than in compartment 2. This model is described by the following equations (variables are defined in Table S1):

$$\frac{d[C_1]}{dt} = E_{AC} + \frac{J_{12}}{V_1}\left([C_2]-[C_1]\right) - \frac{E_1 \cdot [C_1]}{K_{M1}+[C_1]}$$

$$\frac{d[C_2]}{dt} = \frac{J_{12}}{V_2}\left([C_1]-[C_2]\right) - \frac{E_2 \cdot [C_2]}{K_{M2}+[C_2]}.$$

Simulations of this model demonstrate the importance of diffusional barriers or other hindrances to free diffusion in segregating cyclic nucleotide signals.

Kinetic model used to describe difficulties in the measurement of cyclic nucleotide signals

Here we present a realistic mathematical description of the cGMP signaling pathway. In this model, activation of pGC triggers cGMP synthesis. Dephosphorylation of pGC causes receptor desensitization and subsequent reduction in the rate of cGMP synthesis. PDE type 5 (PDE5), which is regulated by cGMP binding to the noncatalytic site and phosphorylation, hydrolyzes cGMP. The sinusoidal regulation of pGC activity (periods [T] of 30, 300, and 3,000 s) used in this model is analogous to oscillations in ANP. These inputs triggered sinusoidal intracellular cGMP accumulation with a lag that varied with stimulus frequency. The amplitude of cGMP oscillations increased with the period of sinusoidal stimulation. The model is described by the following equations (parameter definitions and values are provided in Table S2).

$$[cG_f] = [cGMP] - [PDE5]\left([PDE5\cdot P] + [cG\cdot PDE5\cdot P]\right) - [buf][cG\cdot buf] \quad (1)$$

$$PDE5 = \frac{\left([cG\cdot PDE5\cdot P]+[PDE5\cdot P]\right)[cG_f]E_{PDE5}}{K_{m1}+[cG_f]}$$

$$f = \frac{1}{2}\sin\left(\frac{2\pi t}{\mathrm{T}} - \frac{\pi}{2}\right) + \frac{1}{2}$$

$$\frac{d[cGMP]}{dt} = [pGC\cdot P]E_{pGCbas} + f\times[pGC\cdot P]E_{pGC} - PDE5$$

$$\frac{d[pGC\cdot P]}{dt} = k_{kin}[pGC] - k_{pp2b}[pGC\cdot P]$$

$$\frac{d[pGC]}{dt} = -k_{kin}[pGC] + k_{pp2b}[pGC\cdot P]$$

$$\frac{d[cG\cdot PDE5]}{dt} = k_1[PDE5][cG_f] - \left(k_{-1} + \frac{k_2[cG_f]}{[cG_f]+K_{1/2}}\right)[cG\cdot PDE5] + k_{-2}[cG\cdot PDE5\cdot P]$$

$$\frac{d[cG\cdot PDE5\cdot P]}{dt} = \frac{k_2[cG_f]}{[cG_f]+K_{1/2}}[cG\cdot PDE5] - \left(k_{-2}+k_3\right)[cG\cdot PDE5\cdot P] + k_{-3}[PDE5\cdot P]$$

$$\frac{d[PDE5\cdot P]}{dt} = k_3[cG\cdot PDE5\cdot P] - \left(k_{-3}+k_4\right)[PDE5\cdot P] + \frac{k_{-4}[cG_f]}{[cG_f]+K_{1/2}}[PDE5]$$

$$\frac{d[PDE5]}{dt} = k_4[PDE5\cdot P] - \frac{k_{-4}[cG_f]}{[cG_f]+K_{1/2}}[PDE5] - \left(k_1 + \frac{k_{-4}[cG_f]}{[cG_f]+K_{1/2}}\right)[PDE5][cG_f] + k_{-1}[cG\cdot PDE5]$$

$$\frac{d[buf]}{dt} = -k_{bufF}[cG_f][buf] + k_{bufR}[cG\cdot buf]$$

$$\frac{d[cG\cdot buf]}{dt} = k_{bufF}[cG_f][buf] - k_{bufR}[cG\cdot buf]$$

TABLE S1

Parameters used in simulations of compartmentalized signaling as depicted in Fig. 1

Parameter	Parameter definition	Value	Initial condition	Reference
$[C_1]$	concentration of cAMP in compartment 1[a]		0.1355 µM	
$[C_2]$	concentration of cAMP in compartment 2[a]		0.0045 µM	
V_1	volume of compartment 1	0.3 pL		
V_2	volume of compartment 2	2.7 pL		Feinstein et al., 2012
J_{12}	Flux coefficient between compartments 1 and 2	8×10^{-16} liter/s		Rich et al., 2000, 2001
E_{AC}	AC activity	Basal: 0.03 µM/min Stimulated: 0.66 µM/min		
E_1	Maximal PDE activity in C_1	0.8 µM/min		Unpublished data
E_2	Maximal PDE activity in C_2	4.4 µM/min		Feinstein et al., 2012
K_{m1}	Michaelis constant for PDE activity in C_1	4 µM		Houslay et al., 1998; Richter and Conti, 2004
K_{m2}	Michaelis constant for PDE activity in C_2	4 µM		Houslay et al., 1998; Richter and Conti, 2004

Note that based upon recent studies (unpublished data) PDE activity in compartment 1 is markedly lower than PDE activity in compartment 2.
[a]Initial conditions were estimated by running simulation to equilibrium with basal AC activity.

TABLE S2

Parameters used in simulations describing oscillatory cGMP signals

Parameter	Parameter definition	Value	Initial condition	Reference
E_{pGC}	Maximal pGC activity[b]	Basal: 0.01 µM/min Stimulated: 0.33 µM/min		
T	Period of ANP oscillations	30, 300, or 3,000 s		
E_{PDE5}	Maximal PDE5 activity[a]	2 µM/min		
f_{pGCP}	Fraction of phosphorylated pGC[a]		0.8	
f_{pGC}	Fraction of dephosphorylated pGC[a]		0.2	
$[cGMP]$	Concentration of total cGMP [a]		0.056 µM	
$[cG_F]$	Concentration of free cGMP		Estimated using Eq. 3	
K_{m1}	Michaelis constant for PDE5 activity	4 µM		Francis et al., 2009, and references therein
k_{kin}	Rate constant of pGC phosphorylation	1×10^{-2} s^{-1}		Adapted from Henesy et al., 2012
k_{pp2b}	Rate constant for pGC dephosphorylation	5×10^{-3} s^{-1}		Adapted from Henesy et al., 2012
k_1	Rate constant for cGMP binding to PDE5	1.81×10^4 $M^{-1} \times s^{-1}$		Batchelor et al., 2010, and references therein
k_{-1}	Rate constant for cGMP release from PDE5	0.0868 s^{-1}		Batchelor et al., 2010, and references therein
k_2	Rate constant for cGMP-bound PDE5 phosphorylation	0.246 s^{-1}		Batchelor et al., 2010, and references therein
$K_{1/2}$	cGMP concentration required for half-maximal rate of PDE5 phosphorylation	0.25 µM		Batchelor et al., 2010, and references therein
k_{-2}	Rate constant for cGMP-bound PDE5 dephosphorylation	0.154 s^{-1}		Batchelor et al., 2010, and references therein
k_3	Rate of cGMP binding to phosphorylated PDE5[b]	1.21×10^{-3} $M^{-1} \times s^{-1}$		
k_{-3}	Rate of cGMP release from phosphorylated PDE5[b]	1.13×10^{-5} s^{-1}		
k_4	Rate of PDE5 phosphorylation[b]	1×10^{-2} s^{-1}		
k_{-4}	Rate of PDE5 dephosphorylation[b]	5×10^{-3} s^{-1}		
buf	Buffering capacity of cGMP sensor	0, 0.05, 0.5, or 5 µM		
K_{bufF}	On rate for cGMP binding	1×10^6 $M^{-1} \times s^{-1}s$		
K_{bufR}	Off rate for cGMP binding	1 s^{-1}		

[a]Initial conditions were estimated by running simulation to equilibrium with basal GC activity.
[b]Fit to experimental data.

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