SUPPLEMENTAL MATERIAL

Natriuretic Peptides and Nitric Oxide Stimulate cGMP Synthesis in Different Cellular Compartments

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A compartmental model describing the spatial segregation of cGMP signals was used to predict the efficacy with which cGMP produced by either particulate guanylyl cyclase (pGC) or soluble guanylyl cyclase (sGC) will activate CNG channels (Fig. S1). This model is based upon our previously proposed compartmental model of cAMP signals (Rich et al., 2000; Rich et al., 2001).

In the model, pGC, PDE5, and CNG channels (CNGC) are localized within a subcellular compartment near the plasma membrane (compartment 1), and PDE5 and sGC are localized in the cytosol (compartment 2). Activation of pGC triggers the synthesis of cGMP in compartment 1. This pool of cGMP readily activates CNG channels, but the spatial spread of cGMP out of this compartment is hindered. Similarly, the spatial spread of cGMP from compartment 2 into compartment 1 is hindered. The system is described by the following equations:

\[
\frac{d[C_1]}{dt} = \frac{F_{pGC} + J_{12}}{V_1} ([C_1] - [C_1]) - \frac{E_{PDE1} [C_1]}{K_M (1 + [I]/K_I) + [C_1]} \]  

(1)

\[
\frac{d[C_2]}{dt} = \frac{F_{sGC}}{V_2} ([C_2] - [C_2]) - \frac{E_{PDE2} [C_2]}{K_M (1 + [I]/K_I) + [C_2]} \]  

(2)

\[
\frac{I_{CNG}}{I_{CNG,\text{max}}} = \frac{[C_1]^N}{[C_1]^N + K_{1/2}^N} \]  

(3)

where \(V_1\) and \(V_2\) are the volumes of compartments 1 and 2, \([C_1]\) and \([C_2]\) are the cGMP concentrations, \(J_{12}\) is the flux coefficient between compartments, \(F_{pGC}\) and \(F_{sGC}\) are the synthesis rates of cGMP by pGC and sGC, \(E_{PDE1}\) and \(E_{PDE2}\) are the maximal cGMP hydrolysis rates in compartments 1 and 2, \(K_M\) is the Michaelis constant for PDE5 activity, \([I]\) is the concentration of a competitive PDE inhibitor such as IBMX, and \(K_I\) is the inhibition constant. The parameters \(J_{12} = 8.0 \times 10^{-16} \text{ L/s}, V_1 = 0.040 \text{ pL, and } V_2 = 2.0 \text{ pL}\) are the same as those used previously (Rich et al., 2000; Rich et al., 2001). pGC and sGC activities are considered constant, with \(E_{pGC} = 0\) or 0.083 \(\mu\text{M/s}\) and \(E_{sGC} = 0\) or 0.0017 \(\mu\text{M/s}\). \(K_M\), \(E_{PDE1}\), and \(E_{PDE2}\) are 5 \(\mu\text{M}, 0.33 \mu\text{M/s, and 0.0068 }\mu\text{M/s}\). These values give similar total cGMP synthesis and hydrolysis rates in compartments 1 and 2. The \(K_I\) of IBMX for PDE5 is 10 \(\mu\text{M}, and \([I]\) is either 0 or 100 \(\mu\text{M}. Activation of CNG channels is described by the Hill equation where \(I_{CNG}/I_{CNG,\text{max}}\) is the fraction current through CNG channels, \(K_{1/2}\) is 1 \(\mu\text{M}, and N = 2\).

Simulations of the model predict that activation of pGC will cause increases in cGMP levels within compartment 1, and that these increased cGMP levels will readily activate CNG channels (Fig. S2). However, following activation of sGC in compartment 2, cGMP levels in compartment 1 do not reach high enough levels to significantly activate CNG channels. This is due to both PDE activity and the restricted flux of cGMP between compartments. Inhibition of PDE activity with a competitive inhibitor such as IBMX significantly reduces

![Figure S1. Schematic of the compartmental model used to describe the spatial spread of cGMP signals.](image-url)
the role of PDE activity in compartmentalizing cGMP signals; yet, cGMP synthesized by sGC (in compartment 2) fails to substantially activate CNG channels (Fig. S3). This is primarily due to the restricted flux between compartments 1 and 2. These simulations help to illustrate the roles of restricted diffusion and PDE activity in localizing cGMP signals.

REFERENCES