REGULATION OF GUANYLYL CYCLASE B: ROLES OF ATP AND P-SITE INHIBITORS

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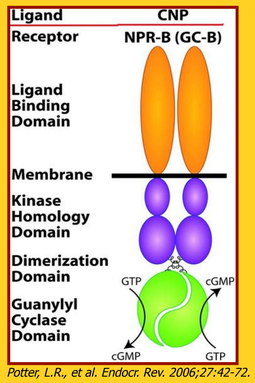
Background: CNP Binds and Activates GC-B

C-type natriuretic peptide (CNP) is a 22-amino acid peptide paracrine factor that regulates long bone growth, vasorelaxation, and axonal guidance. CNP mediates its effects by activating the single membrane-spanning receptor guanylyl cyclase-B (GC-B), which synthesizes the intracellular secondary messenger cGMP that regulates protein kinases, ion channels, and phosphodiesterases **(1)**.



Potter, L.R., et al. Endocr. Rev. 2006;27:42-72.

FIG. 1. Structure of Guanylyl Cyclase-B

Under basal conditions, GC-B is phosphorylated on six known serine and threonine sites in its kinase homology domain **(2)**. Substitution of individual sites with glutamic acid or alanine mimics the effects of a phosphorylated or dephosphorylated residue, respectively, and completely dephosphorylated receptors are not activated by CNP. Conversely, chronic exposure of GC-B to CNP results in a time-dependent decrease in activity that is temporally correlated with receptor dephosphorylation.

FIG. 2. Clinical Significance of GC-B Regulation

Bocciardi et al. Human Mutation (2007) 28: 724.

Hachiya et al. J Clin. Endocrinol. Metab. (2007) 92: 4095.

Upper and Lower Left Panel:
Increased CNP-mediated GC-B activity results in skeletal overgrowth.

Left and Right Top Panel:
Homologous inactivation of GC-B (defects in both alleles) results in dwarfism.

Role of ATP in GC-B Regulation is Controversial

Two Roles of ATP

- **Substrate** → **Maintenance of receptor phosphorylation sites in KHD**
- **Regulatory** → **Stabilization of enzyme to increase its affinity for GTP**

The addition of ATP to broken cell preparations containing GC-B increases the phosphorylation state and maximal velocity of GC-B **(3-7)**. However, AMP-PNP, a nonhydrolyzable ATP analog that cannot be used as a phosphate donor for GC-B, also increases CNP-dependent activity. Additionally, AMP-PNP reduces the Michaelis constant for GC-B without affecting the maximal velocity of GC-B. These observations suggest that ATP increases the maximal velocity of GC-B by increasing the phosphorylation state of the enzyme and reducing the Km of the enzyme through a separate, independent, and currently unknown process.

Methods: Measuring GC-B Activity Over Time

Cocktail solutions containing crude membranes, creatine phosphate, creatine kinase, 0.1mM or 1mM GTP, and the presence or absence of 1mM ATP, were added to stop solution (50mM NaOAC + 5mM EDTA) on ice after being incubated at 37ºC for 0s, 15s, 30s, 1min, 2min, 4min, 8min, 16min, and 32min. Activator mixtures contained 1 µM CNP. Protein concentrations were estimated using Pierce Coomassie Plus Protein Assays, and GraphPad Prism5 was used for data analysis and significance testing **(8)**.

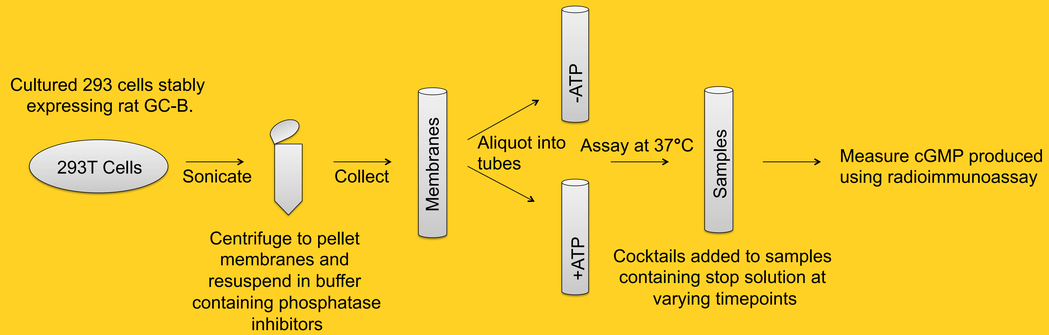
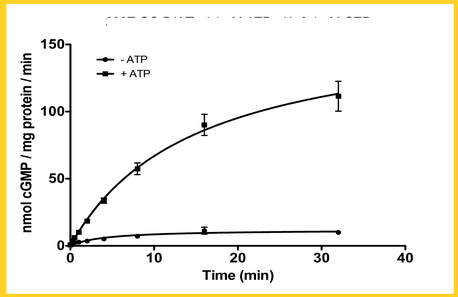


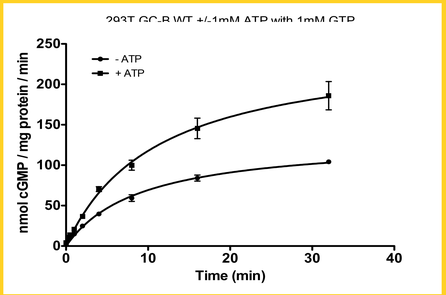
FIG. 3. Effect of ATP on GC-B activity in the presence of low (0.1 mM) substrate concentrations



Time course data depicting CNP-dependent guanylyl cyclase activity over time in the presence or absence of 1mM ATP at 0.1mM GTP. Each point represents 9 replicates.

1mM ATP increases maximal receptor activity ten-fold at low GTP concentrations. In the absence of ATP, GC-B activity declines at a much faster rate (within the first few minutes of receptor activation) than in its presence.

FIG. 4. Effect of ATP on GC-B activity in the presence of high (1 mM) substrate concentrations



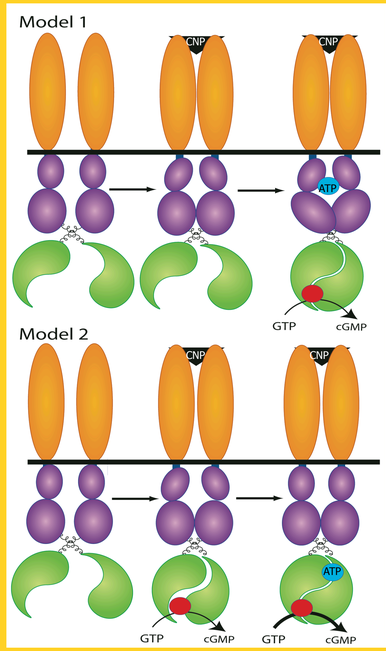
Time course data depicting CNP-dependent guanylyl cyclase activity over time in the presence or absence of 1mM ATP at 1mM GTP. Each point represents 9 replicates.

1mM ATP increases maximal receptor activity two- to three-fold at high GTP concentrations. In the absence of ATP, GC-B activity declines at a similar rate as in its presence.

Conclusions

At 0.1mM GTP, ATP exerts a greater Km effect, increasing the affinity of the catalytic site for GTP; at 1mM GTP, catalytic sites are saturated with substrate, and thus, ATP exerts a less significant Km effect. We propose that the decline in receptor activity over time can be explained by product inhibition. Once a sufficient amount of product is produced, the binding of purine nucleotides and pyrophosphate (PP_i) to the catalytic domain results in receptor deactivation.

Current Receptor Activation Models



Diagrams courtesy of Jerid Robinson (2011).

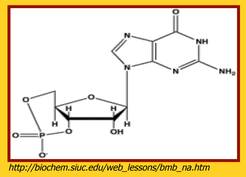
FIG. 5. Model 1

Low levels of cGMP are produced under basal conditions. Hormone binding induces a conformational change that allows ATP to bind to the KHD, which causes the catalytic domains to come together and form the active site, resulting in high levels of cGMP production.

FIG. 5. Model 2

Low levels of cGMP are produced under basal conditions. Hormone binding causes catalytic domains to come together to form the active site, which results in increased maximal velocities. In addition, hormone binding allows ATP to bind to one of the purine sites in the guanylyl cyclase catalytic domain, which increases affinity of the catalytic site for GTP by reducing its Michaelis constant.

Product Inhibition in Guanylyl Cyclases



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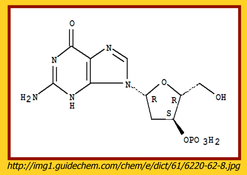

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FIG. 6. Structure of cGMP (left) and 2'd3'GMP (right)

P site inhibitors have been identified in adenylyl cyclases, which are homologous to guanylyl cyclases. The potency of purine nucleotides increases when adenylate cyclase is activated, suggesting its inhibitory mechanism involves direct binding of cAMP product compounds and pyrophosphate (PP_i) to the catalytic domain **(9,10)**. Current experiments are testing whether the product analog, 2'd3'GMP, and/or PP_i inhibit GC-B to explain the time-dependent loss of guanylyl cyclase activity observed in Figures 3 and 4.

References

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