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Regulation of soluble guanylyl cyclase by phosphorylation

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Background

Soluble guanylyl cyclase (sGC) is a receptor for the signaling molecule nitric oxide (NO). Binding of NO to the sGC heme moiety leads to increased cGMP synthesis and activation of cGMP-dependent protein kinase (PKG). As sGC subunits contain putative phosphorylation sites for PKG, we tested whether sGC activity is regulated by PKG.

Results

In vitro kinase assays revealed that the α1, but not the β1, subunit of sGC is a PKG substrate and that the phosphorylation site is located within the first 360 residues of the α1. A constitutively active form of PKG stimulated incorporation of ^{32}P into the α1 subunit in vivo. In addition, PKG could be detected in sGC immunoprecipitates, suggesting that the two proteins interact in cells. Serine to alanine mutation of putative PKG sites revealed that Ser64 is the main phosphorylation site for PKG. After generating a phospho-specific antibody for Ser64, we could demonstrate that phosphorylation of endogenous sGC on Ser64 increases in cells and tissues exposed to NO. Phosphorylation of sGC in cells following SNP stimulation was blocked by the peptide inhibitor of PKG, DT-3. Wild-type (wt) sGC when co-expressed in COS cells with a constitutively active form of PKG exhibited lower basal and NO-stimulated cGMP accumulation. In contrast, the S64A α1/β1 sGC was resistant to the PKG-induced reduction in activity. Moreover, co-cultures of endothelial cells with smooth muscle expressing a phosphorylation-resistant form of sGC, exhibited higher levels of cGMP (both basally and following stimulation with bradykinin) compared to co-cultures expressing wild-type sGC. Phosphorylation of the α1 subunit did not alter its ability to form heterodimers with β1. Using purified sGC and mutants, we observed that the S64D α1 phosphomimetic/β1 dimer exhibited lower Vmax; moreover, the decrease in Km after NO stimulation was less pronounced in S64D α1/β1 compared to wt sGC. Expression of a phosphorylation deficient form of sGC showed reduced desensitization to acute NO exposure and allowed for greater VASP phosphorylation.

Conclusion

We conclude that PKG phosphorylates sGC on Ser64 of the α1 subunit and phosphorylation inhibits sGC activity, establishing a negative feedback loop.