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Protein kinase G phosphorylates soluble guanylyl cyclase and inhibits its activity

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Soluble guanylyl cyclase (sGC) is a receptor for the signaling molecule nitric oxide (NO). Binding of NO to the sGC heme moiety causes up to 400-fold stimulation of its activity, leading to increased cGMP levels and cGMP-dependent protein kinase (PKG) activation. As sGC subunits contain putative phosphorylation sites for PKG, we tested the hypothesis that sGC activity is regulated by PKG. *In vitro* kinase assays revealed that sGC is a PKG substrate. *In vivo*, a constitutively active form of PKG stimulated incorporation of ^{32}P into sGC. We then proceeded to map the exact phosphorylation site by generating serine to alanine mutations of putative PKG sites. Wild-type (wt) sGC co-expressed with a constitutively active form of PKG exhibited lower basal and NO-stimulated cGMP accumulation, while the serine to alanine sGC was resistant to the PKG-induced reduction in activity. In line with these observations, a phosphomimetic serine to aspartate sGC mutant showed reduced ability to synthesize cGMP. Using purified sGC and mutants, we observed that the phosphomimetic sGC mutant exhibited lower Vmax both under basal and NO-stimulated conditions and that the decrease in Km after NO stimulation was less pronounced than that for the wt. Moreover, the phosphorylation deficient sGC exhibited reduced desensitization to acute NO exposure and facilitated greater VASP phosphorylation. We conclude that PKG phosphorylates sGC on serine and phosphorylation inhibits sGC activity, establishing a negative feedback loop.

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