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Protein Kinase G Phosphorylates Soluble Guanylyl Cyclase on Serine 64 and Inhibits its Activity

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Soluble guanylyl cyclase (sGC) is a receptor for nitric oxide (NO). Binding of NO to the sGC heme moiety causes an up to 400-fold stimulation of sGC activity. The subsequent increase in cGMP formation activates a variety of intracellular targets including cGMP-dependent protein kinase (PKG) to mediate, among other things, vascular relaxation and inhibition of platelet aggregation. Based on pharmacological observations it has been postulated that sGC itself might be a substrate for PKG. We set out to investigate whether sGC is phosphorylated by PKG and whether PKG-stimulated sGC phosphorylation alters its activity. *In vitro* kinase assays revealed that the α1, but not the β1, subunit of sGC is phosphorylated by PKG. The ability of PKG to phosphorylate sGC was confirmed in COS cells overexpressing the two proteins (PKG and sGC). Moreover, immunoprecipitation experiments demonstrated that PKG and sGC interact. To map the site of phosphorylation, we generated a series of deletion mutants of the α1 subunit; these experiments showed that the PKG phosphorylation site resides within the first 360 amino acids. Using site directed mutagenesis, we engineered a serine to alanine mutant (S64A) predicted to conform with high probability to the PKG phosphorylation motif based *in silico* analysis. Mutation of serine 64 to alanine abolished ^{32}P incorporation in sGC in cells overexpressing a constitutively active form of PKG, suggesting that this residue is indeed the site of PKG phosphorylation. When wild-type (wt) sGC was co-expressed with PKG in COS cells it exhibited 30-40% lower basal and NO-stimulated cGMP accumulation. In contrast, the S64Aα1/β1 sGC was resistant to the PKG-induced reduction in activity. Moreover, when co-expressed with a β1 subunit the phosphomimetic S64D mutant showed a limited ability to synthesize cGMP. Both the S64A and the S64D mutants were expressed to the same extent as the wt α1 subunit and exhibited similar heterodimerization properties with β1. We conclude that PKG phosphorylates sGC on serine 64 of the α1 subunit inhibiting its activity, thereby leading to the formation of a negative feedback loop. The down regulation of sGC activity by cGMP could dampen excessive stimulation of the NO/cGMP pathway and could contribute sGC desensitization and to the development of nitrate tolerance.