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Thiol-dependent redox modulation of soluble guanylyl cyclase

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Background

Following prolonged exposure to NO, soluble guanylyl cyclase (sGC) becomes desensitized and fails to respond to additional NO stimulation. We showed that sGC is desensitized by S-nitrosylation *in vitro*, in primary smooth muscle cells (SMC) and in tissues and identified two cysteines (Cys) targeted by this post-translational modification that are involved in sGC desensitization [1]. We recently discovered that nitroglycerin (GTN) induces S-nitrosylation of sGC. We also showed that chronic treatment with GTN or acute treatment with S-nitroso-cysteine (CSNO), which lead to impaired relaxation *in vivo*, were accompanied by decreased GTN- or NO-stimulated cGMP production and characterized by strong S-nitrosylation of sGC. These observations suggested that desensitization of sGC by S-nitrosylation could be a mechanism of tolerance [2]. Based on observations by others that chronic GTN treatment increases ROS species and that oxidants exposure of cells impaired sGC response to NO, we hypothesize that desensitization of sGC by redox-dependent modification of its Cys is a mechanism underlying the loss of vascular reactivity in some oxidative vascular diseases.

Results and discussion

We have now identified two additional Cys by Mass Spec that are, at least *in vitro*, S-nitrosylated. Mutational analysis of these Cys-sGC mutants seems to indicate that these four Cys are differentially modified upon exposure to oxidative or nitrosative stress. For example, β1C122 located in the heme-binding domain appears to be both S-nitrosylated and oxidized by CSNO and aldosterone treatment, respectively [3]. Comparison of the kinetics and spectral properties of the purified β1C122A mutant with the WT indicated that S-nitrosylation of C122 does not affect the EC_{50} for NO but reduces the maximal velocity at saturating concentration of NO. Biochemical studies of purified sGC exposed to oxidants (H_2O_2, diamide) under non-reducing conditions confirmed that some of the Cys are engaged in disulfide bond and/or modified by glutathionylation, which correlate with decreased sGC activity. We are now in the process to integrate these *in vitro* findings in various physiologic and pathophysiologic models to determine the mechanisms of loss of vascular reactivity in development of NO resistance, in addition to heme oxidation [4].

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