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cGMP-dependent protein kinase contributes to hydrogen sulfide-stimulated vasorelaxation

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Background and aim. A growing body of evidence suggests that hydrogen sulfide (H_2S) is a signaling molecule in mammalian cells. In the cardiovascular system, H_2S enhances vasodilatation and angiogenesis (Szabo and Papapetropoulos, 2011). H_2S-induced vasodilatation is hypothesized to occur through ATP-sensitive potassium channels; however, recently it has been demonstrated that H_2S also increases cGMP levels in tissues (Bucci et al., 2010). Herein, we studied the involvement of cGMPdependent protein kinase-I in H_2S-induced vasorelaxation.

Methods and results. Pretreatment of aortic rings with sildenafil attenuated NaHS-induced relaxation (Emax 75.2 ± 12.2% vs. 91.3 ± 7.2%, sildenafil and vehicle respectively; n=4), confirming previous findings that H_2S is a phosphodiesterase inhibitor. In line with the notion that H_2S is an inhibitor of phosphodiesterase, vascular tissue levels of cGMP in cystathionine gamma lyase (CSE) knockouts were lower than those in wild-type control mice (aorta 9.55 ± 0.80 pmole/mg vs. 23.40 ± 1.44 pmole/mg protein, CSE-/- mice vs. wild-type mice respectively; n=5; mesenteric artery 0.42 ± 0.04 pmole/mg protein vs. 1.57 ± 0.05 pmole/mg protein, CSE-/- mice vs. wild-type mice respectively; n=5). Treatment of aortic rings with NaHS, a fast releasing H_2S donor, enhanced phosphorylation of vasodilator-stimulated phosphoprotein in a time-dependent manner, suggesting that cGMP-dependent protein kinase (PKG) is activated after exposure to H_2S. Incubation of rings with a PKG-I inhibitor (DT-2) attenuated NaHS-stimulated relaxation (Ec50 $1.9x10^{-4}$M vs. $7.1x10^{-5}$M DT-2 vs vehicle respectively; n=6). Interestingly, vasodilatory responses to a slowly releasing H_2S donor (GYY 4137) were unaffected by DT-2 (46.6 ± 8.8 vs. 32.2 ± 9.4 vehicle and DT-2 respectively, n=7), suggesting that this donor dilates mouse aorta through PKG-independent pathways. In agreement to what was observed with DT-2, dilatory responses to NaHS and L-cysteine (a substrate for H_2S production) were reduced in vessels of PKG-I knockout mice (NaHS, 92.0 ± 3.0 vs. 68.3 ± 3.6 WT and PKG-KO respectively, n=7; L-cys, 23.1 ± 2.1 vs. 10.0 ± 2.8 WT and PKG-KO respectively, n=12).

Conclusion. Our results confirm the role of cGMP in the vascular responses to H_2S and demonstrate that PKG-I regulates H_2S-stimulated vasodilation.