A new gaseous signaling molecule emerges: Cardioprotective role of hydrogen sulfide

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Cardiovascular (CV) research has largely been focused on two endogenously produced gaseous signaling molecules, namely, nitric oxide (NO) and carbon monoxide (CO). These gaseous messengers are both highly conserved evolutionarily and synthesized by endogenous enzyme systems. Extensive research efforts have uniformly demonstrated that CO- and NO-based therapeutics protect the brain, heart, and circulation against a number of CV disease states (1–3). However, controversy exists regarding the therapeutic value of both CO and NO, with some studies citing NO cytotoxicity at very high levels and under certain conditions (2). The toxic effects of NO are related to the highly reactive nature of NO and its interaction with superoxide to form the potent oxidant peroxynitrite ($ONOO^-$) (2). Very recently, a third endogenously produced gaseous signaling molecule, hydrogen sulfide (H_2S), has emerged as a potentially important mediator of CV homeostasis and cytoprotection (3). In this issue of PNAS, Benavides *et al.* (4) reveal that the vasculoprotective and antihypertensive effects of dietary garlic (*Allium sativum*) are mediated in large part via the generation of H_2S.

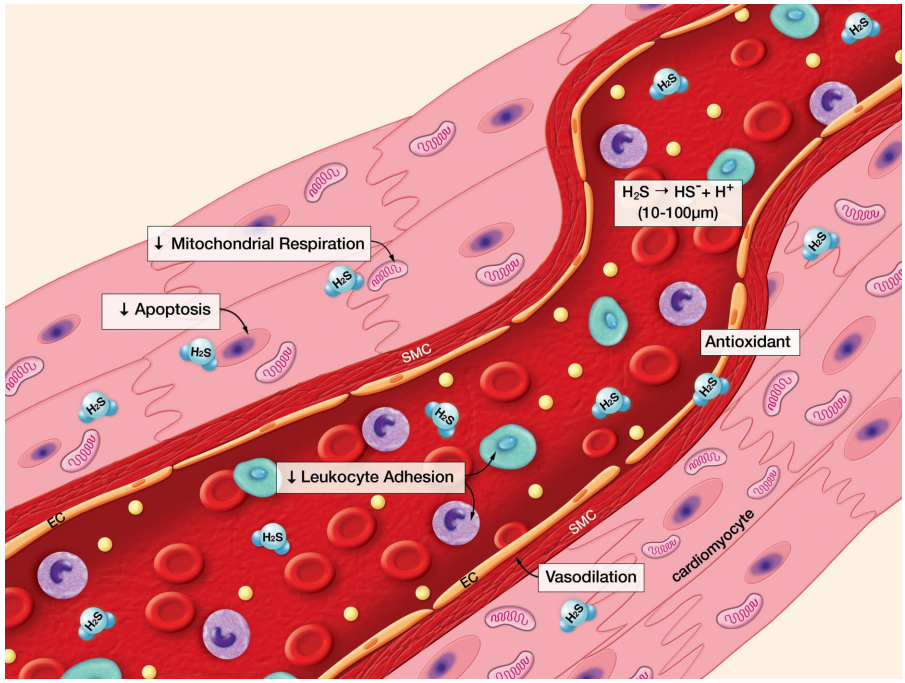

Fig. 1. Summary of the physiological actions of hydrogen sulfide (H_2S). H_2S is produced in micromolar (i.e., 10–100 μM) concentrations in the circulation and exerts a number of critical effects on the CV system. H_2S has been shown to induce vasodilation and inhibit leukocyte-endothelial cell interactions in the circulation. H_2S is a potent antioxidant and inhibits cellular apoptosis. H_2S also has been shown to transiently and reversibly inhibit mitochondrial respiration. Taken together, this physiological profile is ideally suited for protection of the CV system against disease states.

Previous studies indicate that dietary garlic consumption is inversely correlated with the progression and severity of CV disease and that it affects a lower incidence of hypertension (5). However, the mechanism(s) by which consumption of garlic attenuates CV disease has in large part remained a mystery. Benavides *et al.* (4) elegantly demonstrate that garlic-derived organic polysulfides are converted by red blood cells (RBCs) into hydrogen sulfide gas. Interestingly, the garlic bulbs used for this study were obtained from local supermarkets in the city of Birmingham, AL, and then pressed by the investigators to obtain garlic juice on the day of the experiment. The bioconversion of polysulfides to H_2S is enhanced by glucose-maintained cytosolic glutathione levels and depends on reduced thiols in close proximity to the RBC membrane. The authors demonstrate that generation of H_2S after bioconversion of garlic-derived polysulfides results in potent vasorelaxation of isolated blood vessels. The authors propose that H_2S produced from garlic leads to vasorelaxation via vascular smooth muscle K_{ATP} channel-mediated hyperpolarization (3). These novel experimental studies provide important information regarding the mechanisms responsible for garlic-mediated attenuation of hypertension and serve to highlight the potential importance of H_2S therapy in CV disease.

H_2S is well known as a toxic gas with a repulsive odor often used to describe the smell of rotten eggs (3). Experimental studies reveal that H_2S is produced enzymatically at micromolar levels in mammals and exerts a number of physiological actions in the CV system (3, 6, 7). H_2S production has been attributed to two key enzymes in the cysteine biosynthesis pathway, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CGL). CBS and CGL are both heme-containing enzymes whose activity depends on the cofactor pyridoxal 5′-phosphate (6–8). CBS is capable of catalyzing the reaction of cysteine with other free thiols to generate H_2S; likewise, thiocysteine generated by CGL can interact with thiols to generate H_2S (6–8). The endogenous production of H_2S was initially described in the brain and attributed to CBS (7–9). In addition, H_2S is produced in the vasculature by CGL, where it mediates smooth muscle relaxation and subsequent vasodilation (3). At present, the distribution of CGL and CBS throughout the body are not well characterized. Furthermore, very little is currently known regarding the functions of CBS and CGL under physiological and pathophysiological conditions. As this rapidly emerging field advances, we are likely to learn a great

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deal regarding the biochemistry and physiology of these H_2S-producing enzyme systems.

Since the recent discovery that H_2S is a powerful gaseous signaling molecule, studies have begun to characterize the biological profile of this promiscuous gas. Fig. 1 summarizes some of the physiological actions of H_2S in the CV system. H_2S has been shown to relax vascular smooth muscle, induce vasodilation of isolated blood vessels, and reduce blood pressure (3, 10). In this regard, H_2S might be an important regulator of blood pressure. H_2S has also been shown to inhibit leukocyte–endothelial cell interactions *in vivo*. Zanardo *et al.* (11) used intravital microscopy to demonstrate that H_2S donors inhibit leukocyte adherence in the rat mesenteric microcirculation during vascular inflammation, suggesting that H_2S is a potent antiinflammatory molecule. It has also become evident that H_2S is as a potent antioxidant and, under chronic conditions, can up-regulate antioxidant defenses (9). In addition, it has been demonstrated that H_2S effectively inhibits apoptosis of a number of cell types, and this effect has been shown to promote cytoprotection (9).

Research interest in H_2S has also been focused on its profound metabolic effects, which result in a state of suspended animation with drastic reduction in metabolic demand (12). Initiating suspended animation may prove to be an ideal therapy for various shock or trauma states in which dramatic reductions in metabolic demands may be highly protective. Studies investigating the metabolic effects of H_2S have revealed that, similar to NO, H_2S exerts potent effects on mitochondrial function and respiration (10, 13). Physiological concentrations have been shown to dose-dependently and transiently inhibit mitochondrial respiration, an effect that could promote myocardial cell survival during myocardial ischemia and reperfusion (I/R) (10). The mitochondrial effects of H_2S have been shown to be mediated in part by reversible and potent inhibition of cytochrome *c* oxidase (complex IV of the mitochondrial electron transport chain) (10, 13). The defined physiological profile of H_2S makes this novel gaseous signaling molecule an ideal pharmacological candidate for the treatment of CV disease states.

It appears that H_2S possesses all of the positive effects of NO without the capacity to form a toxic metabolite.

A number of recent studies have provided insights into the protective actions of H_2S in the setting of myocardial I/R injury (10, 14–17). Pretreatment with the H_2S donor (NaHS) reduces arrhythmias in isolated hearts subjected to global cardiac I/R and improves myocyte survival (14, 15). Similarly, Johansen *et al.* (16) reported that NaHS pretreatment significantly attenuates myocardial injury in isolated, perfused rat hearts subjected to cardiac I/R. An article by Sivarajah *et al.* (17) represents the first report of the cardioprotective actions of H_2S in an *in vivo* model of myocardial I/R injury. The authors reported a 25% reduction in myocardial infarct size after pretreatment with an H_2S–donor compound. Further support for the cardioprotective effects of H_2S in myocardial I/R injury are provided by Elrod *et al.* (10), who have reported that administration of H_2S at the time of reperfusion dose-dependently attenuates myocardial infarct size and preserves postischemic left ventricular (LV) function in a clinically relevant, *in vivo* model of acute myocardial infarction. Furthermore, cardiac-specific overexpression of the H_2S-generating enzyme CGL significantly reduces myocardial I/R injury and similarly preserves LV function (10). The authors propose that preservation of cardiac mitochondrial structure and function is central in the cardioprotective actions of H_2S in I/R injury.

Recent investigations into the vascular effects of H_2S have revealed that this physiologically produced gas has profound effects on vascular growth or formation and neointimal hyperplasia. Meng *et al.* (18) have very recently demonstrated that H_2S ameliorates the intimal hyperplasia response in blood vessels after balloon-mediated vascular injury. Furthermore, Cai *et al.* (19) have reported that physiologically relevant dosages of H_2S promote a highly robust angiogenic response that is largely dependent on activation of Akt. Thus, H_2S may prove beneficial for the inhibition of coronary artery restenosis and the induction of therapeutic angiogenesis.

Clearly, additional research is warranted to further our understanding of the synthesis, metabolism, and physiological and potentially pathophysiological actions of endogenously produced H_2S in mammalian systems. The biological profile of H_2S strongly suggests that this gaseous signaling molecule is likely to protect a variety of organs despite its prior reputation as a noxious gas with a repulsive odor. It appears that H_2S possesses all of the positive effects of NO without the capacity to form a toxic metabolite such as $ONOO^-$. It is clear that H_2S will provide many years of exciting research opportunities for scientific investigators in diverse disciplines. The paper by Benavides *et al.* (4) sets the stage for future research into the physiological and pharmacological actions of H_2S in the CV system.

1. Baranano DE, Snyder SH (2001) *Proc Natl Acad Sci USA* 98:10996–11002.
2. Elrod JW, Duranski MR, Langston WJ, Greer JJM, Tao L, Dugas TR, Kevil CG, Champion HC, Lefer DJ (2006) *Circ Res* 99:78–85.
3. Pryor WA, Houk KN, Foote CS, Fukuto JM, Ignarro LJ, Squadrito GL, Davies KJ (2006) *Am J Physiol* 291:R491–R511.
4. Benavides GA, Squadrito GL, Mills RW, Patel HD, Isbell TS, Patel RP, Darley-Usmar VM, Doeller JE, Kraus DW (2007) *Proc Natl Acad Sci USA* 104:17977–17982.
5. Banerjee SK, Maulik SK (2002) *Nutr J* 1:4.
6. Kamoun P (2004) *Amino Acids* 26(3):243–254.
7. Boehning D, Snyder SH (2003) *Annu Rev Neurosci* 26:105–131.
8. Abe K, Kimura H (1996) *J Neurosci* 16:1066–1071.
9. Kimura Y, Kimura H (2004) *FASEB J* 18(10):1165–1170.
10. Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, Tao L, Jiao X, Scalia R, Kiss L, Szabo C, *et al.* (2007) *Proc Natl Acad Sci USA* 104:15560–15565.
11. Zanardo RC, Brancaleone V, Distrutti E, Fiorucci S, Cirino G, Wallace JL (2006) *FASEB J* 20:2118–21120.
12. Blackstone E, Morrison M, Roth MB (2005) *Science* 308:518.
13. Nicholls P (1975) *Biochem Soc Trans* 3:316–319.
14. Bian JS, Yong QC, Pan TT, Feng ZN, Ali MY, Zhou S, Moore PK (2006) *J Pharmacol Exp Ther* 316:670–678.
15. Pan TT, Feng ZN, Lee SW, Moore PK, Bian JS (2006) *J Mol Cell Cardiol* 40(1):119–130.
16. Johansen D, Ytrehus K, Baxter GF (2006) *Basic Res Cardiol* 101(1):53–60.
17. Sivarajah A, McDonald MC, Thiemermann C (2006) *Shock* 26:154–161.
18. Meng QH, Yang G, Yang W, Jiang B, Wu L, Wang R (2007) *Am J Pathol* 170(4):1406–1414.
19. Cai WJ, Wang MJ, Moore PK, Jin HM, Yao T, Zhu YC (2007) *Cardiovasc Res* 76(1):29–40.