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COMMENTARY

Involvement of redox-signalling in endogenous hydrogen sulfide production

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Recently, cystathionine-γ-lyase (CSE) was found to provide the major physiological pathway for H_2S, the third member of the gasotransmitter family. In various pathophysiological conditions, H_2S exerted protective effects based on its antioxidant, anti-inflammatory, anti-hypertensive and other regulatory functions. Interestingly, CSE expression had been only poorly studied and only in relation with inflammatory processes. Therefore, the study by Hassan *et al*. in this issue of the *BJP*, provides a considerable advance by furnishing direct experimental evidence for the involvement of redox signalling in the regulation of CSE gene expression. They found that PDGF up-regulated CSE expression and activity that was abolished by antioxidants and by deletion of the transcription factor nuclear erythroid-2-related factor-2 (Nrf2). Furthermore, PDGF induced Nrf2 binding to its consensus sequence that was again reversed by antioxidants. As Nrf2 also governs CO biosynthesis, and PDGF inversely affects H_2S and NO production, these data could indicate a concerted regulation of the three gasotransmitters by redox signalling.

LINKED ARTICLE

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Abbreviations

AP-1, activating protein 1; CBS, cystathionine β-synthase [EC 4.2.1.22]; CSE, cystathionine-γ-lyase [EC 4.4.1.1]; HO-1, heme oxygenase 1 [EC 1.14.99.3]; iNOS, inducible NOS [EC 1.14.13.39]; MST, 3-mercaptopyruvate sulfurtransferase [EC 2.8.1.2]; Nrf2, nuclear erythroid-2-related factor-2; ROS, reactive oxygen species

Hydrogen sulfide has been established as the third gasotransmitter following NO and carbon monoxide based on its presence in serum and various tissues and based on its participation in a variety of cellular functions such as endothelium-dependent vasorelaxation, modulation of neuronal transmission, stimulation of angiogenesis and regulation of insulin release (Hu *et al*., 2011; Whiteman *et al*., 2011; Szabo, 2012). In mammals, H_2S is produced by cystathionine β-synthase [EC 4.2.1.22] (CBS), cystathionine γ-lyase [EC 4.4.1.1] (CSE) and the coordinate actions of 3-mercaptopyruvate sulfurtransferase [EC 2.8.1.2] (MST) and cysteine aminotransferase [EC 2.6.1.3] (Hu *et al*., 2011; Whiteman *et al*., 2011; Szabo, 2012). Recently, CSE was shown to be the major physiological pathway to H_2S, at least, in the periphery, as H_2S levels in serum, aorta and heart of CSE knockout mice were reduced by about 50–80% (Yang *et al*., 2008). CBS is expressed predominantly in the brain where it is regarded as the major H_2S source, while the physiological significance of MST in contributing to the H_2S pools is yet to be established (Hu *et al*., 2011; Whiteman *et al*., 2011; Szabo, 2012). Unlike the other two members of the gasotransmitter family, H_2S does not seem to exert its physiological effects by stimulating soluble guanylyl cyclase. Rather, it S-sulphydrates various proteins such as β-tubulin, actin and glyceraldehyde 3-phosphate dehydrogenase (Mustafa *et al*., 2009) stimulating them. This molecular

mechanism is similar to the S-nitrosylation effect of NO, although S-nitrosylation usually inhibits, while S-sulphydration usually activates the target proteins.

A growing number of papers describe the protective effects of H_2S in various pathophysiological conditions. Although the majority of these reports are about beneficial effects of H_2S in the cardiovascular system (Whiteman *et al.*, 2011), deficiency of H_2S was indicated in the pathogenesis of diabetic endothelial dysfunction, diabetic nephropathy and cardiomyopathy (Szabo, 2012); and its potential as a new therapeutic tool was suggested in neurodegenerative diseases (Hu *et al.*, 2011). These effects of H_2S are usually based on its antioxidant, anti-inflammatory, anti-hypertensive and other regulatory functions. As for the molecular targets and mechanisms involved, K_{ATP} and transient receptor potential channels, Ca^{2+}-sensitive K^+ channels, T- and M-type calcium channels, the transcription factors NF-κB, activating protein 1 (AP-1) and nuclear erythroid-2-related factor-2 (Nrf2), and numerous kinases including p38MAPK, ERK and PKB/Akt were indicated (Li *et al.*, 2011). Effect on these disparate targets could be secondary to the reducing and protein S-sulphydrating activities of H_2S.

Surprisingly, much less work was performed on elucidating the regulation of the genes responsible for the biosynthesis of this remarkable gasotransmitter. Glucocorticoids were reported to inhibit bacterial LPS-induced CSE expression in macrophages (Zhu *et al.*, 2010), and inflammatory cytokines were found to induce CSE expression, thereby enhancing H_2S formation in primary human articular chondrocytes as well as in mesenchymal progenitor cells (Fox *et al.*, 2012), two reports revealing some aspects of CSE regulation in inflammatory processes. However, in the study reported in this issue, Hassan *et al.* (2012) are the first to provide experimental evidence for the involvement of redox signalling in the regulation of CSE gene expression. Importance of this contribution to our understanding of the regulation of H_2S biosynthesis is emphasized by the fact that oxidative stress is known to be a major causative and mediating factor in the pathomechanisms of all the conditions mentioned above, as well as in important physiological processes such as cellular senescence.

For their study, Hassan *et al.* (2012) used rodent glomerular mesangial cells that resemble vascular smooth muscle cells while capable of forming large amounts of NO via the induction of inducible NOS (iNOS) upon cytokine stimulation. PDGF, a highly potent mitogen in mesangial cells, had earlier been found to suppress iNOS expression. In the present study by Hassan *et al.* (2012), the effect of PDGF-BB, the isoform that recognizes both known PDGF receptors, was studied on CSE gene expression in glomerular mesangial cells isolated from wild-type and Nrf2-deficient mice. Extension of the study to Nrf2-deficient cells was prompted by the presence of an Nrf2 consensus binding sequence (GTGACTCAG) 355 bp upstream from the transcriptional start site of the murine CSE promoter. PDGF-BB up-regulated CSE gene expression both at the mRNA and protein levels in a time- and dose-dependent manner, accompanied by increased CSE activity and elevated formation of reactive oxygen species (ROS). However, antioxidants as well as Nrf2 deficiency abolished the up-regulation of CSE by PDGF-BB. Furthermore, PDGF-BB induced binding of Nrf2 to its consensus binding sequence that was reversed by antioxidants (Hassan *et al.*, 2012). All these data provide direct experimental evidence for the first time for the involvement of redox signalling mechanisms in the regulation of CSE gene expression. Supporting, although indirect, independent experimental evidence was also presented by Hassan *et al.* (2012), in that they observed a parallel up-regulation of CSE and Nrf2 proteins in a rat anti-Thy-1-induced proliferative glomerulonephritis model.

Although Nrf2 has been previously suggested as a potential mediator of the cardioprotective effect of H_2S (Li *et al.*, 2011), this suggestion was supported only by indirect experimental evidence. Nrf2 is ubiquitously expressed in animal tissues and seems to operate as a key regulator of the antioxidant response. It mediates the antioxidant response element-dependent transcriptional regulation of phase II enzymes such as NAD(P)H:quinine oxidoreductase 1 and glutathione S-transferase A1, and antioxidative enzymes and proteins such as thioredoxin, haem oxygenase 1 (HO-1) and ferritin (Ma *et al.*, 2006). As HO-1 is a significant source of physiological CO, it is interesting that its expression and that of CSE is regulated by the same transcription factor, Nrf2. Furthermore, as PDGF up-regulates CSE while down-regulating iNOS, at least in rodent glomerular mesangial cells, one can speculate about a concerted, redox signalling-mediated regulation of gasotransmitter biosynthesis. On the other hand, a number of factors, such as physiological relevance of the model, specificity of the intervening pharmacological agents, suitability of 'biomarkers' of H_2S metabolism, should be considered in future experiments. Deviations from the physiologically relevant conditions generated controversy that was only recently resolved (Whiteman *et al.*, 2011). Undoubtedly, much more experimental evidence is needed: The roles of the transcription factors such as NF-κB and AP-1 have yet to be elucidated and the effects of kinase signalling pathways and ROS have to be determined. Specific silencing by, for example, RNA interference technique of the biosynthetic enzymes for H_2S and/or the corresponding regulatory proteins has to be performed. Nevertheless, the study reported in this issue by Hassan *et al.* (2012) has expanded our understanding of the field considerably and presented experimental evidence that should stimulate research in both redox signalling and gasotransmitters.

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Conflicts of interest

None.

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