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Metabolic turnover of hydrogen sulfide

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More than 15 years have passed since hydrogen sulfide (H_2S) emerged as a biological signaling molecule – initially, in the nervous and vascular systems through the modulation of *N*-methyl D-aspartate (NMDA) receptors and the activation of transmembrane receptor potential (TRP)- and ATP-dependent K^+ (K_{ATP})-channels (Abe and Kimura, 1996; Hosoki et al., 1997; Dello Russo et al., 2000; Zhao et al., 2001; Teague et al., 2002; Nagai et al., 2004; Streng et al., 2008), and later, in nearly every organ system (Predmore and Lefer, 2010). H_2S was found to play a role as a cytoprotectant in the nervous system (Kimura and Kimura, 2004; Whiteman et al., 2004); this finding led to the discovery of the cardioprotective effect of H_2S (Elrod et al., 2007) (**Figure 1**). Previous studies have also described that H_2S plays several roles: as a regulator of insulin release, inflammation, and angiogenesis, and as an oxygen (O_2) sensor (Li et al., 2005; Yang et al., 2005; Kaneko et al., 2006; Olson et al., 2006; Zanardo et al., 2006; Cai et al., 2007; Papapetropoulos et al., 2009).

Previous studies report measurement of endogenous concentrations of sulfide by methods involving high concentrations of acids; therefore, contamination by free H_2S released from acid-labile sulfur resulted in an overestimate of the free H_2S levels (50–160 μM; Goodwin et al., 1989; Warenycia et al., 1989; Savage and Gould, 1990). A sulfur/silver electrode has frequently been used for the measurement of sulfide concentrations in biological samples. The electrode measures the level of S^{2-}, and a pKa value of 13.9 results in the replacement of cysteine sulfide groups in proteins with hydroxyl groups, thereby releasing H_2S from proteins. Because tissue and blood samples contain abundant proteins, this method estimates erroneously high concentrations of sulfide (Whitfield et al., 2008). Recently, the basal or steady state endogenous concentrations of H_2S have been re-evaluated using methods that avoid release of contaminant H_2S from proteins; these methods give concentration estimates of 20 nM to a few micromolar in tissue and blood samples (Furne et al., 2008; Ishigami et al., 2009; Wintner et al., 2010).

It is necessary to determine the active state concentrations of H_2S. At least three factors influence H_2S concentration: (1) rate of H_2S production, (2) rate of H_2S metabolism, and (3) storage of H_2S as bound sulfane sulfur and its associated release.

RATE OF H_2S PRODUCTION

H_2S production by three enzymes has been studied extensively; these enzymes are cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MST; Stipanuk and Beck, 1982; Chiku et al., 2009; Shibuya et al., 2009a,b; Singh et al., 2009) (**Figure 1**). CBS and CSE metabolize cysteine and/or homocysteine to release H_2S, whereas 3MST produces H_2S from 3-mercaptopyruvate (3MP), which is produced by the action of cysteine aminotransferase (CAT) on cysteine and α-ketoglutarate (Cooper, 1983; Shibuya et al., 2009a,b). 3MST requires cofactors to reduce a persulfide intermediate generated between a cysteine residue of 3MST and a sulfide provided by 3MP. We recently found that thioredoxin and dihydrolipoic acid (DHLA) are endogenous reducing cofactors that facilitate H_2S release from 3MST (Mikami et al., 2011a). We also found that the 3MST/CAT pathway is regulated by Ca^{2+} (Mikami et al., 2011b; Mikami and Kimura, 2012) and that the activity of CBS is enhanced by *S*-adenosyl methionine (SAM; Abe and Kimura, 1996). Thus, the physiological stimuli that alter the intracellular levels of Ca^{2+} and SAM should be investigated in order to clarify the regulation of H_2S production.

RATE OF H_2S METABOLISM

H_2S is metabolized in the mitochondria, via the sulfide oxidation pathway (Hildebrandt and Grieshaber, 2008). The first step is catalyzed by a membrane-bound sulfide:quinone oxidoreductase (SQR), which oxidizes H_2S to persulfide. SQRs were initially identified in invertebrates, and their mammalian counterpart SQRs were later discovered (Theissen et al., 2003; Theissen and Martin, 2008). In the presence of O_2 and water, sulfur dioxygenase oxidizes persulfide to sulfite, which is combined with another persulfide molecule by sulfur transferase rhodanese in order to producing thiosulfate (**Figure 1**). Because H_2S consumption by a dioxygenase is high in the presence of O_2 (Furne et al., 2008), high H_2S production is offset by rapid H_2S clearance under aerobic conditions, accounting for very low basal levels of H_2S. Small deviations in the rates of H_2S production and clearance may lead to rapid and several-fold changes in the H_2S levels (Vitvitsky et al., 2012).

STORAGE OF H_2S AS BOUND SULFANE SULFUR AND ITS ASSOCIATED RELEASE

In addition to enzymatic regulation of H_2S levels, H_2S may be stored in proteins as bound sulfane sulfur that is divalent sulfur bound mostly to sulfur of cysteine residues. (Ishigami et al., 2009) (**Figure 1**). Exogenously applied H_2S is absorbed and stored as bound sulfane sulfur, and the rate of absorption varies according to tissue types. Cells expressing 3MST and CAT have higher levels of bound sulfane sulfur than control cells (Shibuya et al., 2009a,b). In contrast, cells expressing 3MST mutants – that lack the ability to produce H_2S – retain control-like levels of bound sulfane sulfur. Therefore, H_2S produced by enzymes is stored in cells as bound sulfane sulfur. Bound sulfane sulfur releases H_2S under reducing conditions. In the presence of major cellular reducing substances, glutathione, cysteine, and dihydrolipoic acid (DHLA) at their physiologic concentrations, H_2S is released from lysates of cultured neurons and astrocytes at pH 8.0–8.4 (Ishigami et al., 2009; Mikami et al., 2011a). When neurons are excited, sodium ions enter and potassium ions exit from cells, resulting in high potassium concentrations in the extracellular environment, which

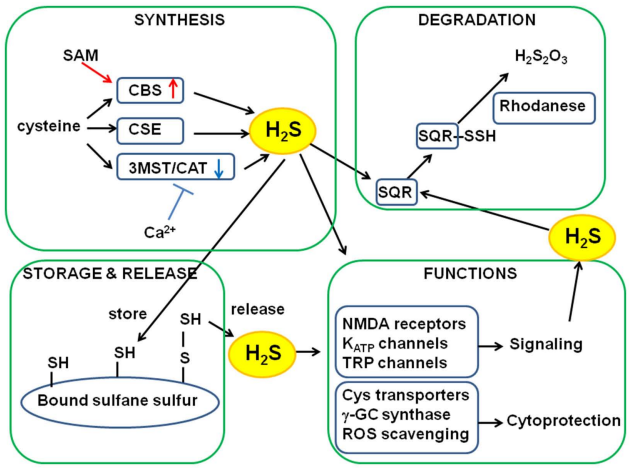

FIGURE 1 | Production, metabolism, storage, and functions of H_2S.

depolarizes the membrane of surrounding astrocytes. To recover from depolarization, Na^+ / HCO_3^- cotransporters are activated in astrocytes (Brookes and Turner, 1994). The entrance of HCO_3^- causes alkalinization. Approximately 10% of the astrocytes shifted their intracellular pH to 8.4, which can induce bound sulfane sulfur to release H_2S (Ishigami et al., 2009).

The concentrations of H_2S can vary locally in restricted areas of cells, while the reported values are those averaged over whole tissues, using tissue homogenates or blood samples (Furne et al., 2008; Ishigami et al., 2009; Wintner et al., 2010). Such local variations in H_2S concentrations must be determined. Recently developed H_2S-sensitive fluorescence probes are able to estimate local H_2S levels in live cells (Lippert et al., 2011; Liu et al., 2011; Peng et al., 2011; Qian et al., 2011; Sasakura et al., 2011). However, the probes bind to H_2S irreversibly; the detectable limit for H_2S is approximately 5–30 μM, i.e., values higher than the basal or steady state concentrations; and the time to reach maximum sensitivity is between 3 min and 1 h. The development of probes able to detect rapid changes in H_2S concentrations is awaited.

The metabolic balance between the production and clearance of H_2S has a considerable effect on its endogenous concentrations. In addition, because of bound sulfane sulfur, which releases and absorbs H_2S, the H_2S concentrations change more rapidly and extensively. The discrepancy between the endogenous basal concentrations and the effective-concentrations of exogenously applied H_2S has been discussed frequently. Some effects of H_2S may be elicited by its entry into cells through the plasma membrane. However, the rate of permeation and how freely and rapidly H_2S diffuses within the cytosol have not been determined. We also need to clarify the H_2S concentrations reached when cells are stimulated and the associated mechanism.

In conclusion, the basal or steady state levels of H_2S have been re-evaluated and found to be much lower than those previously reported. There is a difference between the steady state- and the effective-concentrations of H_2S. Therefore, it is urgent to determine the local concentrations of H_2S achieved when cells are stimulated.

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