British Journal of Pharmacology

British Journal of Pharmacology (2019) **176** 554–570 554

Themed Section: Chemical Biology of Reactive Sulfur Species

REVIEW ARTICLE

Homeostatic impact of sulfite and hydrogen sulfide on cysteine catabolism

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Received 16 March 2018; **Revised** 22 June 2018; **Accepted** 2 July 2018

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Cysteine is one of the two key sulfur-containing amino acids with important functions in redox homeostasis, protein functionality and metabolism. Cysteine is taken up by mammals *via* their diet and can also be derived from methionine *via* the transsulfuration pathway. The cellular concentration of cysteine is kept within a narrow range by controlling its synthesis and degradation. There are two pathways for the catabolism of cysteine leading to sulfate, taurine and thiosulfate as terminal products. The oxidative pathway produces taurine and sulfate, while the H_2S pathway involves different enzymatic reactions leading to the formation and clearance of H_2S, an important signalling molecule in mammals, resulting in thiosulfate and sulfate. Sulfite is a common intermediate in both catabolic pathways. Sulfite is considered as cytotoxic and produces neurotoxic S-sulfonates. As a result, a deficiency in the terminal steps of cysteine or H_2S catabolism leads to severe forms of encephalopathy with the accumulation of sulfite and H_2S in the body. This review links the homeostatic regulation of both cysteine catabolic pathways to sulfite and H_2S.

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Abbreviations

ADO, cysteamine dioxygenase; AdoMet, S-adenosylmethionine; CBS, cystathionine β-synthase; CDO, cysteine dioxygenase; CSA, cysteine sulfinic acid; CSAD, CSA decarboxylase; CSE, cystathionine γ-lyase; GOT, glutamate oxaloacetate transaminase; GSH, γ-glutamylcysteinylglycine; GSSG, glutathione disulfide; Moco, molybdenum cofactor; MPST, 3-mercaptopyruvate sulfurtransferase; PDO, persulfide dioxygenase; PLP, pyridoxal phosphate-dependent; SO, sulfite oxidase; SQR, sulfide : quinone oxidoreductase; SSC, S-sulfocysteine; TST, thiosulfate sulfurtransferase

DOI:10.1111/bph.14464

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Introduction

Cysteine is one of two sulfur-containing amino acids with key functions in cellular redox regulation, metabolism, protein synthesis and functionality. Cysteine plays pivotal roles in protein structure due to its ability to form disulfide bridges in an oxidative environment and its functions as active site residues in various families of enzymes. As a result of their high reactivity, cysteine thiols can react with reactive oxygen species forming sulfenic, sulfinic and sulfonic acids, with the latter two being considered as irreversible oxidation products (Poole and Nelson, 2008; Klomsiri *et al.,* 2011). Cysteine is further used as sulfur source for various types of cofactors, such as coenzyme A, biotin or Fe-S clusters.

Cysteine is able to undergo a large number of different post-translational modifications (Miseta and Csutora, 2000). For example, S-palmitoylation is the only reversible form of lipid modification that is found in many neuronal proteins and is catalysed by a large family of palmitoylating enzymes. Other modifications of cysteine residues are related to the action of gasotransmitters **NO** and **H_2S** forming covalent adducts by S-nitrosylation and S-persulfidation (also termed S-sulfhydration) respectively. Common to all of these modifications is the reactivity and accessibility of a given cysteine residue. In some cases (glyceraldehyde-3-phosphate dehydrogenase or parkin), the same residue can undergo nitrosylation and persulfidation, mediating opposing effects on protein function suggesting important roles of those post-translational modifications as molecular switches (Mustafa *et al.,* 2009; Vandiver *et al.,* 2013).

Cysteine is a semi-essential amino acid, as it can either be obtained from the diet or enzymatically produced from methionine *via* the transsulfuration pathway (Figure 1) (Zou and Banerjee, 2005; Banerjee, 2017). **Methionine** functions as an important methyl group donor in the cell following its conversion to **S-adenosylmethionine**. After methyl group transfer, the product S-adenosylhomocysteine is hydrolysed to **adenosine** and **homocysteine**. While a part of the homocysteine generated is regenerated to methionine, a fraction enters the transsulfuration pathway forming **cystathionine** following the reaction of homocysteine with serine catalysed by **cystathionine β-synthase (CBS)**. The

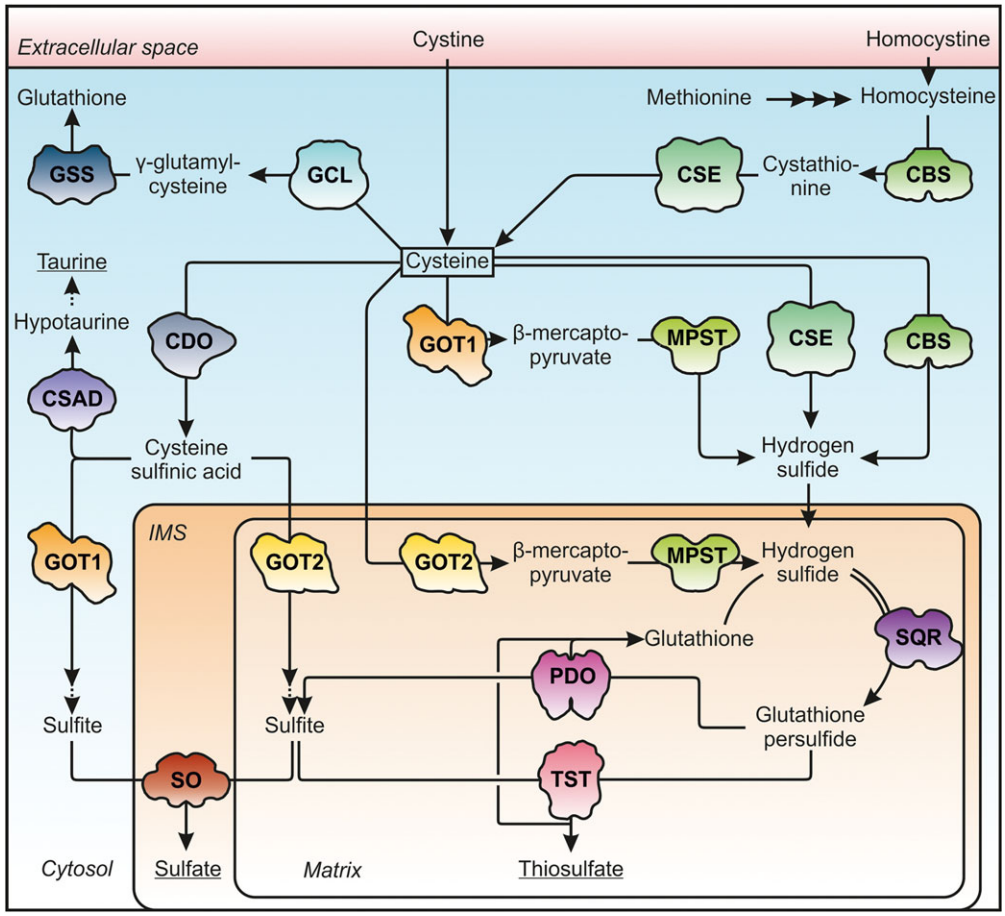

Figure 1

Cysteine anabolism and catabolism pathways. Cysteine is either imported or generated from methionine in the transsulfuration pathway. Cysteine is the rate-limiting substrate for GSH synthesis. Cysteine degradation proceeds *via* formation of CSA or H_2S to sulfate and taurine or sulfate and thiosulfate respectively. Terminal products are underlined. The shapes of the enzymes involved are depicted according to the crystal structures available. CBS, 1M54; CDO, 2IC1; CSAD, 2JIS; CSE, 2NMP; PDO, 4CHL; GCL, glutamate cysteine ligase, 3LVW; GSS, GSH synthetase, 1M0T; GOT1/2, 3II0/5AX8; IMS, intermembrane space; MPST, 4JGT; SQR, 3H8L; SO, 1SOX; TST, 1RHD.

partitioning of homocysteine to the remethylation cycle to form methionine or the transsulfuration pathway has been described as highly dynamic (Finkelstein, 2000). Cystathionine is subsequently cleaved into cysteine and α-ketobutyrate by **cystathionine γ-lyase (CSE)**. Interestingly, both enzymes involved in the transsulfuration pathway are also able to accept cysteine as substrate producing H_2S, an important signalling molecule (Figure 1) (Paul and Snyder, 2012; Kabil and Banerjee, 2014).

The vast majority of 'free' cysteine in cells is bound in **GSH** (γ-glutamylcysteinylglycine), the major antioxidant and most abundant non-protein thiol in mammals. GSH is synthesized from glutamate, cysteine and glycine, by a two-step reaction of glutamyl-cysteine synthetase and GSH synthetase in an ATP-dependent manner (Figure 1). With 0.5–10 mM cytosolic GSH concentrations (Lu, 2013), GSH is considered as the major source and buffer of cysteine while the cellular concentrations of cysteine are kept within a narrow window in the micromolar range (Lee *et al.,* 2004; Vitvitsky *et al.,* 2004). The availability of cysteine is considered as one of the rate-limiting steps in GSH synthesis, thereby impacting cellular redox status as well as diverse cellular functions (reviewed in Deponte, 2013; Lu, 2013).

In the extracellular compartment, cysteine is oxidized to **cystine**, thus representing the major transport form of non-protein-bound cysteine (Ueland *et al.,* 1996). Across membranes, cysteine and cystine are transported by different membrane carriers. In the CNS, glial cells mainly import cystine *via* the cystine-glutamate antiporter (system x_c^-) providing the major route for GSH synthesis in the brain (Sato *et al.,* 2005). In its reduced form, cysteine is transported by **excitatory amino acid transporters**, which are known for their function in glutamate and aspartate clearance. Deficiencies in these transporters have been found to be associated with reduced cellular levels of GSH, oxidative stress and neurodegeneration (Sato *et al.,* 2005; Aoyama *et al.,* 2006).

Alterations in cysteine homeostasis have been associated with various primarily neurodegenerative disorders (see below). Several severe, fast progressing, rare genetic disorders are related to the terminal steps in cysteine degradation and characterized by major alterations in GSH, cysteine/cystine and H_2S levels. More complex disorders, such as Huntington's and Alzheimer's disease, have also been linked to altered cysteine homeostasis (McBean *et al.,* 2015). Finally, positive effects of dietary restriction on longevity have been traced back to cysteine-dependent alterations in the cellular homeostasis of H_2S (Hine *et al.,* 2015; Hine and Mitchell, 2015), thus placing the homeostatic control of cysteine metabolism at the heart of cellular signalling and redox regulation. In this review, we discuss the different pathways of cysteine catabolism and provide evidence that sulfite and H_2S, as key intermediates, have a major regulatory impact on cysteine homeostasis in health and disease.

Oxidative cysteine catabolism

Cysteine, which is not used for protein or GSH synthesis is further metabolized *via* two distinct pathways, the oxidative cysteine catabolism and the H_2S pathway (Figure 1) (Kabil *et al.,* 2011; Stipanuk and Ueki, 2011). Within the oxidative catabolic pathway, cysteine is first converted to **cysteine sulfinic acid** (CSA) *via* oxidation of its thiol group by cysteine dioxygenase (CDO) (Figure 2). CSA is then further

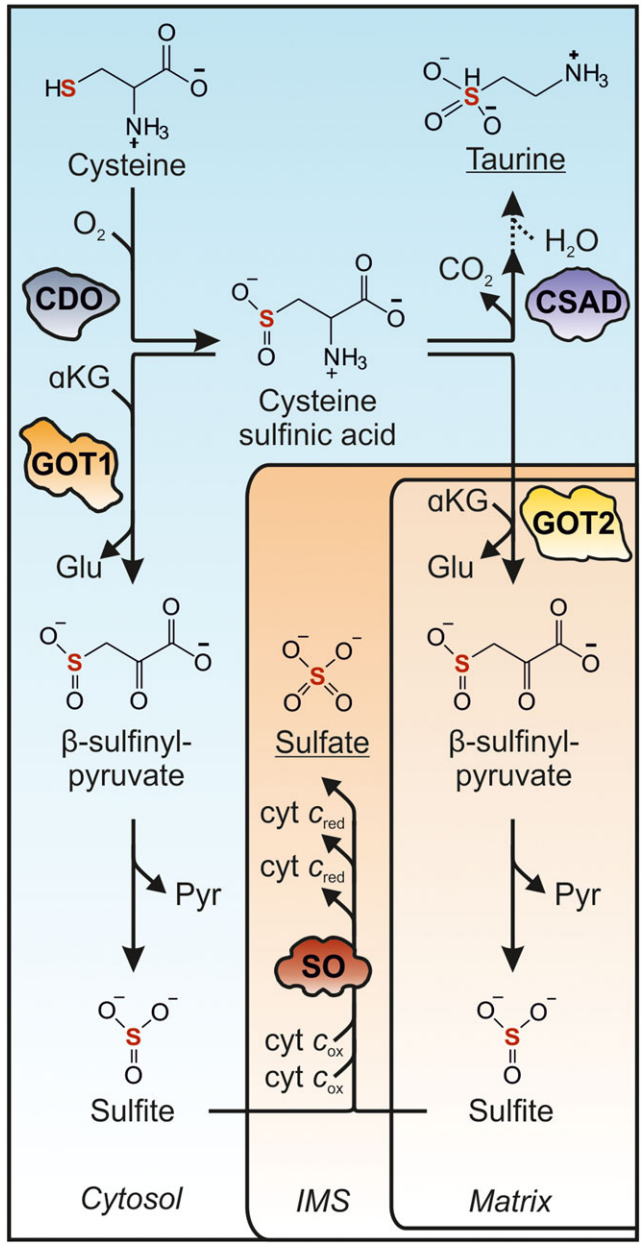

Figure 2

Oxidative cysteine catabolism to taurine and sulfate. Cysteine is converted by CDO (2IC1) to CSA, which is subsequently used for taurine synthesis catalysis by CSAD (2JIS) yielding hypotaurine, which is further converted to taurine. Alternatively, GOT1/2 (3II0/5AX8) catalyse the deamination of CSA to β-sulfinyl pyruvate, which spontaneously decomposes to sulfite and sulfate. Note that, as yet, it is not known which of the two GOT isoforms functions primarily in oxidative cysteine catabolism. Terminal oxidation of sulfite is catalysed by SO (1SOX) localized to the mitochondrial intermembrane space (IMS). The shapes of the enzymes involved are depicted according to the crystal structures available.

processed *via* two different routes, yielding either **taurine** or sulfate as end products. Decarboxylation of CSA by CSA decarboxylase (CSAD) releases hypotaurine, which is further oxidized by a non-enzymatic mechanism to taurine. As the most abundant amino acid derivative in human cells, taurine participates in a broad range of biological processes including bile acid conjugation, antioxidant and anti-inflammatory actions as well as modulation of the CNS (Ripps and Shen, 2012). Beside taurine synthesis, CSA is deaminated to β-sulfinylpyruvate, which spontaneously decomposes to pyruvate and sulfite. It has been shown that glutamate oxaloacetate transaminase [GOT; also named aspartate aminotransferase (AST/AAT)] is able to catalyse this reaction *in vitro* (Singer and Kearney, 1956). Since sulfite is highly toxic, as it inhibits glutamate dehydrogenase (GDH) and malate dehydrogenase (Zhang *et al.,* 2004), fast elimination by oxidation to harmless sulfate is of great importance. This reaction is catalysed by the molybdenum cofactor-containing mitochondrial enzyme sulfite oxidase (SO) (Schwarz *et al.,* 2009). The individual enzymes involved in oxidative cysteine catabolism will be discussed in the following chapters.

Cysteine dioxygenase

In humans, the amount of dietary cysteine and methionine intake can vary greatly, while intracellular cysteine levels are tightly controlled and maintained within a range of 10–25 μM suggesting a homeostatic control of cysteine-producing and cysteine-consuming processes (Lee *et al.,* 2004). It is generally considered that under conditions of cysteine excess, the enzyme CDO plays a key function in the maintenance of cysteine levels (Stipanuk and Ueki, 2011) (Figure 2). Upon increased cysteine availability, CDO concentrations in hepatic and adipose tissue were found to be increased by up to 45-fold (Dominy *et al.,* 2006). In contrast, in the absence of cysteine, CDO levels are kept low by ubiquitination and subsequent proteasomal degradation of the enzyme. In return, ubiquitination is inhibited in the presence of cysteine, allowing for a rapid adjustment of CDO levels and revealing the physiological importance of the underlying regulatory mechanisms (Dominy *et al.,* 2006).

CDO-deficient mice are characterized by high postnatal mortality, growth deficiency and connective tissue disease (Ueki *et al.,* 2011). Biochemically, $CDO^{-/-}$ mice display elevated cysteine and severely lowered taurine levels. The remaining production of taurine (~7%) is derived from cysteamine (Ueki *et al.,* 2011), a catabolic cleavage product of coenzyme A. Interestingly, while taurine supplementation prevents the high mortality rate, it does not impact other characteristic phenotypes. Slightly elevated sulfate and H_2S levels suggest an increased flux of cysteine through the H_2S pathway (see below), which may be connected to the pathology of $CDO^{-/-}$ mice (Ueki *et al.,* 2011; Roman *et al.,* 2013). Interestingly, no deregulation of enzymes involved in the H_2S pathway could be detected (Jurkowska *et al.,* 2014).

Catabolism of cysteine sulfinic acid

The distribution of CSA between taurine- and sulfate-generating pathways likely depends on the expression of CSA-processing enzymes as well as their affinity towards CSA (Figure 2). Interestingly, CSAD and CDO show both high levels in liver and adipose tissue, with only moderate to low levels in other tissues (Stipanuk and Ueki, 2011). The reported K_m values of 0.04–0.17 mM are in line with a high specificity of CSAD towards CSA (Guion-Rain *et al.,* 1975; Oertel *et al.,* 1981). CSAD-deficient mice resemble $CDO^{-/-}$ mice in terms of taurine levels and mortality rate (Park *et al.,* 2014). Oral administration of taurine is sufficient to restore survival and plasma taurine to wildtype levels.

Deamination of CSA was found to be catalysed by GOT, of which two isoforms are known, one localized in the cytosol (GOT1), the other in the mitochondrial matrix (GOT2) (Recasens *et al.,* 1980) (Figure 2). In contrast to CSAD, both GOT1 and GOT2 display a much broader expression pattern with high levels in the liver, heart, kidney, skeletal muscle and red blood cells (Cechetto *et al.,* 2002). GOT1/2 are important components of the malate–aspartate shuttle, which functions in the transfer of reducing equivalents to mitochondria (Bremer and Davis, 1975). In general, GOT1/2 catalyse the reversible transfer of an amino group from an amino acid donor to an α-keto acid. They accept three main amino acid donors – aspartate, CSA and cysteine (Nisselbaum and Bodansky, 1964) – while the amino group is usually transferred to α-ketoglutarate, thereby yielding glutamate.

Based on kinetic data, aspartate is considered to be the main substrate of GOT1/2 (Ubuka *et al.,* 1978; Akagi, 1982). Our own data show a K_m of 1.8 mM (GOT1) and 0.4 mM (GOT2) for aspartate, while the K_m for CSA was found between 10 and 20 mM for both enzyme isoforms (Schwarz *et al.*, unpublished). In addition, GOT1/2 have also been reported to accept cysteine directly for transamination, thereby participating not only in oxidative cysteine catabolism but also within the H_2S pathway (Figure 1) (Ubuka *et al.,* 1978; Akagi, 1982). With K_m values for cysteine of around 2 mM for both enzymes, CSA and cysteine represent substrates with similar affinity towards GOT1/2. In contrast to GOT1/2, CSAD exhibits a much lower K_m for CSA (0.04–0.17 mM) suggesting a larger flux of CSA towards taurine biosynthesis than towards sulfate formation (Stipanuk and Ueki, 2011).

To this day, it is not known which of the two GOT isoforms represents the primary physiological site for CSA deamination. Kinetic data of CSA deamination by GOT1/2 do not allow any conclusion as to which of the two isoforms is physiologically relevant (Figure 2). Notably, the involvement of GOT2 would require the import of CSA into mitochondria *via* a yet unidentified transporter. The rate of this import would determine whether or not GOT2 contributes to any significant deamination of CSA. In the future, the individual contribution of each GOT isoform to cysteine catabolism should be clarified by using specific *in vivo* models. The reaction product of CSA deamination, β-sulfinyl pyruvate, decomposes into pyruvate and sulfite (Singer and Kearney, 1956); the latter requires immediate oxidation by sulfite oxidase (SO) to avoid cellular damage.

Sulfite oxidase

SO catalyses the two-electron oxidation of sulfite to sulfate coupled to the reduction of two molecules of cytochrome *c* (Figures 2 and 3) (Johnson and Rajagopalan, 1980). The structure and reaction mechanism of SO has been extensively reviewed elsewhere (Hille *et al.,* 2014). Vertebrate SO forms homodimers; each monomer harbours an N-terminal

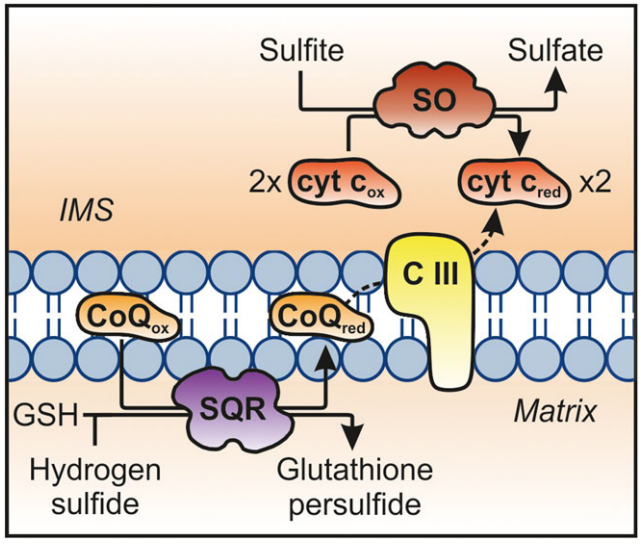

Figure 3

The contribution of SO and sulfide quionone oxidoreductase (SQR) to the mitochondrial electron transport chain. One electron is transferred from H_2S to coenzyme Q *via* SQR (3H8L), which is further transferred to cytochrome c *via* complex III (CIII). Alternatively, two molecules of cytochrome c are reduced by SO (1SOX)-catalysed oxidation of one molecule sulfite to sulfate.

cytochrome b_5-type haem domain, a catalytic molybdenum cofactor (Moco)-containing domain and a C-terminal dimerization domain (Kisker *et al.*, 1997). SO is localized in the mitochondrial intermembrane space where electrons derived from sulfite oxidation are passed to the physiological electron acceptor cytochrome *c* (Cohen *et al.*, 1972). Until now, the contribution of sulfite to mitochondrial respiration was not considered as a source of any significance. However, we have found that sulfite excess (500 μM) increased oxygen consumption in mitochondria in an SO-dependent manner by up to 15% (Schwarz *et al.*, unpublished).

SO is highly expressed in liver and kidney, whereas very low levels are detected in the brain (Belaidi *et al.*, 2015). As a mitochondrial enzyme, SO requires a specific translocation and maturation pathway to gain enzymatic function. This process is highly orchestrated and involves targeting and mitochondrial processing of the SO apo-protein, stepwise integration of Moco and haem cofactors and homodimerization (Klein and Schwarz, 2012).

The catalytic mechanism of SO involves the transfer of two electrons from sulfite to Moco followed by two single-electron transfer steps *via* the haem domain to cytochrome *c*. Electron transfer within SO is dependent on dynamic movements of the haem domain to enable efficient electron transfer (Rapson *et al.*, 2010). These conformational changes have been found to determine a novel, recently identified function of SO: the reduction of nitrite to nitric oxide (Wang *et al.*, 2015). With decreasing intramolecular electron transfer, the rate of nitrite-dependent nitric oxide synthesis increases.

We have recently generated a mouse model of SO deficiency (isolated SO deficiency – iSOD) (Kohl *et al.*, unpublished data), which resembles in large parts mice that are impaired in the biosynthesis of Moco (Lee *et al.*, 2002). As a result, all Moco-dependent enzymes are dysfunctional and affected animals die within 10 days of postnatal life. The fact that SO-deficient mice show a similar survival rate suggests that the lack of SO activity represents the major cause of disease in Moco-deficient mice.

SO not only serves as terminal enzyme in the oxidative cysteine catabolism. Also *via* the H_2S pathways, significant quantities of sulfite are formed that require the oxidation by SO – if sulfite production from H_2S is blocked in rodents, quantities of excreted sulfate are sixfold decreased (Tiranti *et al.*, 2009). Therefore, we will next discuss the generation and catabolism of H_2S before linking the contribution of altered sulfite homeostasis to the overall fluxes of S-containing metabolites.

Enzymatic production of hydrogen sulfide (H_2S) from cysteine

While the oxidative cysteine catabolism primarily serves taurine biosynthesis and is stimulated under cysteine excess, the other important branch of cysteine catabolism – the generation of H_2S and its subsequent breakdown – has gained increasing attention in recent years. H_2S joins the group of physiologically important gasotransmitters and has been found to be involved in multiple cellular functions in cardiovascular systems, neuronal tissues and in the gastrointestinal tract (Abe and Kimura, 1996; Dongó *et al.*, 2017). In the last two decades, H_2S has been shown to function as a potent vasodilator with multiple medical applications arising (Caliendo *et al.*, 2010; Beltowski, 2014), for example, **ATB-346**, a novel H_2S-releasing non-steroid anti-inflammatory drug (Wallace *et al.*, 2010, 2017). H_2S is a mediator of tissue inflammation (Li *et al.*, 2011), and early studies provided evidence for its involvement in numerous neurological processes, such as long-term potentiation in the hippocampus – an important process in memory formation and learning – by enhancing NMDA receptor-mediated responses (Abe and Kimura, 1996). H_2S is closely connected to the post-translational protein modification of cysteines leading to persulfidation, which has been implicated in mediating a protective function on redox-active cysteine residues as well as modulating enzymatic activities (Filipovic *et al.*, 2017). Whereas free H_2S concentrations in tissues are considered to be rather low (<0.05 μmol·kg^{-1}), concentrations of bound H_2S in the blood have been found to be several orders of magnitude higher (Furne *et al.*, 2008; Levitt *et al.*, 2011). Five enzymes in three distinct reaction paths are considered to function in the generation of H_2S (Figures 1 and 4). Four additional enzymes are involved in the oxidative degradation of H_2S to sulfate and thiosulfate, the latter also involves sulfite derived from the oxidative branch of cysteine catabolism. The high number of enzymes and multitude of pathways involved highlight the physiological importance of these pathways. The contribution of the different enzymes in the formation and breakdown of H_2S will be discussed in the following chapters.

Cystathionine β-synthase

CBS is the first enzyme in the transsulfuration pathway catalysing the condensation of homocysteine and serine,

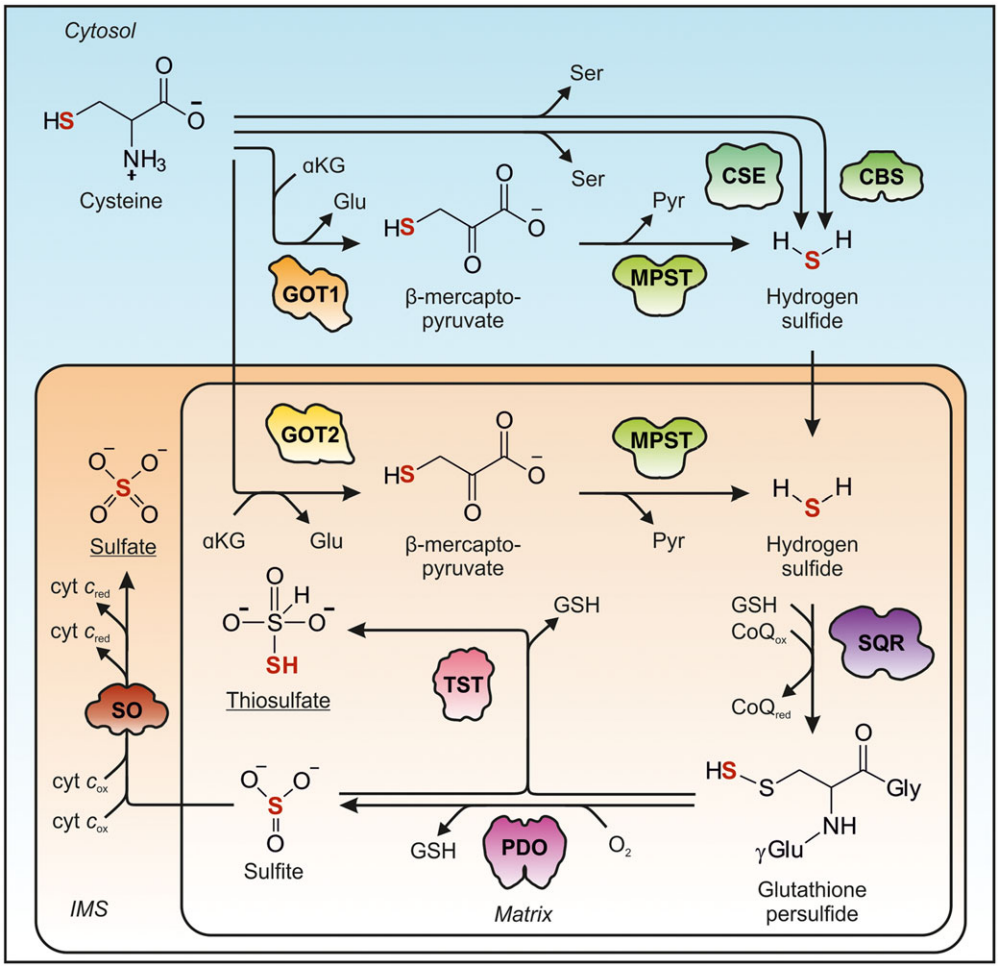

Figure 4

Sulfur flow from cysteine *via* H_2S to sulfate and thiosulfate. Cysteine catabolism *via* H_2S proceeds *via* three distinct pathways that involve either CBS (1M54), CSE (2NMP), or GOT1/2 (3II0/5AX8) and MPST (4JGT). Within the mitochondrial matrix, H_2S is oxidized *via* SQR (3H8L) to GSH persulfide, which is further converted to either sulfite by PDO (4CHL) or thiosulfate by TST (1RHD). The shapes of the enzymes involved are depicted according to the crystal structures available.

yielding cystathionine (Figure 1). Initially described as a cysteine-desulfurating enzyme (Porter *et al.*, 1974), CBS is capable of catalysing multiple reactions towards the generation of H_2S (Figure 4). CBS preferentially accepts cysteine as a substrate, which reacts with water releasing serine and H_2S or – at a lower rate – with homocysteine and cysteine to form cystathionine and H_2S (Chen *et al.*, 2004). Alternatively, cystine may also serve as a substrate to form lanthionine and H_2S. Among these alternative H_2S-producing reactions, only the hydrolytic desulfuration of cysteine proceeds with a rate that exhibits physiological relevance (Yadav *et al.*, 2016). CBS is also capable of producing a persulfide by converting cystine to a cysteine persulfide, pyruvate and ammonia. The latter reaction, however, likely only takes place under oxidative stress when intracellular cystine concentrations are elevated (Yadav *et al.*, 2016).

CBS is localized in the cytosol and expressed in several tissues, including neuronal, specifically in glial cells and astrocytes (Enokido *et al.*, 2005). Although CBS is strongly expressed in, that is, hepatic tissues, its relative activity towards H_2S production is considered to be tissue-specific and relatively low in non-neuronal tissues (Yang *et al.*, 2008) (see below). A recent study suggests that hepatic H_2S production by CBS is strongly dependent on the concentration of S-adenosylmethionine (AdoMet), an allosteric activator of CBS, and serine, a substrate of CBS (Majtan *et al.*, 2018). CBS regulation of both the transcriptional and post-translational level is complex. CBS is a pyridoxal phosphate-dependent (PLP) haem enzyme and responds to an oxidative environment *via* its haem domain, which directly influences the reactivity of the phosphate of PLP, inducing an increase in activity (Banerjee *et al.*, 2003). Several dedicated CBS-deficient mouse models have been generated and replicate the human disease phenotype and biomarker level abnormalities further described below (Watanabe *et al.*, 1995; Gupta *et al.*, 2009; Maclean *et al.*, 2010). It is noteworthy that homozygous *Cbs*-deficient pups display high pre- and neonatal mortality due to liver failure, highlighting the importance of CBS in the hepatic transsulphuration pathway (Watanabe *et al.*, 1995). The translational aspects of CBS-deficient mice

to the human disease phenotype have been reviewed extensively elsewhere (Kruger, 2017). Neither sulfate nor thiosulfate levels have been reported in these mice; however, since CBS is the major H_2S-generating enzyme in neuronal tissues, it is reasonable to assume that H_2S synthesis and downstream pathways are affected.

Cystathionine γ-lyase

CSE primarily functions in the trans-sulfuration pathway by catalysing the downstream reaction of CBS, the hydrolytic cleavage of cystathionine into cysteine, β-ketobutyrate and ammonia (Figure 1). Additionally, CSE is considered as an important H_2S-producing enzyme (Kabil *et al.,* 2011) catalysing five different reactions all leading to the generation of H_2S (Figure 4). However, only two of these reactions are likely relevant under physiological cysteine and homocysteine concentrations: (i) the CBS-like reaction of cysteine and water to H_2S and serine has been estimated to account for up to 70% of CSE-derived H_2S, and (ii) the remaining 30% are covered by the reaction of homocysteine to H_2S and α-ketobutyrate (Yadav *et al.,* 2016), while all other reactions produce only insignificant amounts of H_2S. In addition, CSE is able to produce cysteine and homocysteine persulfides, with a rate again considered to be marginal (Yadav *et al.,* 2016; Filipovic *et al.,* 2017).

CSE is a PLP cytosolic enzyme strongly expressed in endothelial tissues of blood vessels (Morikawa *et al.,* 2012). While in the past, the contribution of CSE to neuronal H_2S synthesis received little attention, recent studies have found a significant impact of CSE-dependent H_2S production in murine brains under both healthy and disease conditions (Paul *et al.,* 2014). The basal expression levels of CSE are controlled by a specificity protein 1, a ubiquitously expressed transcription factor mainly involved in cellular development (Ishii *et al.,* 2004). Under conditions of cysteine deprivation and ER stress, increased presence of the activating transcription factor 4 increases expression of CSE drastically, which is believed to provide sufficient supply of cysteine for GSH biosynthesis (Dickhout *et al.,* 2012).

To study the function of CSE in a physiological context, two mouse models have been developed and investigated (Yang *et al.,* 2008; Ishii *et al.,* 2010). In general, only mild neurological phenotypes have been reported for both models. CSE-deficient mice require dietary cysteine supplementation (provided as bioavailable *N*-acetyl-cysteine) to avoid the development of a lethal myopathy and excessive oxidative damage (Ishii *et al.,* 2010; Yamada *et al.,* 2012). They also display increased serum cystathionine and homocysteine levels and decreased taurine levels, which can be correlated to an impaired cysteine biosynthesis, ultimately reducing the flux of cysteine towards the oxidative pathway. Sulfate and thiosulfate levels have not been reported for these mouse models, but are likely to be decreased, as the production of the precursor to both metabolites – H_2S – in the heart and endothelial tissues is impaired (Yang *et al.,* 2008). Additionally, protein persulfidation levels in the lung, heart, kidney and brain are significantly decreased under CSE deficiency (Wedmann *et al.,* 2016), in addition to the inability of these mice to produce cysteine from methionine.

Mercaptopyruvate sulfurtransferase

A third pathway capable of producing H_2S is dependent on several enzymatic activities. First, a transamination reaction by GOT1/2 converts cysteine and α-ketoglutarate to **3-mercaptopyruvate** and glutamate. Under which conditions both enzymes are able to accept cysteine as substrate remains unknown, given that the K_M values for cysteine are in the low millimolar range (Schwarz *et al.,* unpublished data). In a subsequent reaction, **3-mercaptopyruvate sulfurtransferase** (MPST) catalyses the reductive desulfuration of 3-mercaptopyruvate, a reaction that also requires thioredoxin, yielding pyruvate and H_2S (Nandi *et al.,* 2000). As described above, human GOT1 and GOT2 show similar kinetic properties, whereas rat liver mitochondrial MPST activity (5.2 $U{\cdot}mg^{-1}$) was found to be threefold higher than that of the cytosolic fraction (1.7 $U{\cdot}mg^{-1}$) (Nagahara *et al.,* 1998). The fractional contribution of cytosolic and mitochondrial MPST to the overall H_2S production remains to be elucidated.

MPST expression can be ubiquitously detected, with highest levels in the liver, large intestine and kidney (Shibuya *et al.,* 2013), coinciding with GOT expression. Recently, another source for β-mercaptopyruvate has been reported: the conversion of D-cysteine to β-mercaptopyruvate, hydrogen peroxide and ammonia *via* D-amino acid oxidase, a peroxisomal enzyme (Shibuya *et al.,* 2013). D-cysteine is mostly taken up *via* the diet, and administration of D-cysteine *via* an oral diet to rats has previously been found to lead to an increase in sulfate levels (Krijgsheld *et al.,* 1981). MPST contains two rhodanese-like domains, and, as such, MPST shares the scope of its substrates with other rhodaneses. MPST transfers the sulfur atom of β-mercaptopyruvate to a cysteine residue on its C-terminal rhodanese domain, forming a persulfide. This persulfide can be transferred to cyanide yielding thiocyanide or – more likely – the persulfide is transferred to thioredoxin, releasing H_2S through auto-oxidation (Yadav *et al.,* 2013). Thioredoxin has also been shown to be the primary persulfidation-resolving enzyme in cells (Dóka *et al.,* 2016; Wedmann *et al.,* 2016).

MPST-deficient mice have been generated and display increased anxiety-related behaviour, with no observed physical abnormalities (Nagahara *et al.,* 2013); a comparison to the phenotype of the rare cases of human mercaptolactate-cysteine disulfiduria is difficult to establish due to the low number of cases reported. Mental retardation, however, seems to be a common phenotype for this disease and was related to the anxiety phenotype of MPST-deficient mice (Nagahara *et al.,* 2013). While primary neurons derived from these mice display decreased H_2S and polysulfide (H_2S_n) production in response to β-mercaptopyruvate, urinary or blood plasma thiosulfate and sulfate levels have not yet been reported (Kimura *et al.,* 2015).

In the light of the three routes of H_2S formation, the majority of H_2S is formed within the cytosol and only a minor fraction is produced in mitochondria. In contrast, the major H_2S-dependent post-translational modification, protein persulfidation, has been mainly detected in mitochondria (Wedmann *et al.,* 2016). Although all three H_2S-producing enzymes are capable of H_2S generation with significant rates under substrate saturation conditions, their relative contribution to the free and bound H_2S pools vary between different

tissues and is expected to be highly dependent on the local concentration of substrate and the expression levels of CBS, CSE and MPST in the respective cell type (Kabil *et al.,* 2011). One reason that the action of H_2S is highly controlled in a special and temporal manner resides in its efficient oxidation, which will be discussed in the following chapters.

Mitochondrial oxidation of hydrogen sulfide

The biogenesis of H_2S takes place primarily in the cytosol, yet its degradation is restricted to the mitochondrial matrix and the intermembrane space (Figures 1 and 4). H_2S is exceptionally adept at diffusing through biological membranes and therefore should easily reach the mitochondrial matrix (Riahi and Rowley, 2014). There are several enzymes involved in H_2S oxidation resulting in the formation of sulfate and thiosulfate as terminal products (Kabil and Banerjee, 2014). While different routes of H_2S oxidation have been proposed, only a few are expected to take place under substrate concentrations that are physiologically relevant. While sulfate is essential for several cellular processes and present in high micromolar concentrations in mammalian tissues (Markovich, 2001), thiosulfate has numerous medical implications, including being an antidote to cyanide poisoning. Furthermore, thiosulfate has been shown to be non-toxic to neurons, although causing nausea and headache when rapidly infused intravenously (Baskin *et al.,* 1992; Kumar *et al.,* 2017).

Sulfide : quinone oxidoreductase

The key enzyme in H_2S oxidation is sulfide : quinone oxidoreductase (SQR), which is associated with the matrix side of the inner mitochondrial membrane (Figure 4) and harbours a flavine adenine dinucleotide (FAD) cofactor. It oxidizes H_2S to a zero-valent sulfur by generating a protein-bound persulfide, which is further transferred to an acceptor. In a two-step reaction, the electron from the sulfur atom is first transferred to the FAD cofactor and then to coenzyme Q located in the mitochondrial inner membrane, thus feeding into the electron transport chain of mitochondria (Marcia *et al.,* 2010). Therefore, both cysteine degradation intermediates (sulfite and H_2S) represent inorganic substrates for the electron transport chain in vertebrates. The final acceptors of the SQR-bound persulfide are controversial (Filipovic *et al.,* 2017), but GSH and sulfite have been proposed as the most likely candidates, while other acceptors discussed in the field (Libiad *et al.,* 2014) exhibit mitochondrial concentrations that makes their physiological relevance questionable. Due to the high concentration of GSH (in contrast to low sulfite in healthy individuals), one can expect GSH as primary acceptor for the SQR persulfide, thus producing GSH persulfide. However, it is worth mentioning that the k_{cat} and K_M determined for the sulfite-dependent reactivities of SQR and SO are nearly identical. Therefore, under conditions of low mitochondrial GSH levels, SQR could take over a significant share of sulfite clearance from SO (Jackson *et al.,* 2012).

Persulfide dioxygenase

The first path to oxidize the terminal sulfur of the GSH persulfide is dependent on persulfide dioxygenase (PDO), also known as the ethylmalonic encephalopathy 1 (ETHE1) protein. Consistent with the localization of its substrate, PDO is located within the mitochondrial matrix (Figure 4) and appears to be most strongly expressed in liver and muscle tissues (Hildebrandt *et al.,* 2013). For catalysis, the GSH persulfide is coordinated to a mononuclear iron in the active site of PDO, followed by stepwise oxidation of the terminal sulfur atom and an H_2O-dependent displacement of the generated sulfur dioxide, converting it to sulfite. PDO is only capable of oxidizing the persulfide of GSH, but not of cysteine or homocysteine (Kabil and Banerjee, 2012). It was also proposed that PDO may convert thiosulfate, oxygen and water to two sulfite moieties (Kabil and Banerjee, 2012); however, *in vitro* studies could not provide evidence for such a reaction under physiological pH (Kabil and Banerjee, 2012). The reaction of PDO holds interesting implications for the oxygen-dependence of the H_2S oxidation, and its enzymatic reaction may very well be restricted under hypoxic conditions.

ETHE1$^{-/-}$ mice have been reported to largely mimic the human disease phenotype, displaying increased C4- and C5-acylcarnitine levels as well as strongly increased tissue, serum and urinary thiosulfate levels (Tiranti *et al.,* 2009). H_2S levels have also been found to be increased, with mice showing signs of H_2S-dependent inhibition of cytochrome c oxidase (Tiranti *et al.,* 2009). Interestingly, urinary sulfate concentrations have been demonstrated to be approximately sixfold decreased in these mice (Tiranti *et al.,* 2009), providing evidence for a quantitative flux of sulfite towards thiosulfate *via* rhodanese.

Thiosulfate sulfurtransferase: rhodanese (TST)

The second path to further metabolize GSH persulfide produced by SQR is catalysed by rhodanese (also labelled TST), the second member of the sulfurtransferase superfamily present in this pathway. Rhodanese is most prominently expressed in hepatic and gastrointestinal tissues and localizes to the mitochondrial matrix. The TST reaction mechanism has been described as largely similar to MPST, with the exception that rhodanese does not rely on thioredoxin to resolve its enzyme-bound persulfide (Huang and Yu, 2016). Rhodanese is capable of catalysing the transfer of a sulfur atom from a GSH persulfide to sulfite, thereby generating thiosulfate and recovering GSH (Figure 4), as well as the reverse reaction (Hildebrandt and Grieshaber, 2008). Kinetic studies of the enzyme support the view that, under physiological concentrations of substrates, rhodanese preferentially runs in the forward reaction producing thiosulfate (Libiad *et al.,* 2015). However, *in vitro*, it was shown that rhodanese is able to generate H_2S and oxidized GSH in the presence of GSH persulfide and reduced GSH, which may play a significant role in hepatic H_2S production (Libiad *et al.,* 2015).

Deficiencies in cysteine catabolism

In the past, different approaches have been followed to investigate the *in vivo* regulation of cysteine catabolism. Hepatocytes isolated from rats fed with diets of varying sulfur

amino acid content were used to estimate the cysteine-flux *via* the different catabolic pathways. Under the conditions of a basal diet, the majority of metabolized cysteine primarily enters the H_2S pathway. However, with increasing sulfur amino acid intake, the amount of cysteine catabolized *via* the oxidative pathway increases, as demonstrated by an increased CDO activity. Within the oxidative pathway, it is considered that about 60–70% of CSA is converted into taurine (Bagley and Stipanuk, 1995). These correlations were mainly reported for the liver, the most prominent site of cysteine catabolism; however, cysteine flux might differ in other tissues. Besides feeding studies, major insights into the homeostatic regulation of cysteine catabolism were obtained from specific deficiencies in cysteine catabolism leading to rare and severe inborn errors of metabolism (Bagley and Stipanuk, 1995).

Alterations and deficiencies in the oxidative cysteine catabolism

For most enzymes participating in the oxidative cysteine catabolism, there are no human mutations reported, indicating that the respective enzymes might be essential. This hypothesis is supported by different mouse models; for example, CDO, CSAD and SO knockout mice display a high mortality rate (Ueki *et al.,* 2011; Park *et al.,* 2014). For GOT1/2, a mouse model is lacking and the only known human mutation is a heterozygous single nucleotide polymorphism in the *GOT1* gene, leading to a decrease in plasma GOT activity (Shen *et al.,* 2011). Remarkably, no association between the mutation and metabolic traits including alterations in other subclinical markers could be identified. CDO, GOT2 and CSAD have not been associated with any genetic alterations in humans.

Within the oxidative pathway of cysteine catabolism, the only enzyme for which a well-known human disease has been reported is SO (Shih *et al.,* 1977; Schwarz, 2016). SO deficiency can either be caused by mutations in the *SUOX* gene (iSOD) or by mutations in genes (*MOCS1, MOCS2, MOCS3, GPHN*) required for the biosynthesis of Moco (Moco deficiency – MoCD), the active site cofactor of four Mo-containing enzymes. Aside from SO, other Moco-dependent enzymes in mammals include xanthine oxidase, aldehyde oxidase and the mitochondrial amidoxime reducing components 1 and 2 (Schwarz and Belaidi, 2013). On a biochemical level, MoCD and iSOD patients are only distinguishable through accumulation of xanthine and hypoxanthine, as well as the absence of uric acid in MoCD, but not iSOD patients (Sass *et al.,* 2010; Schwahn *et al.,* 2015). MoCD and iSOD patients present in their neonatal period with feeding difficulties, intractable seizures with an exaggerated startle reaction followed by severe neurological abnormalities, lens dislocation and head dismorphism. More than 150 MoCD cases and approximately 40 cases of iSOD have been reported (Schwarz, 2016). Disease progression is accompanied by psychomotor retardation due to progressive cerebral atrophy and ventricular dilatation. Patients that survive the acute neonatal period lack neuronal development and usually die within their first years of life (Schwarz and Veldman, 2014). As most of the symptoms of MoCD are mirrored in iSOD, SO is considered as the most important Moco-dependent enzyme in humans. Therefore, sulfite accumulation presents the primary cause of neurodegeneration in both disorders, although the cytosolic or mitochondrial origin of sulfite remains to be elucidated.

An accumulation of sulfite (0.1–1 mM) has been shown to impair important cellular functions like inhibition of GDH and malate dehydrogenase, decreasing NADH concentrations and mitochondrial membrane potential, and ultimately ATP production (Vincent *et al.,* 2004; Zhang *et al.,* 2004). Sulfite appears to act predominantly on mitochondria, as it was demonstrated that sulfite diminishes mitochondrial respiration, membrane potential and Ca^{2+} retention capacity, presumably by damaging the electron transport chain (Zhang *et al.,* 2004). In line with this, the administration of sulfite leads to increased ROS levels in different neuronal and kidney cell lines (Vincent *et al.,* 2004; Zhang *et al.,* 2004). Furthermore, GDH, a central metabolic enzyme, is inhibited by sulfite in a dose-dependent manner. It has been speculated that inhibition of GDH leads to a decreased flux of the tricarboxylic acid cycle, which might further contribute to the reduced ATP production (Zhang *et al.,* 2004). Within the extracellular space, sulfite can reduce disulfide-bridges of proteins, thus affecting protein folding, stability and activity. Sulfite-dependent cleavage of disulfide-bridges produces S-sulfonated species, such as S-sulfocysteine (SSC) or S-sulfonated transthyretin (Kishikawa *et al.,* 2002).

Given the high plasma concentrations of cystine, the reaction of sulfite with cystine to SSC can be seen as the first scavenging mechanism for sulfite (Figure 5). SSC accumulation is inversely associated with a dramatic reduction in cysteine levels in iSOD and MoCD patients (Belaidi *et al.,* 2012; Schwarz and Belaidi, 2013). While these processes likely take place throughout the body, the local damage in the brain suggests a specific sensitivity of neurons towards those metabolic changes. As a result of low cystine, GSH levels may also be reduced, which particularly impacts neuronal redox homeostasis. While no patient data regarding GSH levels under disease conditions exist, *in vitro* studies show that glutathione disulfide (GSSG), but not GSH, levels were found to be reduced when rat hepatocytes were acutely incubated with high dosages of sulfite (Niknahad and O'Brien, 2008). Furthermore, cystine depletion and glutamate excitotoxicity were found to decrease cellular GSH concentrations (Kato *et al.,* 1992).

SSC is structurally similar to glutamate and – once accumulated in the brain – able to bind to NMDA receptors, likely in the same manner as other amino acids previously discovered to be excitotoxic, including homocysteic acid (Olney *et al.,* 1975). In this context, we have studied SSC-mediated neuronal cell death and found an NMDA receptor-mediated calcium influx followed by calpain activation and proteolytic cleavage of synaptic proteins. Therefore, the accumulation of neuronal SSC represents a major contribution to neurodegeneration in iSOD and MoCD, triggering excitotoxicity (Kumar *et al.,* 2017). Since, under physiological conditions, SO expression and activity in the brain is extremely low compared to the kidney or liver (Belaidi *et al.,* 2015), it remains to be elucidated to what extent SSC formed in the periphery is able to cross the blood–brain barrier or if sulfite derived from the periphery or from the brain reacts with neuronal cysteine (Figure 5).

Besides reduced plasma cysteine/cystine levels under MoCD conditions, a reduction of homocysteine further

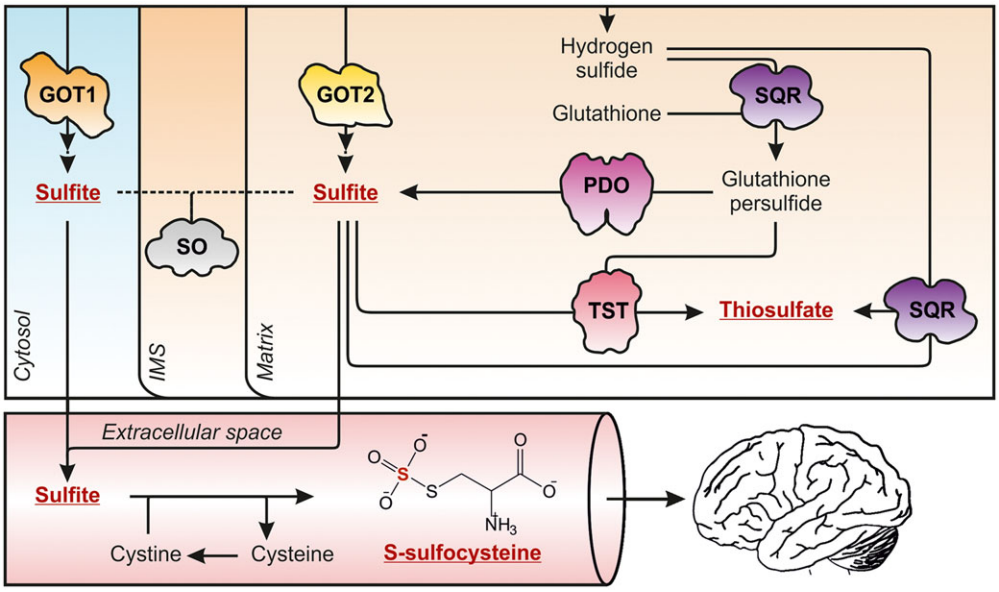

Figure 5

Sulfite excess facilitates the production of S-sulfonates and causes brain damage. Missing SO activity results in the accumulation of cytotoxic sulfite, which diffuses out of the cell into the blood stream where it reduces disulfide bridges to form S-sulfonates. S-sulfocysteine (SSC) is the product of sulfite-dependent cleavage of cystine. Within the brain, SSC is – like its structural homologue glutamate – excitotoxic. To what extent SSC can cross the blood–brain-barrier or is formed in the brain is currently unknown. Under sulfite excess, an alternative reaction of SQR with H_2S and sulfite leads to the formation thiosulfate. GOT1/2, 3II0/5AX8; PDO, 4CHL; SQR, 3H8L; SO, 1SOX; TST, 1RHD.

underlines the severe homeostatic dysregulation caused by sulfite accumulation (Sass *et al.,* 2003). Finally, the observed accumulation of thiosulfate suggests an increased sulfur flux *via* the H_2S pathway (Figures 1 and 5), which we will discuss next.

Metabolic disorders in H_2S metabolism

In humans, several inherited enzyme deficiencies have been associated with H_2S metabolism, which vary in both their presentation and severity (Schwarz and Veldman, 2014). While some known syndromes, such as CSE deficiency, have little to mild symptoms, others, such as *ETHE1* deficiency or MoCD and iSOD, are severe disorders with a mostly lethal outcome. The severity of a given disease is closely related to the respective metabolite(s) that primarily accumulate in the absence of the defective enzyme, while the lack of downstream products is less harmful.

A deficiency of CSE leads to cystathioninuria, sometimes also termed cystathioninaemia, which is described as a benign biochemical anomaly rather than a disease (Mudd *et al.,* 2001). CSE deficiency has no or very diffuse clinical symptoms under normal conditions and is characterized by high plasma levels and urinary excretion of cystathionine. In contrast, an impairment of CBS activity may lead to a more pronounced effect in classical homocystinuria including an increased risk of thrombosis, embolism, skeletal abnormalities, developmental delay and hepatic steatosis. Patients may display drastically increased serum methionine and S-adenosylmethionine as well as homocysteine levels; the severity of the disease is often correlated with the increase in plasma homocysteine levels (Barić and Fowler, 2014). CBS deficiency, however, has been shown to be symptomatically diverse, with differences between pyridoxine-responsive and non-responsive patients, which also influences biomarker levels and has been reviewed elsewhere (Kery *et al.,* 1994; Huemer *et al.,* 2015). For MPST, only very few deficiency or insufficiency cases have been reported. The increased presence of a specific, eponymous biomarker is the primary method for the identification of human mercaptolactate-cysteine disulfidura (Crawhall *et al.,* 1971), with patients displaying either severe phenotypes related to mental retardation and facial abnormalities (Crawhall *et al.,* 1968; Ampola *et al.,* 1969), or displaying essentially no phenotype (Niederwieser *et al.,* 1973). The degree of severity is likely related to the level of insufficiency. Whether the phenotype is a result of the presence of this specific metabolite or due to the reduced production of H_2S during the prenatal period is currently unclear (Nagahara *et al.,* 2013). Furthermore, no symptoms or cases concerning a rhodanese deficiency have been reported, likely due to the fact that the most prominent reaction catalysed by rhodanese – the production of thiosulfate – can also be performed, albeit to a lesser degree, by SQR. The detoxification of cyanide to thiocyanide may also be performed by other rhodanese-domain-containing enzymes like MPST, for which this capacity has indeed been reported (Yadav *et al.,* 2013). Interestingly, rhodanese has been identified as a candidate in obesity resistance, linking sulfur catabolism to other metabolic processes (Morton *et al.,* 2016).

More severe disorders of cysteine catabolism impair reactions related to the detoxification of H_2S. The toxic properties of sulfite and related metabolites have been discussed already. Again, the role of H_2S in disease pathology is complex. On the one hand, several beneficial physiological functions have been described for H_2S including up-regulation of

antioxidant systems, anti-inflammatory and cytoprotective pathways as well as modulation of the nervous system by enhancement of NMDA-receptor-mediated responses (reviewed in Tiranti and Zeviani, 2013). On the other hand, when present in high micromolar concentrations, H_2S has also been reported to interfere with a variety of cellular functions; impaired mitochondrial respiration *via* inhibition of cytochrome *c* oxidase is among the most pronounced phenotypes associated with H_2S toxicity (Tiranti *et al.*, 2009). Exposure to gaseous H_2S can lead to anoxic brain injury, pulmonary oedema and ultimately death in humans (Yalamanchili and Smith, 2008). While brain hypoxia and acute respiratory syndrome are the most likely causes of death after acute H_2S exposure, chronic exposure to low levels leads to irritant effects including upper airway inflammation (Tiranti and Zeviani, 2013).

The most prominent disease associated with an increase in H_2S levels is ethylmalonic encephalopathy (*ETHE1* deficiency) or PDO deficiency. The disease is invariably fatal and characterized by early-onset encephalopathy, chronic diarrhoea, microangiopathy and excretion of ethylmalonic acid in urine (Tiranti *et al.*, 2004). Another molecular hallmark of the disease is H_2S-mediated inhibition of cytochrome c oxidase, which represents a cause of the accumulation of C4- and C5-acylcarnitines. Interestingly, while urinary and tissue thiosulfate levels are increased in *ETHE1*-deficient mice, urinary sulfate levels are decreased sixfold (Tiranti *et al.*, 2009) underscoring a major flux of sulfur *via* the H_2S pathway. The strong accumulation of thiosulfate suggests an increased flux of sulfur *via* the thiosulphate sulfurtransferase (TST) enzyme due to the accumulation of persulfidated GSH.

More recently, coenzyme Q deficiency, a classical mitochondrial disorder, has been added to the list H_2S-related disorders, as it was found to negatively affect the activity of the H_2S-metabolizing enzyme SQR (Ziosi *et al.*, 2016; Luna-Sánchez *et al.*, 2017). While no direct deficiency of SQR has been described for humans, an impaired production of coenzyme Q, the cofactor of SQR, appears to correlate with low SQR protein levels (Kühl *et al.*, 2017). Coenzyme Q deficiency causes a variety of severe symptoms including encephalomyopathy, severe infantile multi-systemic disease, cerebellar ataxia, isolated myopathy and steroid-resistant nephrotic syndrome (Desbats *et al.*, 2015). The pathogenesis is complex, which is likely related to the different functions of coenzyme Q. Consistent with the reduced enzymatic capacity of SQR, kidney H_2S levels are increased whereas urinary thiosulfate levels are decreased (Ziosi *et al.*, 2016). Interestingly, kidney levels of enzymes downstream of SQR were also reduced, suggesting a regulatory mechanism that controls expression of H_2S-catabolizing enzymes in general (Ziosi *et al.*, 2016).

The homeostatic link between oxidative and H_2S-dependent cysteine catabolism in health and disease

Both the oxidative cysteine catabolism and the H_2S pathway converge in the formation of sulfite as a terminal intermediate leading to the formation of sulfate and thiosulfate. Therefore, the accumulation of sulfite, caused by iSOD/MoCD, impacts on metabolite and protein levels of both branches of cysteine catabolism in multiple ways.

The scavenging reaction of sulfite with cystine results in a depletion of cystine. As a result, cellular cysteine is reduced as found under conditions where the cystine/glutamate transporter **xCT** if deficient, causing a reduction in plasma GSH concentrations (Sato *et al.*, 2005). To what extent GSH levels are reduced in patients of iSOD and MoCD remains to be determined. Besides the multiple functions in redox signalling, mitochondrial GSH levels also control the rate of H_2S catabolism. Therefore, a sulfite-dependent depletion of GSH should ultimately limit the capacity of mitochondria to oxidize H_2S. In addition to sulfite-dependent inhibition of mitochondrial respiration, it is reasonable to assume that increasing H_2S levels further exaggerates metabolic deficits in iSOD and MoCD.

Mitochondria play a central and critical role in cysteine metabolism in health and disease. On the one hand, reducing equivalents derived from oxidative reactions in both H_2S and sulfite oxidation contribute to mitochondrial respiration (Figure 3). On the other hand, dysfunctions in sulfite and H_2S oxidation severely impact mitochondrial function and thereby contribute to mitochondria-driven signalling and cell death (Zhang *et al.*, 2004; Tiranti *et al.*, 2009). Sulfite-induced mitochondrial damage might be associated with increased taurine levels in iSOD/MoCD patients (Belaidi and Schwarz, 2013). The primary source of taurine biosynthesis under healthy conditions is CSA, which is predominantly produced under cysteine excess. However, since iSOD/MoCD patients display very low cystine/cysteine levels, an alternative pathway for taurine must be upregulated. Hypotaurine can also be produced by cysteamine dioxygenase (ADO) using cysteamine as a substrate (Dominy *et al.*, 2007). However, studies using $CDO^{-/-}$ or $CSAD^{-/-}$ mice show that ADO only accounts for about 10% of overall taurine biosynthesis under normal conditions (Ueki *et al.*, 2011; Park *et al.*, 2014). One possibility is that the degradation of coenzyme A to cysteamine is increased under mitochondrial stress (Robishaw and Neely, 1985) given that mitochondrial function has been shown to be impaired under sulfite stress (Salman *et al.*, 2002; Zhang *et al.*, 2004). As coenzyme A concentrations are in the low millimolar range, an increased degradation of this important coenzyme, which involves the release of cysteamine and its further deamination by ADO, might represent a source of increased taurine production in iSOD/MoCD patients.

Another aspect of iSOD is the accumulation of thiosulfate, suggesting an increased flux of accumulated sulfite *via* rhodanese towards thiosulfate (Touati *et al.*, 2000). As previously discussed, the production of thiosulfate may be accomplished by both SQR and rhodanese. One line of argument is that an increased thiosulfate production might also deplete persulfidated GSH, the product of the SQR-dependent oxidation of H_2S and a possible substrate for rhodanese (Hildebrandt and Grieshaber, 2008). The other is that increased thiosulfate excretion could indicate an increased H_2S flux, which is used in the SQR-dependent production of thiosulfate (Jackson *et al.*, 2012; Libiad *et al.*, 2014). Both hypotheses suggest that iSOD might cause a reduction in H_2S levels, and therefore, some aspects of the disease symptoms may be associated with reduced H_2S production. The SQR-dependent

thiosulfate production may gain further importance in iSOD when considering the reduced plasma cystine levels in iSOD patients (Touati *et al.,* 2000; Rocha *et al.,* 2014), since cysteine/cystine levels regulate GSH homeostasis (Yu and Long, 2016). With a K_M for sulfite similar to SO (Jackson *et al.,* 2012), a reduction in its primary substrate GSH would likely cause a switch from the production of persulfidated GSH to the production of thiosulfate (Figure 5), which further supports the view of a depletion of H_2S in iSOD/MoCD.

An alternative scenario could also explain high thiosulfate levels: the low concentration of cystine in iSOD patients (Touati *et al.,* 2000; Rocha *et al.,* 2014) may trigger an up-regulation of the transsulfuration pathway *via* CSE (Yu and Long, 2016; Paul *et al.,* 2018), which is an important factor in H_2S biosynthesis. As a result, high H_2S levels would contribute to increased thiosulfate production under sulfite excess. In conclusion, the complex and fast progressing neurodegenerative phenotype in iSOD and MoCD needs an out-of-box analysis of the entire cysteine metabolism to understand the hierarchical order of degenerative events. To what extent H_2S accumulation or depletion contributes to iSOD/MoCD pathophysiology needs further investigation using SO-deficient animal models.

Thiosulfate accumulation is also a hallmark of *ETHE1* deficiency. Loss of PDO activity in these patients leaves TST as the sole GSH persulfide degrading enzyme. The observed accumulation of thiosulfate demonstrates an increased flux of H_2S towards thiosulfate, accompanied by a loss in sulfate excretion (Tiranti *et al.,* 2009), suggesting that sulfite is primarily incorporated into thiosulfate. The fact that H_2S accumulates in *ETHE1* deficiency suggest that either TST activity or sulfite availability limits GSH persulfide turnover. Given that *N*-acetylcysteine supplementation partially suppresses the phenotype, an increased availability of GSH would allow more H_2S to be scavenged (Viscomi *et al.,* 2010). An alternative explanation might be that increased cysteine levels down-regulate CSE activity, thus producing less H_2S and therefore less thiosulfate.

Treatment options for sulfite and H_2S toxicity disorders

iSOD is currently a non-treatable disorder. In some cases, dietary restriction in the intake of methionine and cysteine showed therapeutic effectiveness in patients with mild phenotypes. For example, a patient harbouring a mutation in the SO N-terminal haem domain (H143N) presented at the age of 12 months with acute left hemiparesis, generalized mild hypotonia and developmental delay. Biomarkers (cystine, SSC and taurine) were typical for iSOD. The introduction of a low-protein diet with a sixfold reduction in methionine and cysteine intake triggered a sustained reduction of pathological biomarkers (SSC, taurine), followed by clinical improvement, partial shrinking of lesions in the basal ganglia and achievements of additional motor milestones (Del Rizzo *et al.*, 2013). Given the fact that the H143A mutation – affecting one of the two haem-coordinating histidines in SO – does not affect the catalytic Moco domain, one can speculate that in the absence of a functional haem domain, the catalytic cycle of SO is closed by the use of O_2 as alternative electron acceptor (Belaidi *et al.,* 2015), thus retaining a partial capacity to reduce excess sulfite. The accompanied formation of ROS might however accumulate over time and cause other deficits.

MoCD represents another form of iSOD, accompanied by the loss of all Mo-enzymes (Schwarz *et al.,* 2009). Both disease symptoms and progression are highly similar suggesting iSOD as major disease-causing mechanism in MoCD, which was also mirrored by SO-deficient mice. For a subgroup of MoCD patients that carry mutations in the first biosynthetic step of Moco (*MOCS1*, MoCD type A), a substitution therapy with the Moco-intermediated cyclic pyranopterin monophosphate (cPMP) has been established in mice (Schwarz *et al.,* 2004) and translated to a first patient (Veldman *et al.,* 2010). In the following years, more than 20 MoCD patients have been administered cPMP showing that initiation of treatment before the manifestation of neurological symptoms has been a prerequisite for treatment success and near normal development (Schwahn *et al.,* 2015). Therefore, patient diagnosis and symptomatic treatment of SSC-evoked seizures by use of NMDA receptor blockers is considered as key to extend the window of treatability to improve clinical outcome for these patients (Kumar *et al.,* 2017).

Beside dietary restriction of methionine and cysteine in mild forms of iSOD and MoCD, no other treatment options are available today. Based on the homeostatic impact of sulfite and H_2S on cysteine catabolism discussed here, additional treatment strategies need to be considered. We suggested earlier that an enzyme substitution therapy with SO might be an option; however, the production of H_2O_2 as a side product of SO-mediated sulfite oxidation in the presence of oxygen is challenging (Belaidi *et al.,* 2015). Alternatively, to reduce sulfite accumulation and cysteine depletion, blocking CDO activity or GOT1/2 activity might shift the flux of sulfur into H_2S pathways. Considering the scenarios mentioned above, this approach would work, if H_2S depletion would accompany iSOD. Crossing CDO and SO-deficient mice to create double-knockout animals may answer this question, if additional taurine supplementation can be warranted. Alternatively, the scavenging of excess sulfite would restore cystine/cysteine and regenerate the intracellular GSH pool as a primary antioxidative barrier.

Similar to iSOD/MoCD, thiosulfate is also accumulated in *ETHE1* (PDO) deficiency. As discussed above, *N*-acetylcysteine treatment diminished the disease phenotype, suggesting a decrease in H_2S formation. An alternative approach towards the productive elimination of excess H_2S in *ETHE1* deficiency might be an increase in sulfite availability by reducing SO activity. Therefore, crossing $SO^{-/-}$ and $ETHE1^{-/-}$ mice, if viable, could answer this question. In the case of a positive outcome, further increased thiosulfate formation would be expected. From a treatment point of view, the administration of tungstate is well known to inhibit SO activity in a dose-dependent manner (Kumar *et al.,* 2017).

Concluding remarks

Altered sulfite and H_2S metabolism is associated with various types of neurodegenerative disorders ranging from rare inborn errors in metabolism to complex neurodegenerative disorders such as Huntington's, Parkinson's and Alzheimer's disease (McBean *et al.,* 2015; Salmina *et al.,* 2015). The

recognized dramatic impact of sulfite, the common intermediate in oxidative cysteine and H_2S catabolism on the overall homeostatic balance of cysteine, GSH, homocysteine and thiosulfate, collectively suggests an important effect of sulfite on H_2S levels and H_2S-related signalling such as protein persulfidation. Therefore, future studies focusing on metabolic fluxes under different nutrient conditions and enzymatic activities will help to develop a better understanding of which thiol-species contribute to a particular aspect of a given type of neurodegenerative disorder. Studying rare, mostly severe, metabolic disorders will allow us to extrapolate common principle from extreme cases towards multifactorial disorders. In this context, the beneficial effect of dietary restriction in general and low sulfur diet more specifically suggests the key importance of a tight control of cysteine, H_2S, sulfite and GSH persulfide levels for the homeostatic balance in cell signalling and survival.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b).

Acknowledgements

Continues support for our research (G.S.) by the German Science Foundation (DFG) is gratefully acknowledged. Current projects on cysteine catabolism are supported by DFG grants SFB1218-B08 (to G.S.) and Center for Molecular Medicine Cologne grant C13 (to G.S.).

Conflict of interest

G.S. is inventor on a patent for the treatment of molybdenum cofactor deficiency type A using cPMP and CEO of Colbourne Pharmaceuticals GmbH that consults Alexion Pharma in the clinical development of cPMP therapy.

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