British Journal of Pharmacology

British Journal of Pharmacology (2019) **176** 583–593 583

Themed Section: Chemical Biology of Reactive Sulfur Species

REVIEW ARTICLE

Regulators of the transsulfuration pathway

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Received 16 February 2018; **Revised** 19 June 2018; **Accepted** 30 June 2018

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The transsulfuration pathway is a metabolic pathway where transfer of sulfur from homocysteine to cysteine occurs. The pathway leads to the generation of several sulfur metabolites, which include cysteine, GSH and the gaseous signalling molecule hydrogen sulfide (H_2S). Precise control of this pathway is critical for maintenance of optimal cellular function and, therefore, the key enzymes of the pathway, cystathionine β-synthase and cystathionine γ-lyase, are regulated at multiple levels. Disruption of the transsulfuration pathway contributes to the pathology of several conditions such as vascular dysfunction, Huntington's disease and during ageing. Treatment with donors of hydrogen sulfide and/or stimulation of this pathway have proved beneficial in several of these disorders. In this review, we focus on the regulation of the transsulfuration pathway pertaining to cysteine and H_2S, which could be targeted to develop novel therapeutics.

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Abbreviations

MPST, 3-mercaptopyruvate sulfurtransferase; ATF4, activating transcription factor 4; CDO, cysteine dioxygenase; CSE, cystathionine γ-lyase; FXR, farnesoid X receptor; GH, growth hormone; GPABR1, GPCR for secondary bile acids; H_2S, hydrogen sulfide; HD, Huntington's disease; HO2, haem oxygenase 2; Keap1, kelch-like ECH-associated protein 1; mHtt, mutant huntingtin; NMDA, N-methyl D-aspartate; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PLP, pyridoxal 5′-phosphate; SAM, S-adenosyl-methionine; SP1, specificity protein 1

DOI:10.1111/bph.14446

Introduction

The transsulfuration pathway plays a central role in sulfur metabolism and redox regulation in cells. In mammals, the pathway involves the transfer of sulfur from **homocysteine** to **cysteine** *via* **cystathionine** and is the only route for biosynthesis of cysteine (Figure 1). Homocysteine, which is derived from dietary methionine, is converted to cystathionine by **cystathionine β-synthase (CBS)**, which is acted on by **cystathionine γ-lyase (CSE)** to generate cysteine. In prokaryotes, fungi and plants, the pathway can operate in the opposite direction to synthesize methionine from cysteine and was initially referred to as the transsulfuration pathway, whereas in mammals, it was designated as the reverse transsulfuration pathway (Steegborn *et al.*, 1999; Wang, 2012). For the sake of clarity, this review will refer to the pathway in mammals as the transsulfuration pathway as the conversion from cysteine to methionine does not occur here.

In addition to its essential role in protein synthesis, cysteine is also a component of the major antioxidant **GSH** and a potent antioxidant itself. Disruption of cysteine and GSH metabolism has been frequently linked to aberrant redox homeostasis and neurodegeneration (McBean *et al.*, 2015; Paul *et al.*, 2018). Cysteine and GSH play central roles in maintaining thiol redox balance in the brain with the

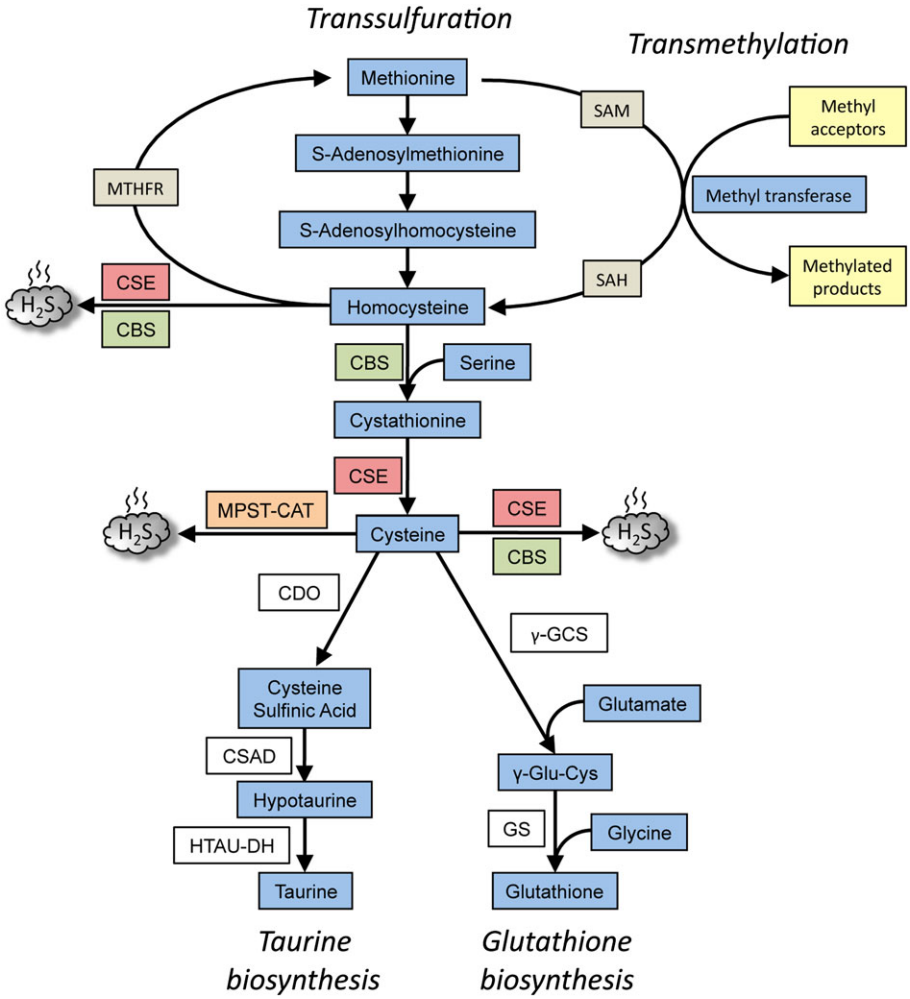

Figure 1

Overview of the transsulfuration pathway. The pathway results in the generation of cysteine from homocysteine, which in turn, is derived from dietary methionine in mammals. CBS condenses homocysteine with serine to generate cystathionine, the substrate for CSE, to generate cysteine. CSE can generate H_2S from either cysteine or homocysteine. While CSE can utilize cysteine to generate H_2S, CBS uses a combination of cysteine and homocysteine to generate H_2S. A third H_2S-generating enzyme, MPST, in conjunction with cysteine amino acid transferase (CAT) utilizes cysteine to generate H_2S. The transsulfuration pathway intersects with the transmethylation pathway at homocysteine, which can be remethylated back to methionine by N5,N10-methylenetetrahydrofolate reductase (MTHFR). The cysteine generated by the pathway can be chanelled into GSH synthesis by the action of the enzymes, γ-glutamyl cysteine synthetase (γ-GCS) and glutathione synthetase (GS) or converted to other sulfur-containing molecules such as taurine. Taurine is generated by the action of three enzymes, CDO, cysteine sulfinic acid decarboxylase (CSAD) and hypotaurine dehydrogenase (HTAU-DH).

transsulfuration pathway being a major source of cysteine in astrocytes (McBean, 2012; McBean, 2017). Both CSE and CBS play important roles in the regulation of redox balance. It has been reported that approximately 50% of the cysteine generated by the transsulfuration pathway is utilized for GSH biosynthesis in hepatic cells (Mosharov *et al.*, 2000; Banerjee and Zou, 2005). In the mouse brain, the activity of the pathway is lower as compared to the liver, but the flux can be increased by oxidative stress (Vitvitsky *et al.*, 2006; Diwakar and Ravindranath, 2007). Cysteine is also the precursor of the gaseous signalling molecule **hydrogen sulfide** (H_2S) and other sulfur metabolites (Paul and Snyder, 2012; Paul and Snyder, 2015a; Paul and Snyder, 2015b). Besides GSH and H_2S, cysteine is converted to the sulfur containing molecule **taurine** by the action of the enzyme cysteine dioxygenase (CDO) to form **cysteinesulfinic acid**, which can then be decarboxylated to hypotaurine by cysteinesulfinic acid decarboxylase, and the hypotaurine generated, oxidized to taurine (Stipanuk and Ueki, 2011) (Figure 1). Since CDO acts directly on cysteine, it can modulate H_2S production by influencing substrate availability. Mice lacking CDO have elevated cysteine and H_2S production capacity (Jurkowska *et al.*, 2014; Rose *et al.*, 2017). Taurine plays a role in osmoregulation, immunomodulation, neuromodulation, Ca^{2+} homeostasis, ocular function and possesses antioxidant and anti-inflammatory effects (Schaffer and Kim, 2018). An intact taurine biosynthetic pathway responsive to hypertonic conditions has been demonstrated in neurons, consistent with its role in osmoregulation (Vitvitsky *et al.*, 2011).

This pathway is intimately linked to the transmethylation pathway *via* homocysteine, which can be remethylated to generate methionine or be irreversibly converted to cysteine (Figure 1). This article will focus on the regulation of the transsulfuration pathway pertaining to cysteine and H_2S metabolism and its role during normal and pathological conditions.

Enzymes of the transsulfuration pathway

CSE and CBS, the key enzymes regulating the flux through the transsulfuration pathway, are regulated at multiple levels. CBS (also called l-serine hydrolyase) catalyses the first committed step of transsulfuration by condensing serine with homocysteine to generate cystathionine in a β-replacement reaction. CBS is believed to function predominantly in the nervous system, although it is active in the periphery in the liver and kidneys. CBS is a cytosolic homotetrametic enzyme of about 63 kDa subunits that binds two cofactors, **pyridoxal 5′-phosphate** (PLP) and haem (Meier *et al.*, 2001). The enzyme is activated allosterically by S-adenosyl-methionine (SAM) and also by a cleavage at its carboxyl terminal at R413 to generate a 45 kDa monomer, which is not only twice as active as the full length form but also refractory to SAM-mediated activation (Kery *et al.*, 1998; Majtan *et al.*, 2014). CBS can also utilize cysteine instead of serine to generate H_2S and cystathionine. In addition, CBS can act on two molecules of cysteine to produce H_2S. However, the preferred substrates for generation of H_2S by CBS are cysteine and homocysteine. CSE can use homocysteine alone to generate H_2S and is responsible for the clearance of homocysteine under conditions of hyperhomocysteinemia, unlike CBS which is unresponsive under these conditions (Chen *et al.*, 2004; Singh *et al.*, 2009). Over 150 mutations in the CBS protein have been reported, of which several, especially those causing misfolding, are linked to enzyme activity (Kozich *et al.*, 2010). CBS mice rarely live past two weeks and exhibit a variety of abnormalities, which include growth retardation, severe hepatopathy, vascular abnormalities, dislocation of the eye-lens and skeletal deformities (Watanabe *et al.*, 1995; Kruger, 2017). Human subjects with CBS mutations exhibit several neurological deficits including anxiety, depression, obsessive–compulsive behaviour and psychosis (Abbott *et al.*, 1987).

CSE, encoded by the gene *Cth*, also known as γ-cystathionase, cysteine lyase, cysteine desulfhydrase, cystathionase, cystathioninase, cystine desulfhydrase, homoserine deaminase or homoserine dehydratase, is the second enzyme in the pathway. CSE utilizes cystathionine generated by CBS to generate cysteine, a semi-essential amino acid. As CSE is the only enzyme that can directly generate cysteine *de novo* in mammals, its depletion is deleterious (Ishii *et al.*, 2010; Mani *et al.*, 2011). In AIDS patients, the very low levels of CSE render cysteine an essential amino acid (Martin *et al.*, 2001). In addition to loss of CSE function, loss of CBS also leads to lowered cysteine synthesis in cells (Gupta and Kruger, 2011). CSE is predominantly expressed in the periphery, although it is now recognized to be functional in the brain with neuroprotective roles (Vitvitsky *et al.*, 2006; Diwakar and Ravindranath, 2007; Paul and Snyder, 2012; Paul *et al.*, 2014; Paul and Snyder, 2018). The crystal structure of human CSE revealed that the enzyme is a homotetramer with each subunit being 45 kDa, and with the cofactor PLP bound to each subunit (Sun *et al.*, 2009). PLP interacts with the carboxylate of Asp^{187} of CSE *via* hydrogen bonding and mutation of this residue to Ala abolishes catalytic activity, as assessed by H_2S production. Unlike CBS, which is constitutively expressed, CSE is inducible by a wide variety of stimuli ranging from oxidative and endoplasmic reticulum stress to nutrient deprivation. CSE, like CBS, can also generate H_2S. In the liver, CSE is the dominant enzyme for generation of H_2S although CBS is also expressed at high levels (Kabil *et al.*, 2011b). Loss of CSE can lead to oxidative stress, aberrant stress responses, vascular deficits and hyperhomocysteinemia (Yang *et al.*, 2008; Sbodio *et al.*, 2016; Sbodio *et al.*, 2018). The third enzyme that produces H_2S, **3-mercaptopyruvate sulfurtransferase (MPST)**, is part of the cysteine catabolic pathway, generating the gaseous signalling molecule in concert with cysteine amino acid transferase (Shibuya *et al.*, 2009).

Signalling by H_2S

H_2S exerts its effects on cellular physiology in several ways. Similar to **NO**, H_2S can modify reactive cysteine residues on proteins, a post-translational modification termed sulfhydration or persulfidation, analogous to nitrosylation mediated by NO. In the case of persulfidation, –SH groups of

reactive cysteine residues are converted to persulfide or –SSH groups (Mustafa *et al.*, 2009; Paul and Snyder, 2015a; Filipovic *et al.*, 2018). Sulfhydration is a physiological modification that is reversible by endogenous reductants, such as the thioredoxin system (Krishnan *et al.*, 2011; Doka *et al.*, 2016). Sulfhydration is more prevalent than nitrosylation with about 50% of hepatic proteins being sulfhydrated (Mustafa *et al.*, 2009). In several instances, sulfhydration and nitrosylation can target the same cysteine residue but have opposite effects. For example, the glycolytic enzyme GAPDH is activated by persulfidation but inhibited by nitrosylation (Hara *et al.*, 2005; Mustafa *et al.*, 2009). Thus, mice lacking CSE have reduced levels of sulfydration and lower GAPDH activity. Sulfhydration can modulate several physiological processes including responses to inflammatory stimuli, oxidative stress, neuronal signalling pathways and vasodilatation (Mustafa *et al.*, 2011; Paul and Snyder, 2012; Sen *et al.*, 2012; Vandiver *et al.*, 2013; Yang *et al.*, 2013).

H_2S also has roles independent of sulfhydration, for example, in cellular bioenergetics. At lower concentrations (0.1–1 μM), the H_2S donor NaHS can stimulate electron transport in rat isolated liver mitochondria, whereas higher concentrations (3–30 μM) suppress mitochondrial activity (Modis *et al.*, 2013). H_2S can directly donate electrons to the mitochondrial electron transport chain (ETC) at complex II and increase mitochondrial cAMP, whereas at higher concentrations, it disrupts the ETC by inhibiting mitochondrial cytochrome c oxidase (Szabo *et al.*, 2014). Another mode by which H_2S acts is by binding to metal centres of metalloenzymes (Pietri *et al.*, 2011). One of the several haem proteins acted on by H_2S is GC C, where a reduction in the ferric (Fe^{3+}) haem to the ferrous (Fe^{2+}) form enhances its interaction with NO, which activates the enzyme (Zhou *et al.*, 2016). H_2S can also interface with NO signalling by forming nitrosothiols, which act as signalling molecules (Whiteman *et al.*, 2006; Filipovic *et al.*, 2012).

Regulation of the transsulfuration pathway at the post-translational level

As H_2S and cysteine participate in a wide variety of physiological processes, precise control of their production is critical for the maintenance of optimal cellular function. Thus, it is not surprising that the transsulfuration pathway is subject to regulatory controls at multiple levels. Both CBS and CSE undergo several post-translational modifications that can alter their enzymatic activity or sub-cellular localizations (Figure 2). One of the less studied aspects of the transsulfuration pathway is the elucidation of conditions leading to cysteine synthesis by CSE as opposed to the production of H_2S, GSH or taurine. The haem in CBS plays a key role in switching the transsulfuration pathway from cysteine production to H_2S generation (Kabil *et al.*, 2016). When haem is bound by endogenous ligands, the production of cystathionine occurs due to the higher intracellular levels of serine and its greater affinity for CBS as compared to cysteine. Similarly, the affinity of cystathionine for CSE is greater than that of cysteine, favouring the generation of cysteine as opposed to H_2S. During conditions of stress, when NO or **carbon monoxide** (CO) levels rise, causing inhibition of CBS due to the formation of nitrosyl or ferrous carbonyl CBS, homocysteine accumulates, leading to the production of H_2S by CSE (Banerjee, 2017). Enzymes which catabolize cysteine also have an effect on the production of H_2S.

Regulation of CBS

The haem cofactor in the N-terminal domain of CBS can bind CO or NO, which can inhibit its activity (Singh *et al.*, 2007). This inhibition has relevance in the regulation of cerebral microvasculature. During normoxia, CO produced by the O_2-dependent enzyme **haem oxygenase 2 (HO2)** binds to the haem of CBS and keeps the enzyme suppressed. HO2 is localized to the endothelial cells of the cerebral vasculature,

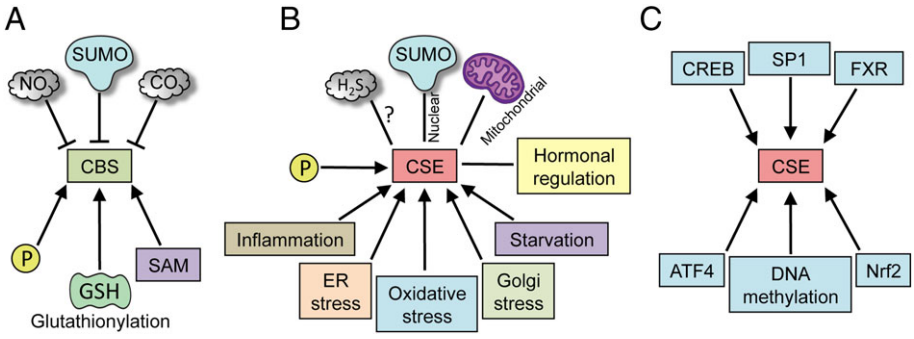

Figure 2

Regulation of CSE and CBS. CBS and CSE are regulated at multiple levels. (A) Post-translational regulation of CBS. CBS is a constitutively expressed enzyme and can be regulated by several post-translational modifications (PTMs) such as sumoylation, phosphorylation and glutathionylation. It is allosterically regulated by SAM, which activates it and increases its stability. The haem centre in CBS can bind NO and CO. (B) CSE is a highly inducible protein, which is regulated by a wide variety of stimuli such as inflammation, ER stress (which can cause translocation to the mitochondria), oxidative stress and Golgi stress, starvation and by hormones. CSE is also modified by PTMs such as phosphorylation, sulfhydration and sumoylation (which may cause translocation to the nucleus). (C) Unlike CBS, whose activity in the cell is regulated predominantly by PTMs, the effects of CSE are exerted mainly by regulation of its expression at the transcriptional level. Several transcription factors, such as the cAMP-response element binding protein (CREB), SP1, FXR, activating transcription factor 4 and Nrf2, have binding sites on the CSE promoter. In addition, the expression of CSE is also regulated by methylation of its promoter.

which is in close proximity to the astrocytes where CBS is localized (Enokido *et al.*, 2005; Morikawa *et al.*, 2012). During hypoxia, when O_2 becomes limiting, activity of HO2 and CO production drops, relieving the inhibition on CBS, which can then produce H_2S to mediate arteriolar vasodilation (Morikawa *et al.*, 2012). Although it is not known whether the CO mediating CBS inhibition originates from the neuronal or endothelial pools HO2, it is evident that two gaseous signalling molecules interact to modulate a major cerebral function. Another mode by which CBS can be modulated is by phosphorylation. For instance, phosphorylation of Ser^{227} of human CBS in human urothelium occurs in a cGMP/PKG-dependent manner to increase H_2S production (d'Emmanuele di Villa Bianca *et al.*, 2016). The activity of CBS can also be modulated allosterically by SAM, a component of the transmethylation pathway (Ereno-Orbea *et al.*, 2014). In addition, SAM stabilizes CBS. Methionine restriction leads to a significant decrease in CBS protein levels due to decrease in SAM concentrations and destabilization of CBS (Prudova *et al.*, 2006). CBS has also been reported to be a substrate for sumoylation by the E3 SUMO ligase, human polycomb group protein 2, which decreases its activity (Agrawal and Banerjee, 2008). However, glutathionylation stimulates the activity of human CBS (Niu *et al.*, 2015). CBS activity increases the flux *via* the transsulfuration pathway, which leads to GSH production, which becomes oxidized and depleted during oxidative stress. Thus, it seems reasonable that CBS would be modified by GSH to stimulate production of cystathionine, the precursor for cysteine, whose availability governs the rate of GSH production (Figure 2A).

Regulation of CSE

Like CBS, CSE can also be regulated by post-translational modifications. Phosphorylation sites have been reported on CSE, which modulates its activity. In response to bile acid receptor activation, CSE is phosphorylated *via* the Akt pathway, which increases its catalytic activity (Renga *et al.*, 2015). CSE has been reported to be sumoylated, which has been speculated to be responsible for its nuclear localization (Agrawal and Banerjee, 2008). Apart from induction, translocation of CSE from the cytosol to other cellular compartments such as mitochondria in response to stress has been observed, adding an additional layer of regulation to the regulation of H_2S production (Fu *et al.*, 2012). Sulfhydration of CSE itself has been reported by our laboratory; however, the significance of the modification is yet to be explored (Mustafa *et al.*, 2009) (Figure 2B).

Regulation of the transsulfuration pathway at the transcriptional level

Between the two enzymes, CSE and CBS, CSE is highly inducible and is regulated in response to a wide variety of extrinsic and intrinsic signals. CSE is induced by oxidative stress, ER stress, Golgi stress, mitochondrial stress, inflammation and starvation, among several others (Harding *et al.*, 2003; Kandil *et al.*, 2010; Sen *et al.*, 2012; Sbodio *et al.*, 2016; Sbodio *et al.*, 2018) (Figure 2B). Expression of CSE is regulated by the specificity protein 1 (SP1) under basal conditions (Yang *et al.*, 2011; Zhang *et al.*, 2011).

CSE is a highly inducible protein and can be regulated by several factors depending on the cell type and tissue (Figure 2C). In the liver, the bile-acid-activated **farnesoid X receptor** (FXR) regulates CSE by binding to the CSE promoter thereby regulating H_2S production and hepatic microcirculation (Renga *et al.*, 2009). FXR binds the sequence, AGTTCAgTGTACCT, with two inverted repeats separated by one nucleotide and increases CSE expression. These studies showed that mice lacking FXR display decreased levels of CSE and H_2S in the systemic circulation. The GPCR for secondary bile acids, GPABR1, activates the cAMP/PKA pathway, which results in the phosphorylation of the cAMP response element binding protein and binding to the CSE promoter and increased transcription of CSE. Agonists of GPABR1 reverse the vasoconstriction induced by noradrenaline and methoxamine on isolated rat livers *via* H_2S production. Inhibiting CSE activity with **propargylglycine** reverses these effects. CSE is also regulated during inflammation, for example, during LPS stimulation of macrophages, which leads to increased association of SP1 with the CSE promoter. This leads to elevated H_2S production and sulfhydration of the p65 subunit of the transcription factor NF-κB and increases its recruitment to promoters of anti-apoptotic genes (Sen *et al.*, 2012). Apart from inflammation, CSE is inducible by oxidative stress and the CSE promoter harbours a binding site for the master regulator of oxidative stress response, nuclear factor (erythroid-derived 2)-like 2 (Nrf2) (Martin *et al.*, 2007; Hassan *et al.*, 2012; Guo *et al.*, 2014). In addition, Nrf2 function is also regulated by H_2S produced by CSE. Treatment of cells with NaHS, the H_2S donor, improves the stability of Nrf2 and also sulfhydrates **Kelch-like ECH-associated protein 1** (Keap1), the repressor of Nrf2 (Hourihan *et al.*, 2013). An independent study also reported sulfhydration of Keap1 leading to Nrf2 activation (Yang *et al.*, 2013). Nrf2 has been reported to have binding sites, which function as antioxidant response elements, with the sequences, 5′-GTGATCTAGCA-3′ and 5′-ATGAGG CAGCT-3′, for the upstream regions of CBS and CSE respectively (Hassan *et al.*, 2012; Hourihan *et al.*, 2013). Thus, Nrf2 can elevate the expression of both CSE and CBS. The induction of CSE by oxidative stress is not surprising as it is a central part of a pathway that leads to the synthesis of two major antioxidants, cysteine and GSH, in addition to H_2S, which can mediate the antioxidant stress response *via* sulfhydration of proteins involved in this response (Yang *et al.*, 2013).

Another stress stimulus that up-regulates CSE expression and H_2S production is nutrient restriction and amino acid starvation. The transcription factor involved in response to amino acid starvation is activating transcription factor 4 (ATF4), which binds to the CSE promoter in addition to promoters of other amino acid biosynthetic and transport genes (Harding *et al.*, 2003).

The expression of CSE can also be modulated by several hormones. In the long-lived Ames dwarf mice, which have reduced growth hormone (GH) and thyroid-stimulating hormone signalling, as well as in mice lacking GH receptors, CSE and CBS levels are elevated and H_2S production is increased. GH and TH negatively regulate hepatic H_2S

production through distinct mechanisms involving the hypothalamic–pituitary axis (Hine *et al.*, 2017).

In addition to regulation by various transcription factors, the CSE promoter has been found to be controlled by DNA methylation wherein cytosine is modified to 5-methylcytosine (Giannakopoulou *et al.*, 2017) (Figure 2C). Several CpG islands (regions rich in cytosine and guanosine residues) have been identified in the CSE promoter, which are hypermethylated in patients with coronary artery disease. Interestingly, there was a gender-specific difference in methylation status, with hypermethylation being observed only in males. These patients exhibited a decrease in H_2S production and increase in cytosine methylation of the *Cth* promoter.

Dysregulation of the transsulfuration pathways and therapeutic opportunities

As the transsulfuration modulates several physiological processes, its dysregulation can lead to deleterious effects. The levels of H_2S are highly regulated during normal cellular processes with controls operating at multiple levels as discussed above. Too much and too little H_2S production can have damaging consequences for cells. Thus, the dose–response of H_2S can explain its variable effects reported in literature. During ageing and other pathophysiological conditions, the fine balance of H_2S can be upset. A few of the conditions associated with a dysregulated transsulfuration pathway are described below.

Vascular abnormalities

Like NO, H_2S plays an important role in the vascular system with effects on BP, vasorelaxation and angiogenesis (Szabo, 2017; Gheibi *et al.*, 2018). Lower H_2S production has been associated with myocardial ischaemia, angina and cardiovascular disease (Polhemus *et al.*, 2014). Depletion of CSE and H_2S causes a range of vascular deficits ranging from hypertension and impaired vasorelaxation to impaired angiogenesis (Yang *et al.*, 2008; Coletta *et al.*, 2012; Cindrova-Davies *et al.*, 2013). The cGMP signalling pathway, which plays a central role in vasodilatation and smooth muscle relaxation, is disrupted in mice lacking CSE. H_2S can not only inhibit **PDEs** which degrade cGMP but also stimulate GCs which synthesize cGMP. Several of the PDE inhibitors, such as sildenafil citrate and tadalafil, which are currently marketed to treat erectile dysfunction and cardiovascular deficits, stimulate H_2S production (Salloum *et al.*, 2009; Fusco *et al.*, 2012). In addition to the generation of H_2S, CSE and CBS can also be considered to be homocysteine-clearing enzymes. Depletion of both CBS and CSE causes hyperhomocystenaemia, which is an independent risk factor for developing cardiovascular deficits and neurodegenerative disorders such as Alzheimer's disease (Mattson and Shea, 2003; Smith *et al.*, 2018). Thus, in addition to generating cysteine, the transsulfuration pathway utilizes homocysteine, whose accumulation can be harmful, by activating the **NMDA receptors** causing excitotoxicity and elevated oxidative stress (Lipton *et al.*, 1997).

Huntington's disease

We have recently shown that the transsulfuration pathway is disrupted in the autosomal dominant neurodegenerative disorder, Huntington's disease (HD) leading to lowered cysteine and H_2S levels (Paul *et al.*, 2014; Paul and Snyder, 2014). HD is triggered by the expansion of polyglutamine repeats in the protein huntingtin, leading to motor, cognitive and psychiatric disturbances (Group, 1993). At the molecular level, mutant huntingtin (mHtt) aggregates and affects multiple cellular processes including transcriptional regulation, response to stress and autophagy (Bates *et al.*, 2015). In cell cultures and mouse models of HD as well as in patient samples, the levels of CSE are drastically diminished, due to the sequestration of SP1 by mHtt (Paul *et al.*, 2014). In addition to impaired SP1 function, transcriptional regulation of CSE in response to amino acid starvation is also affected in HD (Sbodio *et al.*, 2016) as demonstrated in striatal progenitor cells, ST*Hdh*$^{Q7/Q7}$ and ST*Hdh*$^{Q111/Q111}$ (referred to as *Q7* and *Q111* cells: Figure 3). The transporters for cystine and cysteine are also dysregulated in HD (Li *et al.*, 2010; Frederick *et al.*, 2014). Together, the lack of cysteine and GSH cause an elevation of ROS, which prevents the stress response elicited by ATF4 in a vicious cycle, which mediates cell death. As a result of these abnormalities, the Q7 cells are unable to grow under cysteine-deprived conditions. Accordingly, stimulating the transsulfuration pathway affords therapeutic benefits. Recently, we showed that the Golgi stress response can be harnessed to up-regulate cysteine and H_2S biosynthesis. Similar to ER stress and oxidative stress responses, the Golgi stress response can elicit the induction of CSE (Sbodio *et al.*, 2018). Golgi stressors, such as the ionophores monensin and nigericin, can activate the integrated stress response by activating the phosphorylation of PKR-like ERK, which then leads to the induction of ATF4 and CSE. Thus, pretreatment of Q111 cells with monensin promotes growth in cysteine-free conditions *via* induction of CSE (Figure 3).

Ageing

It has been shown that increased flux *via* the transsulfuration pathway delays ageing and increases lifespan. In the long-lived Ames dwarf mice, an enhanced expression and activity of CSE was observed, which correlated with increased GSH levels (Uthus and Brown-Borg, 2003). The positive impact of the pathway on longevity has also been observed in the fruit fly, *Drosophila melanogaster* (Kabil *et al.*, 2011a). More recently, it was demonstrated that H_2S was responsible for increased longevity afforded by caloric restriction in yeast, *C. elegans*, *Drosophila* and mice (Hine *et al.*, 2015). In Werner's syndrome, an autosomal recessive disease characterized by accelerated ageing is caused by a mutation in the Werner protein, which leads to defects in genomic instability and aberrant DNA repair; CSE and CBS are down-regulated and this may contribute to the elevated oxidative stress associated with the disease (Murano, 1995). The administration of H_2S donors has proved beneficial in a cell culture model of Werner's syndrome leading to reversal of abnormal morphology and protein aggregation associated with the disease (Talaei *et al.*, 2013).

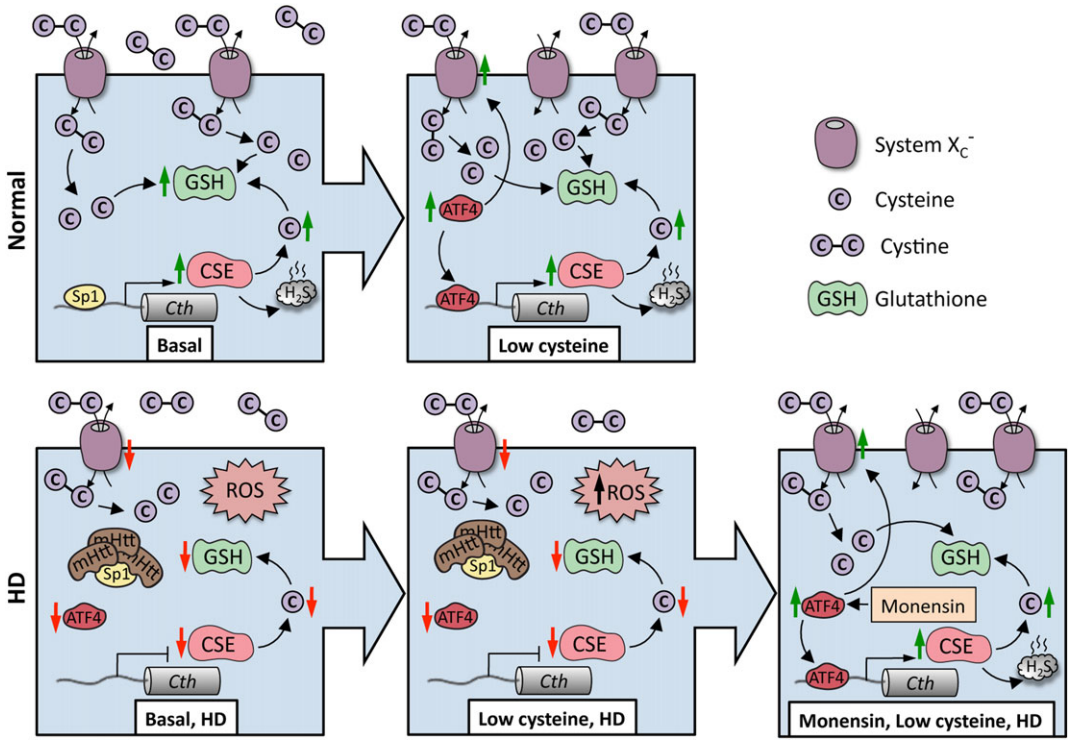

Figure 3

Huntington's disease (HD) as an example of disrupted cysteine metabolism. In normal striatal cells, during basal conditions, the expression of CSE is controlled by the transcription factor SP1, which has binding sites in the CSE (*Cth*) promoter, resulting in cysteine (denoted as C) and GSH production. When cysteine becomes limiting, ATF4 is induced leading to an elevated expression of CSE as well the transporters for cystine (denoted as C–C) (System X_c^-). System X_c^- imports cystine, the oxidized form of cysteine, which is subsequently reduced to cysteine inside cells. Both the basal as well as ATF4-mediated induction of CSE in response to cysteine deprivation are compromised in HD. SP1 is sequestered by mHtt, leading to diminished expression of CSE, causing an increase in ROS. Initially, ATF4 is functional, but during disease progression, a further increase in ROS prevents this response, leading to a decline in its induction. Stimulating the transsulfuration pathway *via* the Golgi stress response (as shown in the case of monensin, an ionophore and Golgi stressor) can protect HD cells and prolong survival.

Strategies to combat dysregulated transsulfuration

As discussed in the preceding sections, aberrant redox homeostasis occurs in a wide variety of diseases and during normal ageing. In cases where elevated CBS or CSE expression occur, use of specific inhibitors for these enzymes may be beneficial as in the case of Down syndrome, where trisomy of chromosome 21, where *Cbs* is located, causes excess H_2S production (Kamoun *et al.*, 2003). However, specific inhibitors of CBS are currently unavailable (Asimakopoulou *et al.*, 2013). In conditions where impaired transsulfuration occurs and causes redox imbalance due to suboptimal expression of CSE, several strategies can be followed to mitigate abnormalities. A case in point is HD, where decreased CSE expression causes decreased cysteine and H_2S production, elevated oxidative stress and associated abnormalities. Several approaches can be taken to counteract the disrupted redox balance in HD (Figure 4). Supplements of cysteine or its precursors mitigated symptoms and delayed disease progression in mouse models of HD (Paul *et al.*, 2014). Another approach is to mitigate oxidative stress by administration of antioxidants such as ascorbate and N-acetyl cysteine. In HD, the response of cytoprotective pathways decline due to oxidative stress and the administration of antioxidants have proved beneficial (Wright *et al.*, 2015; Sbodio *et al.*, 2016). Stimulating the transsulfuration pathway as a whole by inducing mild stress such as Golgi stress can induce CSE expression to increase flux through the pathway and precondition cells to withstand future insults (Sbodio *et al.*, 2018). Another as yet unexplored option is to induce the expression of CSE and/or CBS by modulating epigenetic changes such as DNA methylation or histone modifications (Figure 4).

Summary

It is increasingly evident that the transsulfuration pathway plays a central role in the maintenance of redox homeostasis and integration of stress responses. Both cysteine and H_2S participate in a plethora of signalling processes. In order to better understand the molecular mechanisms underlying the action of these versatile sulfur-containing molecules, precise measurement of their levels is necessary. Thus, the development of more specific and sensitive reagents to detect these signalling molecules is necessary. Confusion in the field is especially caused by the lack of methods to accurately estimate endogenous concentrations of H_2S. In addition to these

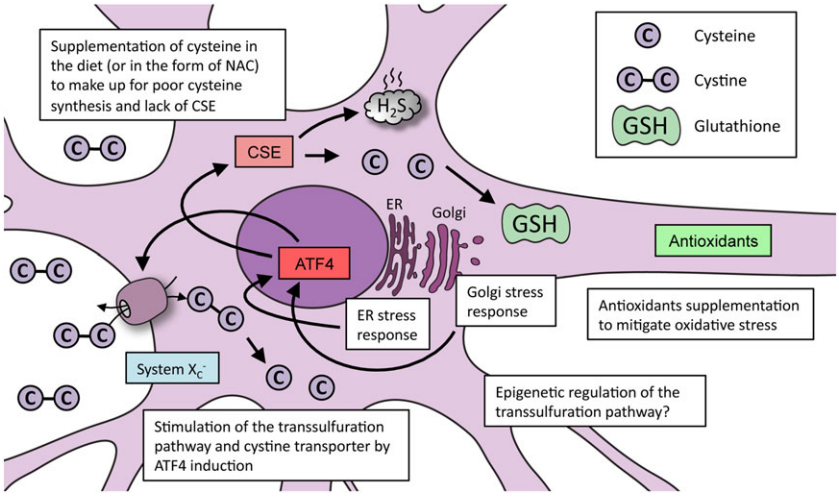

Figure 4

Strategies used to promote redox balance in cells *via* cysteine metabolism: Huntington's disease as an example. In conditions involving elevated oxidative stress caused by cysteine imbalance (as exemplified by Huntington's disease), several approaches can be followed to improve cell survival. Supplementation of cysteine or N-acetyl cysteine (NAC) *via* the diet can reverse abnormalities in cells. Mitigating oxidative stress, which improves stress-response pathways, can promote optimal functioning of the transsulfuration pathway. Up-regulating the expression of CSE *via* the stress-responsive transcription factor ATF4 can correct abnormalities associated with cysteine deprivation. Mild forms of ER and Golgi stress can elicit cytoprotective responses, which may provoke cellular adaptations that can help protect against future damaging insults. In addition to these strategies, altering the epigenetic state of the CSE and CBS promoters may also induce their expression.

aspects, the generation of tissue-specific knockout models of CSE, CBS and MPST would prove invaluable to assess the roles of the three enzymes in normal signalling pathways. In order to identify pathways regulated by H_2S, proteomic approaches in conjunction with metabolomics would yield information on nodes for therapeutic intervention. Screening for compounds, both endogenous and exogenous, that stimulate the transsulfuration pathway would lead to a better understanding of the homeostatic regulation of processes controlled by H_2S and the development of novel precision therapeutics.

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,c,d).

Conflict of interest

The authors declare no conflicts of interest.

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