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REVIEW ARTICLE

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The role of S-nitrosoglutathione reductase (GSNOR) in human disease and therapy

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ABSTRACT

S-nitrosoglutathione reductase (GSNOR), or ADH5, is an enzyme in the alcohol dehydrogenase (ADH) family. It is unique when compared to other ADH enzymes in that primary short-chain alcohols are not its principle substrate. GSNOR metabolizes S-nitrosoglutathione (GSNO), S-hydroxymethylglutathione (the spontaneous adduct of formaldehyde and glutathione), and some alcohols. GSNOR modulates reactive nitric oxide (•NO) availability in the cell by catalyzing the breakdown of GSNO, and indirectly regulates S-nitrosothiols (RSNOs) through GSNO-mediated protein S-nitrosation. The dysregulation of GSNOR can significantly alter cellular homeostasis, leading to disease. GSNOR plays an important regulatory role in smooth muscle relaxation, immune function, inflammation, neuronal development and cancer progression, among many other processes. In recent years, the therapeutic inhibition of GSNOR has been investigated to treat asthma, cystic fibrosis and interstitial lung disease (ILD). The direct action of •NO on cellular pathways, as well as the important regulatory role of protein S-nitrosation, is closely tied to GSNOR regulation and defines this enzyme as an important therapeutic target.

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S-nitrosoglutathione; S-nitrosoglutathione reductase; alcohol dehydrogenase; nitric oxide; S-nitrosation; ADH5

Introduction

S-nitrosoglutathione reductase (GSNOR) is an important regulator of human health and disease. The modulation of protein S-nitrosation by GSNOR contributes to a host of maladies and can be exacerbated by the dysregulation of GSNOR. In recent years, much effort has been dedicated to identifying a safe and efficacious means to alter GSNOR activity. A myopic investigation of GSNOR would reveal little more than its inherit ability to metabolize S-nitrosoglutathione (GSNO) (Jensen *et al.*, 1998), S-hydroxymethylglutathione (HMGSH) (Hedberg *et al.*, 2000), and a handful of alcohols (Adinolfi *et al.*, 1984; Jensen *et al.*, 1998). If we look beyond the direct actions of the enzyme itself, it quickly becomes apparent that GSNOR influences several downstream and parallel pathways (Figure 1). One of the most important is GSNOR's regulation of GSNO, and by extension, nitric oxide (•NO) and protein S-nitrosation. •NO is a reactive nitrogen species (RNS) that is critical to the normal function of most cell types (Beckman & Koppenol, 1996; Moncada *et al.*, 1991; Radi *et al.*, 1991; Salvador Moncada, 1994). It is a powerful smooth muscle relaxing agent (Bradley *et al.*, 1998; Buxton *et al.*, 2001; Ricciardolo *et al.*, 2004; Tomita *et al.*, 2002), cardiopulmonary regulator (Liu *et al.*, 2004; Tamargo *et al.*, 2010), neuroeffector (Bredt & Snyder, 1992; Corti *et al.*, 2014) and immune system modulator (MacMicking *et al.*, 1997). •NO is likely carried as GSNO from endothelium, and other sources, and acts as a stable •NO reserve (Broniowska *et al.*, 2013; Smith & Marletta, 2012). GSNO can transfer its •NO moiety to a cysteine thiol, resulting in the posttranslational modification (PTM) S-nitrosation/S-nitrosylation (Stamler *et al.*, 1992). S-nitrosation describes a thiol (e.g. cysteine) converted to a S-nitrosothiol (RSNO) by a one-electron oxidation from the •NO radical (Smith & Marletta, 2012). The term nitrosylation describes addition of an •NO group to a metal centered protein such as guanylyl cyclase (Martínez-Ruiz & Lamas, 2004). Researchers have used both terms to describe •NO addition to a protein thiol. We employ S-nitrosation to refer to protein modifications on cysteine residues. Protein S-nitrosations are also referred to in the literature in a fashion that takes into account protein and non-protein nitrosations (e.g. RSNO). We employ the term RSNO as it appears in the literature.

Alcohol dehydrogenase family overview

The alcohol dehydrogenase (ADH) family of enzymes have been investigated for well over a century

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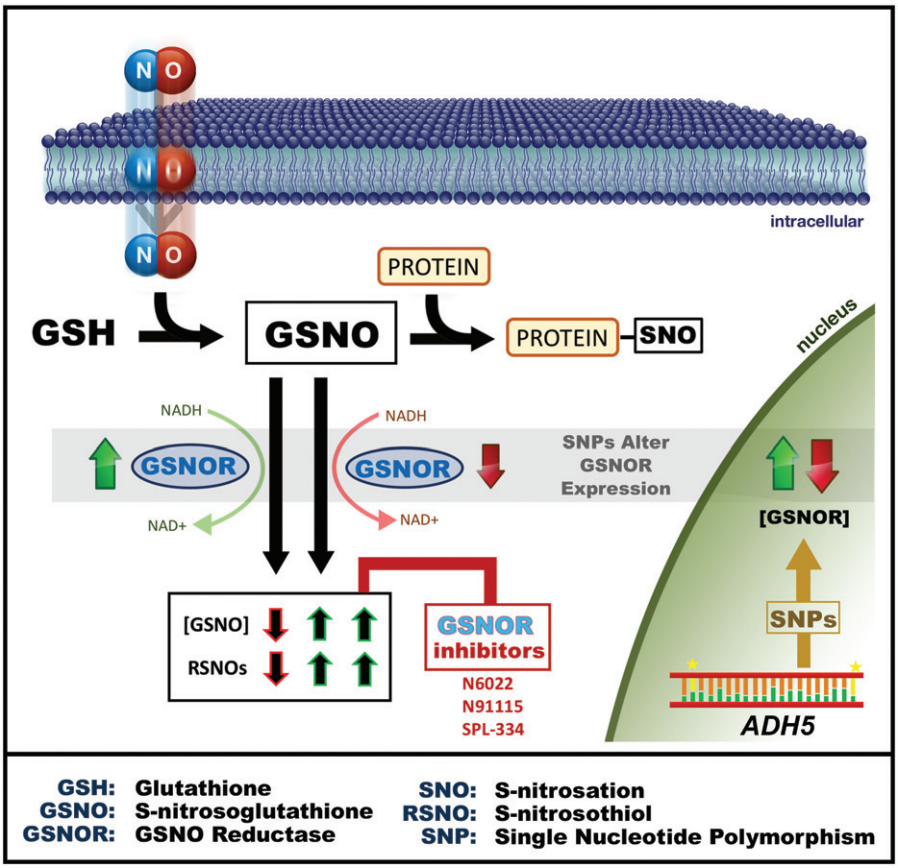

Figure 1. GSNOR in the cell. One of the principle functions of GSNOR is to metabolize GSNO. SNPs in ADH5 can affect expression of GSNOR in the cell, which in turn alters the concentration of GSNO and total levels of RSNOs (term to include nitrosation of cysteine residues, e.g. SNO). Inhibitors of GSNOR increase available GSNO and increase total RSNOs (see colour version of this figure at www.tandfonline.com/ibmg).

(Battelli & Stern, 1910; Daniel, 1909; Lutwak-Mann, 1938). They are evolutionarily conserved from bacteria to man (Gonzàlez-Duarte & Albalat, 2005; Liu *et al.*, 2001) and are categorized into five distinct classes that contain seven known isoforms (Table 1). ADH enzymes perform several important functions in human cells. The most well studied of these is the metabolism of short chain alcohols. Ethanol, being of significant cultural relevance due to its widespread consumption and abuse (Oscar-Berman & Marinkovic, 2003), has led to an extensive investigation of the entire ADH family. Most ADH enzymes have some affinity for ethanol. In hepatocytes, ADH1A (formerly ADH1), ADH1B (formerly ADH2) and ADH1C (formerly ADH3), are responsible for the oxidative catabolism of ethanol to acetaldehyde before further processing in the Krebs cycle, or elimination (Cederbaum, 2013). ADH4, a class II ADH (Svensson *et al.*, 2001) whose sequence is 70% homologous to ADH1, catalyzes the oxidation of retinol (Vitamin A), and bolsters ethanol metabolism in the liver (Ramchandani *et al.*, 2001). Numerous single nucleotide polymorphisms (SNPs) in the genes encoding the ADH family affect the rate of ethanol metabolism. These SNPs have been linked to some forms of alcoholism and cancer (Edenberg, 2007; Hurley & Edenberg, 2012). Other ADH SNPs have been correlated with schizophrenia, Parkinson's disease, asthma and autism in certain populations (Bowers *et al.*, 2011; Buervenich *et al.*, 2000; Wu *et al.*, 2007; Zuo *et al.*, 2013). GSNOR (ADH5), the focus of this review, is differentiated from other ADH enzymes in that primary short chain alcohols, in particular ethanol, are not its principal substrate. ADH6 has been identified in both fetal and adult livers, but its function remains unclear as this enzyme has yet to be isolated for biochemical analysis (Edenberg, 2007; Östberg *et al.*, 2016). A recent examination of ADH6 has provided evidence that it may act as an S-nitroso-CoA reductase (Anand *et al.*, 2014). Similarly, ADH7's function remains elusive. Available data suggest ADH7 may serve a role in seemingly disparate cellular functions and diseases, such as: first pass gastric metabolism of ethanol (Lee *et al.*, 2006), retinol metabolism (Chase *et al.*, 2009), Parkinson's disease (Buervenich *et al.*, 2000) and even personality traits in some individuals with substance dependence (Luo *et al.*, 2008). Clearly, the ADH family of enzymes performs a diverse and important role in

Table 1. ADH variants. ADHs are most commonly recognized as highly effective metabolizers of ethanol. ADH5 evolved independently from class I and class II ADHs varies from other AHDs in that GSNO and HMGSH are its primary substrates. The identification and analysis of other ADHs continue.

Gene name	Principle substrate	Uniprot Identifier	Subunits	Enzyme class
ADH1A	Ethanol	P07327	α	I
ADH1B (formerly *ADH2*)	Ethanol	P00325	β	I
ADH1C (formerly *ADH3*)	Ethanol	P00326	γ	I
ADH1{D-H)[a]	Unkown	–	–	–
ADH4	Ethanol/Retinol	P08319	π	II
ADH5	GSNO/HMGSH	P11766		III
ADH6	Ethanol/S-nitroso-CoA[b]	P28332	μ/σ[c]	V
ADH7	Retinol	P40394	σ	IV
ADH{8-14)[a]	Unkown/Retinol[b]	–		–

Source: Modified from Edenberg (2007).
[a]Non-human.
[b]Limited evidence.
[c]cDNA data.

the cellular metabolism of endogenous and exogenous chemicals. Here we focus on the function, significance and therapeutic potential of modulating GSNOR activity.

Nomenclature of alcohol dehydrogenases

The ADH family of enzymes has had several overlapping naming schemes in the past (Holmquist & Vallee, 1991; Staab *et al.*, 2008). This has led to ambiguity in the literature and is due in part to the fact that naming assessments have historically been guided by substrate specificity, phylogenic classification and publication date. GSNOR was not disambiguated from glutathione-dependent formaldehyde dehydrogenase (FDH) until 1989 when it was found that these two proteins were in fact the same enzyme (Koivusalo *et al.*, 1989). A formal attempt to reconcile the nomenclature began in 1999 when it was proposed that ADH proteins use numeric Arabic designators to identify each class of enzyme (Duester *et al.*, 1999). In recent years, the research community has generally adopted the gene naming guidelines put forth by the Human Genome Organization's Gene Nomenclature Committee (Wain *et al.*, 2002). Of all the ADH enzymes, GSNOR naming is particularly convoluted in this respect. While this protein is still sometimes referred to in the literature by its nonstandard name, ADH3 (as in class III ADH), the official gene designator is now *ADH5*, and the protein is GSNOR, ADH5 or alcohol dehydrogenase 5 (class III) χ-polypeptide. It can also be found in the literature under several other monikers: formaldehyde dehydrogenase (FDH or FALDH); alcohol dehydrogenase X (ADHX); alcohol dehydrogenase class-3 (ADH-3); χχ-ADH (homodimeric *chi* ADH); ADH 5; glutathione-dependent formaldehyde dehydrogenase (GSH-FDH); and S-(hydroxymethyl) glutathione dehydrogenase (EC 1.1.1.284). For purposes of clarity, this review will address the gene as *ADH5*, and the protein as ADH5 or GSNOR.

ADH5: structure/localization

ADH5, the gene that encodes GSNOR, is located on the reverse strand of chromosome 4 (4q23 – chr4:99993567 – 10000985) (Smith, 1986). *ADH5* is tandemly aligned in the same orientation as the other genes that encode for the entire family of ADH enzymes. Phylogenic analysis of the *ADH5* locus revealed that GSNOR evolved independently from class I and II ADH (Adinolfi *et al.*, 1984), and it is highly conserved across most vertebrate species (Foglio & Duester, 1996). GSNOR has a molecular weight of 39,724 Da and is translated to a 374 amino acid enzyme (UniProtKB identifier: P11766) *via* 9 exons (Hur & Edenberg, 1992). Glu-67 and Arg-368 are highly conserved essential amino acids important to the catalytic mechanism of this enzyme (Sanghani *et al.*, 2006). Splice variants of *ADH5* exist and result in the production of truncated proteins; however, their functional relevance has not been documented (Höög *et al.*, 2001).

GSNOR functions as a homodimer (Figure 2) (Yang *et al.*, 1997) and is localized to the nucleus and cytoplasm (Fernández *et al.*, 2003). Amino acid substitutions in the subunit interacting portions of the coenzyme-binding domain prevent heterodimeric variants from being generated with other ADH enzymes (Julià *et al.*, 1988). Each subunit binds a catalytic and structural Zn^{2+} cofactor (Kaiser *et al.*, 1988; Östberg *et al.*, 2016), for a total of four Zn^{2+} ions per functional enzyme. In addition to Zn^{2+}, GSNOR also requires a coenzyme that can vary based upon the substrate. These include: nicotinamide adenine dinucleotide (NAD+), its reduced form NADH, $NADPH + H^+$ or NAD(P) + (Gupta & Igamberdiev, 2015; Hedberg *et al.*, 2003; Jensen *et al.*, 1998; Sanghani *et al.*, 2000).

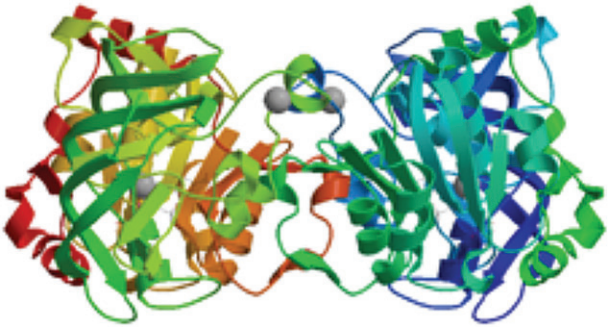

Figure 2. GSNOR quaternary model derived from X-ray diffraction (2.7 Å) and displayed as a functional χχ homodimer with (2) Zn + ions and (1) NADH co-enzyme per subunit. swissmodel.expasy.org SMTL id 1teh.1 (see colour version of this figure at www.tandfonline.com/ibmg).

In general, ADH enzymes are highly expressed in the liver, the upper digestive tract and the kidneys (Zuo *et al.*, 2013). *ADH5* RNA has been recognized in all major human tissue types with protein expression highest in smooth muscle, liver, epididymis, kidney and testis (Giri *et al.*, 1989). GSNOR is an important negative regulator of neuronal differentiation during development (Wu *et al.*, 2014) and is the only known ADH enzyme present in the brain (Beisswenger *et al.*, 1985; Galter *et al.*, 2003). Conversely, GSNOR protein expression is negligible or non-existent in skeletal muscle, lymph nodes, spleen, bone marrow, cerebellum and the lateral ventricle (If & Wb, 2017).

Substrates

S-nitrosoglutathione/formaldehyde

As with most enzymes, GSNOR has a varying degree of affinity for several substrates. The two primary targets of GSNOR are GSNO, and HMGSH, the spontaneous adduct of formaldehyde and glutathione. HMGSH binds at the zinc active site and interacts with the highly conserved residues Arg114/115, Asp55, Glu57 and Thr46 (Engeland *et al.*, 1993; Sanghani *et al.*, 2002). That being said, the rate of substrate conversion (K_{cat}) is about 20-fold higher for GSNO over HMGSH (Green *et al.*, 2012; Hedberg *et al.*, 2003; Salisbury & Bronas, 2015; Sanghani *et al.*, 2000; Staab *et al.*, 2008). Both reactions are dependent on an abundant source glutathione (GSH) in the cell. GSH is the major thiol in mammalian cells and while concentrations can reach as high as 10 mM (Bateman *et al.*, 2008), they are typically 1 mM. Under stress conditions, the concentration can fluctuate dramatically and drive GSNO toward atypical reactions (Figure 3) (Salisbury & Bronas, 2015; Staab *et al.*, 2009). The enzymatic activity of human recombinant GSNOR for GSNO exhibits a K_m of approximately 27 μM and a k_{cat} value of between 2400 and 12,000 min^{-1} (Fernández *et al.*, 2003; Hedberg *et al.*, 2003).

Alcohols

GSNOR more readily acts upon alcohols of greater chain length than class I ADH enzymes (Figure 4). This is due in part to a longer span between the binding and active site of the enzyme (Salisbury & Bronas, 2015), as well as amino acid substitutions that affect binding affinity (Julià *et al.*, 1988; Östberg *et al.*, 2016). As a result of these evolutionary divergences, GSNOR is not optimized for metabolizing short-chain alcohols. Consequently, it is not a misnomer to identify GSNOR as an ADH. GSNOR metabolizes both ethanol and medium/long change alcohols (preferring a double-bond in the beta position). The active site of GSNOR cannot be saturated by ethanol (Beisswenger *et al.*, 1985), and the high activity of class I ADH enzymes toward ethanol minimizes the functional role of ethanol metabolism by GSNOR. Several K_m values for EtOH (all >2 M) (Lee *et al.*, 2003; Sharma *et al.*, 1989) have been reported in the literature, with a k_{cat} of 33 ± 3 min^{-1} (Beisswenger *et al.*, 1985; Lee *et al.*, 2003). GSNOR's ability to metabolize EtOH is far surpassed by those of class I ADH enzymes whose K_m values range from 0.05 to 40 mM. As such, medium and long chain alcohols (>4 carbons) (Holmquist & Vallee, 1991; Salisbury & Bronas, 2015; Theorell *et al.*, 1969; Wagner *et al.*, 1984) are more freely oxidized by GSNOR (Staab *et al.*, 2009).

Other substrates

As with most enzymes, the entire cohort of ADH5 substrates is not fully known. Additional classes of molecules such as ω-hydroxy fatty acids (Achkor *et al.*, 2003; Boleda *et al.*, 1993; Moulis *et al.*, 1991) exhibit a limited affinity for the enzyme. The ability of GSNOR to metabolize retinol remains in question. ADH7 (a class IV ADH) is the primary ADH accountable for retinol metabolism (Cederbaum, 2013), but there is evidence to support GSNOR's contribution in the retinoid-signaling pathway. Studies have shown that *ADH5*$^{-/-}$ null mice exhibit reduced retinoic acid production (Molotkov *et al.*, 2002), and the presence of *ADH5* transcript in human fetal lungs correlates with a decrease in the presence of retinol (Coste & Labbe, 2011). Ultimately, the exact nature of relationship between GSNOR and retinol is still under investigation (Boleda *et al.*, 1993; Cañestro *et al.*, 2010; Gonzàlez-Duarte & Albalat, 2005).

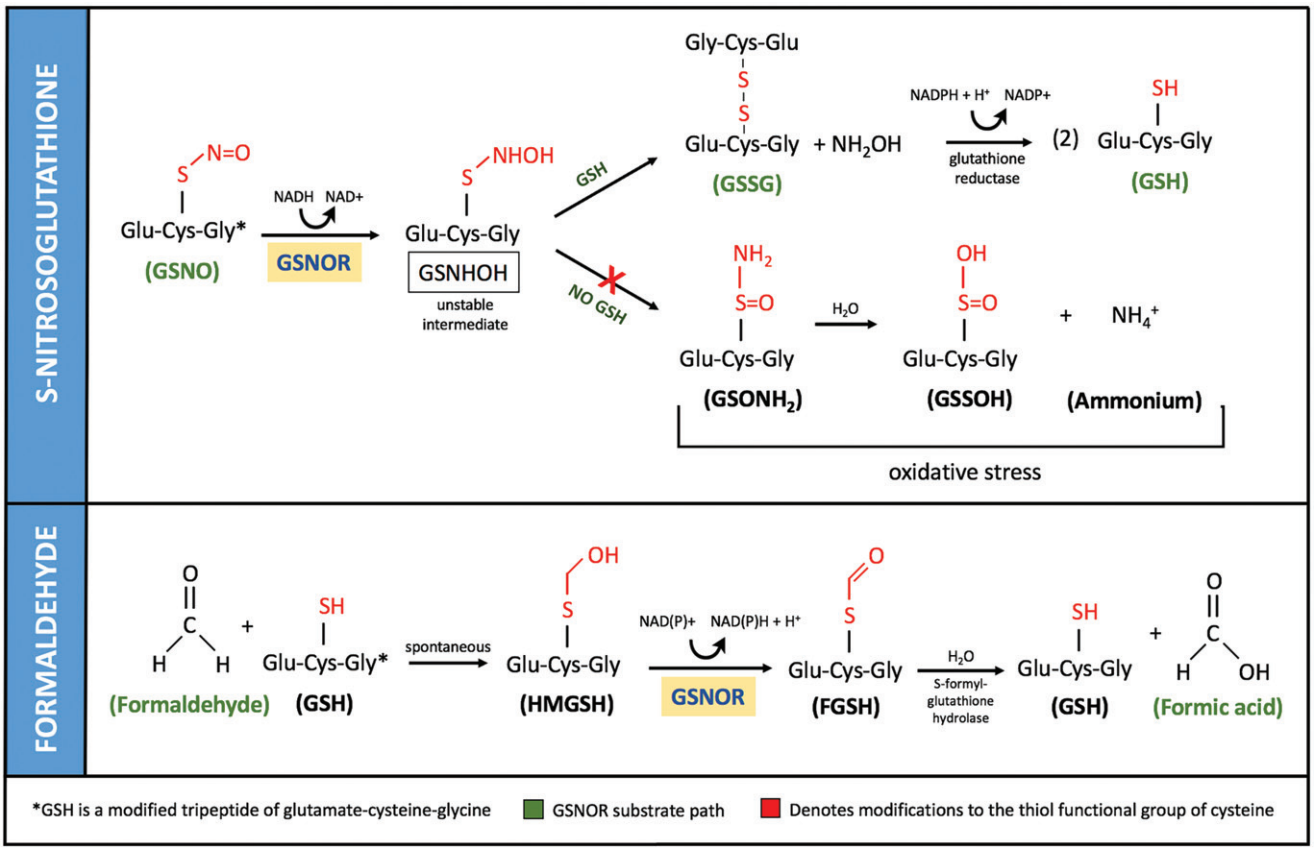

Figure 3. GSNO and HMSGH metabolism by GSNOR – GSNOR metabolizes multiple substrates. S-nitrosoglutathione (GSNO), one of the primary substrates for GSNOR, is first enzymatically degraded to an unstable intermediate, N-hydroxysulfinamide (GSNHOH). In the presence of additional glutathione (GSH), GSNHOH will be converted to glutathione disulfide (GSSG). Under certain condition, such as high levels of oxidative stress, GSH will not be sufficiently available, and other products, such as glutathione sulfinamide (GSONH2) and glutathione sulfinic acid (GSSOH) will be formed. GSNOR can also oxidize the spontaneous adduct of formaldehyde and GSH, S-(hydroxymethyl)glutathione (HMGSH), to S-formylglutathione (FGSH) (see colour version of this figure at www.tandfonline.com/ibmg).

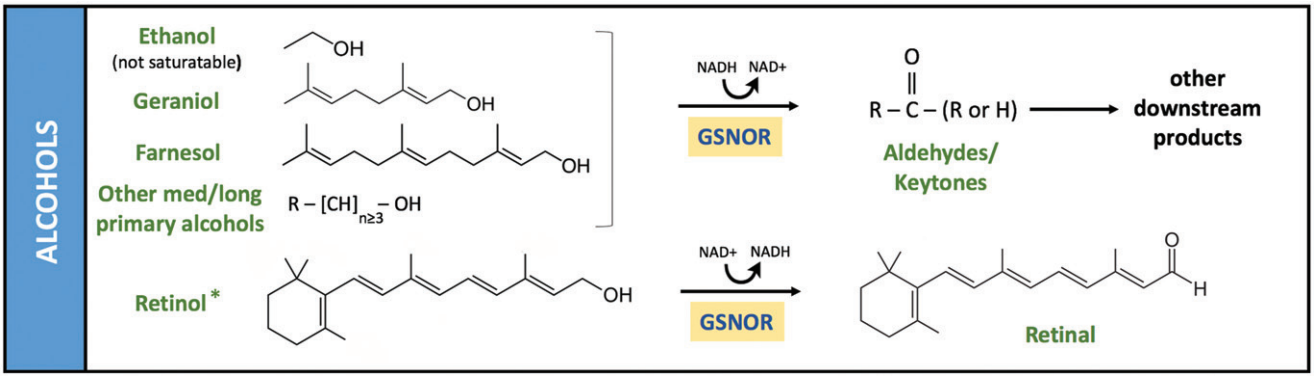

Figure 4. Alcohol metabolism by GSNOR: GSNOR can metabolize medium and long chain primary alcohols to aldehydes and/or ketones before being further processed by other enzymes It preferentially metabolizes medium and long chain alcohols with a double-bond on the beta carbon, however, its specificity for all alcohols is much lower than for GSNO. *Evidence supporting GSNOR's ability to metabolize retinol to the aldehyde retinal is limited (see colour version of this figure at www.tandfonline.com/ibmg).

GSNOR: health and disease

GSNOR is integral to the modulation of •NO in the cell. •NO is produced enzymatically in many cell types (Schmidt & Walter, 1994). Free •NO is a highly reactive uncharged radical with a half-life of ~1–5 second *in vivo* (Kelm & Schrader, 1990), and will often establish a stable RSNO equilibrium with GSH in the form of GSNO (Wink & Mitchell, 1998). •NO, and by extension, GSNO,

plays a critical role in smooth muscle relaxation (Bradley *et al.*, 1998; Buxton, 2004; Liu *et al.*, 2016; Ricciardolo *et al.*, 2004; Tomita *et al.*, 2002) cardiopulmonary regulation (Rastaldo *et al.*, 2007; Sears *et al.*, 2004; Tamargo *et al.*, 2010), neuronal signaling (Shahani & Sawa, 2011), as well as dozens of other intra/extracellular functions (Pacher *et al.*, 1995; Salvador Moncada, 1994). The dysregulation of •NO production and metabolism can lead to drastic changes in protein S-nitrosation (Foster *et al.*, 2003, 2009), an important PTM, and can have numerous other downstream consequences.

Oxidative/nitrosative stress

The dysregulation of GSNO through aberrant GSNOR modulation, when combined with oxidative stress, can further exacerbate disease. During conditions of cellular stress RNS, such as peroxynitrite (ONOO–), are formed when •NO reacts with superoxide ($O_2^{\bullet -}$) (Squadrito & Pryor, 1998). Not only does oxidative stress commandeer available •NO and GSH (Rahman & MacNee, 2000), but peroxynitrite can cross the cell membrane and react directly with protein thiols (Alvarez & Radi, 2003), which may prevent S-nitrosation. RNS also induce S-glutathionylation of protein thiols (Dalle-Donne *et al.*, 2009), further depleting the GSH pool (Klatt & Lamas, 2000). Decades of research have left little question as to detrimental effects of oxidative/nitrosative stress (Dalle-Donne *et al.*, 2006; Guzik *et al.*, 2002; Münzel *et al.*, 1997), and the mechanistic underpinnings of this process have been thoroughly investigated (Apel & Hirt, 2004; Valko *et al.*, 2007). For the purpose of this review, it should be noted that this process can alter the levels of •NO and GSH in the cell, which in turn can affect •NO/GSNO signaling.

GSNO and S-nitrosation

Any investigation into the modulation/activity of GSNOR would not be complete without mention of S-nitrosation. The study of this PTM and its influence on normal cell-signaling and disease has significantly impacted research and medicine for over 25 years (Broniowska & Hogg, 2012; Foster *et al.*, 2009; Stamler *et al.*, 1992).

The detection and quantitation of RSNOs in biological systems are inherently challenging. The biotin switch technique (Jaffrey & Snyder, 2001), in which S-nitrosated cysteines are reduced and biotinylated, provides a simple and elegant method for the qualitative detection of S-nitrosated proteins. An analysis of a wide variety of RSNO measurement techniques, including the biotin switch, has established that artifacts are common when measuring RSNOs and it is not always possible to identify which thiols have been S-nitrosated (Giustarini *et al.*, 2003). Newer techniques have become available in recent years (Chen *et al.*, 2013; Devarie-Baez *et al.*, 2013), such as tandem mass spectrometry (MS/MS) of S-nitrosated protein thiols (Murray *et al.*, 2012; Ulrich *et al.*, 2013), that are highly quantitative. Beyond the problem of quantitation, it has been proposed that other thiol modifications such as dithiol/disulfide exchange, S-glutathionylation and oxidation, may affect signaling more readily than do RSNOs (Lancaster, 2008), and should be investigated along with S-nitrosation.

As with phosphorylation, S-nitrosation regulates cellular mechanisms and affects protein–protein interactions. The intracellular availability of •NO and its functional derivatives, like GSNO, affect protein S-nitrosation (Broniowska & Hogg, 2012; Hess *et al.*, 2005; Thomas & Jourd'heuil, 2012). GSNOR is a potent negative regulator of GSNO in smooth muscle (Que *et al.*, 2009). The aberrant expression of ADH5, as with many ADH subclasses, is associated with disease (Jelski & Szmitkowski, 2008; Jelski *et al.*, 2009; Laniewska-Dunaj *et al.*, 2013). In fact, the deletion of the *ADH5* gene increases both the levels of GSNO and total protein S-nitrosation *in vivo* (Liu *et al.*, 2001). Protein S-nitrosation is of intense interest to researchers and clinicians as the hypo/hyper-S-nitrosation of a diverse set of proteins, spanning nearly every tissue types, can have a drastic effects in disease (Foster *et al.*, 2009). Some of these include: Type 2 diabetes (Carvalho-Filho *et al.*, 2005), sickle cell anemia (Bonaventura *et al.*, 1999, 2002), ventricular arrhythmia in individuals with the Duchenne muscular dystrophy (Fauconnier *et al.*, 2010), cell death and survival pathways (Iyera *et al.*, 2011), post-infarct cardio-protection (Methner *et al.*, 2014), pregnancy/parturition (Ulrich *et al.*, 2013) and many others. Interestingly, GSNOR itself is a cysteine rich protein that is S-nitrosated by GSNO, which in turn initiates a feedback loop that affects GSNOR expression (Guerra *et al.*, 2016) and activity (Brown-Steinke *et al.*, 2010). Although it is beyond the scope of this review, it should be noted that GSNOR dysregulation in plants can result in significant biotic and abiotic nitrosative events that affect growth, development and survival (Leterrier *et al.*, 2011; Shi *et al.*, 2015; Yun *et al.*, 2016).

GSNOR dysregulation

GSNOR dysregulation has been implicated in numerous disease states (Figure 5). The use of models and *ADH5*$^{-/-}$ knockout animals has uncovered surprising and valuable data related to GSNOR function. RSNO levels, as

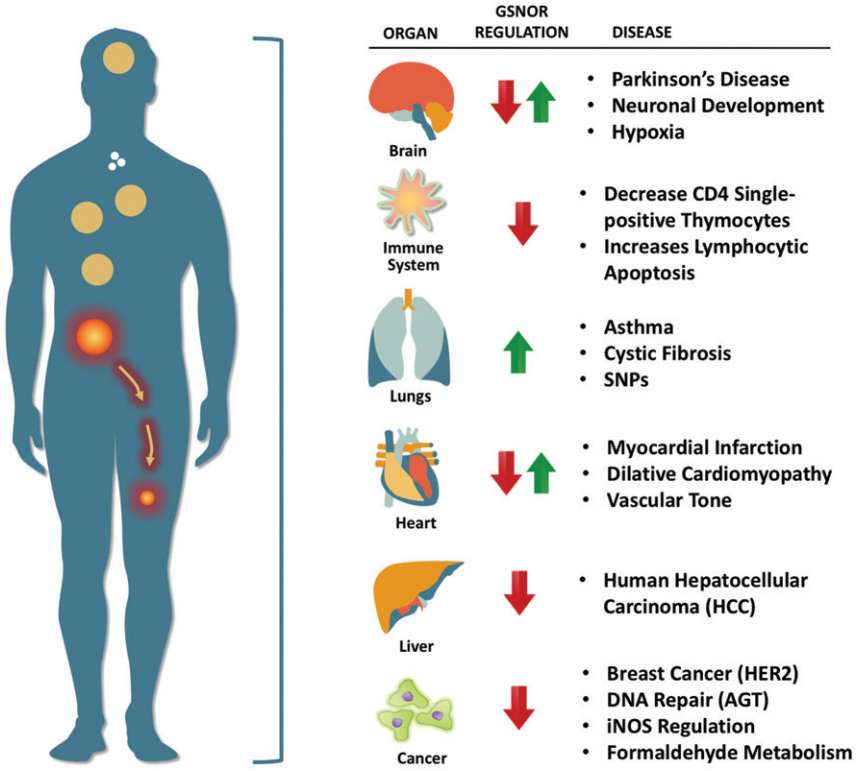

Figure 5. The dysregulation of GSNOR can initiate or exacerbate many disease states. This is due in part to GSNOR's indirect function as a S-nitrosothiol modulator, as well as its ability to mediate canonical NO cell signaling through GSNO metabolism. GSNOR inhibitors are being actively investigated to treat certain disorders in which increased NO availability would be beneficial (see colour version of this figure at www.tandfonline.com/ibmg).

well as canonical NO-mediated pathways, are severely altered when GSNOR activity is modulated.

Cardiovascular health

One of the major organs affected by GSNOR is the heart and surrounding vascularity. It has long been known that •NO and S-nitrosation protect the body from cardiovascular disease. Following myocardial infarction, *ADH5*$^{-/-}$ mice exhibit enhanced cardiac regenerative capabilities as a result of increased cardiac stem cell turnover (Hatzistergos *et al.*, 2015), as well as a reduction in myocardial infarct size and higher coronary vascular density (Lima *et al.*, 2009). Moreover, de-S-nitrosation of cardiac ryanodine receptor 2 (RyR2) in *ADH5*$^{-/-}$ mice results in decreased peripheral vascular tone due to calcium "leak" (Beigi *et al.*, 2012). In skeletal muscle only about 1 in 50 cysteines on the ryanodine receptor are S-nitrosated, indicating that this PTM, even when conservatively distributed, can drastically alter protein function (Sun *et al.*, 2001). Taken together this data suggests that RyR2 S-nitrosation modulates calcium storage in the sarcoplasmic reticulum. There is clearly a complex relationship between the correlative observation of an increase in S-nitrosation and GSNOR dysregulation.

Immune system

GSNOR performs an important protective role in the immune system's development of lymphocytes. *ADH5*$^{-/-}$ KO mice show increased RSNO production that decreases CD4 single-positive thymocyte development, and increases lymphocytic apoptosis (Yang *et al.*, 2010). Damage to immune cells from nitrosative stress in *ADH5*$^{-/-}$ mice results in a significant increase in the animal's susceptibility to pulmonary infection by *K. pneumoniae* as well as multi-fold increases of the bacteria in the spleen and blood, resulting in increased inflammation (Tang *et al.*, 2013a, 2013b). Enhanced nitric oxide synthase (NOS) 2 activity in monocytes and macrophages increases •NO production and elicits a cytostatic or cytotoxic response against bacteria, viruses and other intruders, but also increases inflammation (MacMicking *et al.*, 1997). The bronchoalveolar lavage fluid of asthmatics consists of high macrophage levels as well as significantly increases GSNOR activity (Que *et al.*, 2009). Inhibiting GSNOR in these patients increases total RSNOs and restores inflammatory markers to near baseline levels while limiting ova-induced NFκB activation (Blonder *et al.*, 2014). Ultimately, the balance between GSNOR activation and inhibition is critical in maintaining balance in the immune system.

Brain development and function

GSNOR regulation in the brain affects a broad swath of cellular functions ranging from neural development and maturation to other neurodegenerative diseases more typically associated with adult and geriatric populations. These disease states are often the result of aberrant protein S-nitrosation caused by the dysregulation of GSNOR. For instance, in developing and adult mouse brains the overexpression of GSNOR results in decreased neuronal differentiation in part due to de-S-nitrosation of histone deacetylase 2 (HDAC2) (Wu *et al.*, 2014). Conversely, *ADH5*$^{-/-}$ mice exhibit neuromuscular atrophy as a result of a decrease in muscle mass, while also presenting with neuropathic behavior (Montagna *et al.*, 2014). In *Drosophila*, GSNOR overexpression results in visual pattern memory defects which can be rescued by co-expression of cyclic-GMP dependent protein kinase G (PKG) (Hou *et al.*, 2011). This occurs independently from neuronal development and implies an adjacent regulatory role for GSNOR in the PKG phosphorylation pathway. Neuronal homeostasis is also affected by GSNOR. In a Parkinson's disease model using neuronal (SH-SY5Y) cells, a decrease in GSNOR availability results in activation of nuclear factor Nrf2 ((erythroid-derived 2)-like 2), which regulates the expression of antioxidant proteins (Rizza *et al.*, 2015). Interestingly, GSNOR may also affect the phosphorylated state of platelet-derived growth factor receptor-β (Palmer *et al.*, 2015) in the brainstems of mice during hypoxic exposure. When these data are considered as a whole, it is apparent that deviating GSNOR activity and expression from baseline can have drastic consequences in both the developing and mature brain.

Cancer

The link between GSNOR dysregulation and cancer is not well understood. GSNOR deficiency has been known to affect the rate of genomic mutations in mice by increasing the frequency of A:T to T:A transposition (Leung *et al.*, 2013). This may be the result of a GSNOR-mediated reduction in activity of the DNA repair protein O6-alkylguanine-DNA alkyl transferase which can lead to an increase in the rate of human hepatocellular carcinoma (HCC) (Tang *et al.*, 2012; Wei *et al.*, 2010, 2011). Pharmacologic inhibition of inducible NOS (iNOS) when GSNOR is down-regulated shows strong potential as a therapeutic for those patients with HCC (Tang *et al.*, 2013a). As with HCC, some types of breast cancer are linked to a decrease in GSNOR expression. Specifically, high levels of human epidermal growth factor receptor 2 (HER2) expression in breast tumors is associated with low GSNOR expression and an increase in apoptosis-related protein S-nitrosation (Cañas *et al.*, 2016). This study also determined that an increase in GSNOR expression in *HER2* tumors correlates with higher patient survival and begs the question as to whether or not NOS inhibition would also serve this population well. These examples are of course complicated by the fact that •NO is a pleiotropic regulator of gene function and the modulation of GSNO by GSNOR can have both cytostatic and cytotoxic effects on tumor survival (Xu *et al.*, 2002). To this point, GSNOR is effective at removing formaldehyde, a known carcinogen, from the cell; however, *ADH5* polymorphisms do not significantly affect an individual's capacity to protect against DNA damage when exposed to formaldehyde (Xie *et al.*, 2010). Furthermore, *ADH5*$^{-/-}$ mice are known to generate DNA damage when formaldehyde forms and adduct with guanine to create N2-hydroxymethyl-*d*G which can result dysfunction of hepatocytes and nephrons (Pontel *et al.*, 2015).

Asthma and single nucleotide polymorphisms

SNPs can alter the transcriptional output of a gene as well as the structure/function of proteins they encode. Several SNPs in the promoter and 3′ UTR of the *ADH5* gene can result in the aberrant expression of GSNOR (Choudhry *et al.*, 2010). Of particular interest is the observation that airway hyperesponsivity in wild-type mice correlates with increased expression of GSNOR and decreased RSNO production, while *ADH5*$^{-/-}$ mice are protected from airway hyperresponsiveness and maintain higher total RSNO levels (Que *et al.*, 2005). In humans, GSNOR upregulation can lead to changes in airway smooth muscle tone in asthmatics (Henderson & Gaston, 2005; Wu *et al.*, 2007). A study involving Mexican children with asthma who possess SNPs in the promoter region of *ADH5* at suspected NF-κB binding sites (rs2602899 and rs2851301), were found to exhibit a decreased relative risk of asthma due to suppressed GSNOR production (Wu *et al.*, 2007). Interestingly, alternative SNPs (rs1154404 and rs28730619) were associated with an increase in childhood asthma risk, although the mechanism behind this correlation has not been determined (Wu *et al.*, 2007). Another study in African American children found that SNPs in *ADH5* and the β2 adrenergic receptor gene are associated with acute response to asthma-specific therapy (Moore *et al.*, 2009).

Looking beyond GSNO-mediated relaxation of airway smooth muscle we may also consider GSNOR's ability to

metabolize formaldehyde, a chemical known to induce bronchoconstriction after long term exposure at low concentrations (Leikauf, 1992). It has been suggested that the presence of formaldehyde in airway smooth muscle may stoichiometrically favor bound NADH/GSNOR, thereby increasing GSNOR metabolism of GSNO, and by extension, promote smooth muscle contraction (Thompson & Grafström, 2007).

Regardless of the mechanism driving GSNOR-mediated consumption of GSNO in airway smooth muscle, it is easy to see why the inhibition of GSNOR has been of particular interest to researchers for its therapeutic potential as a smooth muscle relaxant.

Myoendothelial junctions

GSNOR plays an interesting role at myoendothelial junctions (MEJ) where it co-localizes with the hemichannel Connexin-43 (Cx43). Cx43 hemichannels form gap junctions between cells by linking to hemichannels in opposing membranes to couple endothelial and vascular smooth muscle cells and when Cx43 is S-nitrosated, this pore allows for the free movement of inositol trisphosphate from vascular smooth muscle to endothelial cells. Due to the co-localization of GSNOR and Cx43 at the MEJ, basal •NO availability at this site is blunted, which in turn increases the likelihood that Cx43 will not be S-nitrosated (Straub *et al.*, 2011). This decreases channel permeability until Ca^{2+} levels increase as a result of smooth muscle cell stimulation, which in turn activates eNOS and increases the probability of Cx43 S-nitrosation.

Myometrium

•NO is an important mediator of relaxation in the myometrium. It has been well established that •NO relaxes vascular and gastrointestinal smooth muscle by activating soluble guanylyl cyclase (sGC), which in turn converts guanosine triphosphate to cyclic guanosine monophosphate (cGMP), activating PKG, which in turn dephosphorylates the regulatory light chain (MYL9) of myosin *via* the amplified phosphatase activity of MYPT1 (pS695) (Grassie *et al.*, 2011; Nakamura *et al.*, 2007; Puetz *et al.*, 2009; Roux *et al.*, 2012). This is not the dominant •NO-mediated relaxation pathway in uterine smooth muscle, however, •NO can relax the myometrium even when sGC has been inhibited (Buxton *et al.*, 2010). The pathway through which •NO relaxes the myometrium independent of cGMP is unknown, but it is likely that the S-nitrosation of contractile proteins plays a role. It has been determined that the state of labor (full term versus preterm) can vastly alter the S-nitrosated protein landscape in uterine smooth muscle after exposure to GSNO (Ulrich *et al.*, 2012). It is also well known that S-nitrosation can vary significantly based upon the cytoplasmic availability GSNOR (Broniowska & Hogg, 2012; Hess *et al.*, 2005; Thomas & Jourd'heuil, 2012). Regardless of the pathway through which •NO acts to relax uterine smooth muscle, it does beg the question as to whether or not inhibiting GSNOR, and thereby increasing intracellular availability of GSNO, may serve as an effective tocolytic strategy by promoting uterine quiescence through •NO-mediated relaxation pathways. This notion is supported by data showing an increased expression of GSNOR in patients delivering spontaneously preterm.

Therapeutic inhibition of GSNOR

GSNOR is an attractive therapeutic target. GSNOR inhibition increases GSNO availability in the cell and in turn facilitates •NO-mediated signaling pathways. Dozens of small molecules have been identified that can inhibit GSNOR to varying degrees (Green *et al.*, 2012; Jiang *et al.*, 2016; Sanghani *et al.*, 2009; Sun *et al.*, 2011a, 2011b, 2012). Two of these, N6022 (3-(5-(4-(1H-imidazol-1-yl) phenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl) propionic acid) and N91115 from Nivalis Pharmaceuticals show promise as potentially safe and effective GSNOR inhibitors that have undergone clinical trial for both the treatment of mild asthma (clinicaltrials.gov – NCT01316315), and cystic fibrosis in individuals who are heterozygous for the cystic fibrosis transmembrane conductance regulator (CFTR) gating mutation CFTRΔF508 + (clinicaltrials.gov – N6022: NCT01746784; N91115: NCT02724527). Endogenous GSNO levels are low in the airways of cystic fibrosis patients (Grasemann *et al.*, 1999) and GSNOR inhibition is an appealing alternative to the direct administration of GSNO (Snyder *et al.*, 2002; Zaman *et al.*, 2001, 2013). N6022 is well tolerated with minimal side effects, even at high concentrations, in both animals (Blonder *et al.*, 2014; Colagiovanni *et al.*, 2012) and humans (clinicaltrials.gov – NCT01147406, NCT01746784). Another GSNOR inhibitor, SPL-334 (4-{[2-[(2-cyanobenzyl) thio]-4-oxothieno[3,2-d]pyrimidin-3(4H)-yl]methyl}benzoic acid) from SAJE Pharmaceuticals (Baltimore, MD), is being tested as a therapeutic to treat allergic asthma and interstitial lung disease (ILD). Using an allergic asthma mouse model, intranasally administered SPL-334 decreased CD4+ Th2 cytokines, eosinophils, and mitigated the lung inflammatory response (Ferrini *et al.*, 2013). Likewise, in a mouse model of ILD, SPL-334 functions as both a prophylactic agent and a therapeutic to

attenuate profibrotic cytokines and collagen accumulation in the lungs (Luzina *et al.*, 2015). Unlike N6022 and N91115, SPL-334 is not in human clinical trials.

FDA-approved drugs are also being tested as potential GSNOR inhibitors. Nebivolol, a β_1-adrenergic receptor antagonist used for the management of hypertension, has been shown to increase total RSNO levels in animal and cell models (Jiang *et al.*, 2016). Our own investigation of GSNOR fails to confirm Nebivolol as an inhibitor of GSNOR in an enzyme activity assay. Since there are no FDA-approved GSNOR inhibitors, the repurposing of existing therapeutic agents that inhibit GSNOR and/or modulate GSNO and RSNOs is of interest.

When considering GSNOR inhibitors as therapeutic agents, it should be taken into consideration that enzymes other than GSNOR modulate •NO availability in the cell. •NO is critical to the normal function of most cell types, and as is often the case, there are multiple concurrent and complementary mechanisms to regulate •NO and RSNOs (Benhar *et al.*, 2009; Liu *et al.*, 2001). Two of the most well-known are thioredoxin-1 (Sengupta & Holmgren, 2012a, 2012b) and carbonyl reductase (Bateman *et al.*, 2008). NOS, the predominate source of •NO in the body, can also be dysregulated in certain disease states, as can its substrate, L-arginine (Ckless *et al.*, 2007). For instance, after stimulation of the cavernous nerve in *ADH5*$^{-/-}$ mice, eNOS phosphorylation did not increase as predicted (Musicki *et al.*, 2016). Modulating GSNOR activity may insufficiently control, or even aggravate some conditions if these alternate •NO-regulators are the source of the disorder. Unfortunately, direct application of endogenous •NO-donors, such as GSNO, Cys-NO or SNO-albumin, as well as some exogenous donors, are of limited clinical value because they either degrade rapidly, cause intolerable side effects, or lead to a toxic systemic build up nitrates (Al-Sa'doni, 2005).

The therapeutic inhibition of GSNOR to treat •NO-mediated disorders should be weighed carefully against potential contraindications. For example, the inhibition of GSNOR may increase a patient's susceptibility to bacterial or viral infection. The inhibition of GSNOR will also increase total RSNO levels and this can have adverse effects in the body, especially if the drug is administered systemically and not targeted to a specific tissue type through means such as liposomal delivery. GSNOR regulation varies widely in different cancer types (Cañas *et al.*, 2016; Tang *et al.*, 2013a). Inhibiting GSNOR may lead to a further increase in GSNO at the tumor site which can favor angiogenesis (Prudente *et al.*, 2017). Conversely, with disorders such as asthma and hypertension, GSNOR inhibition results in the desired relaxation of the smooth muscle.

Conclusions

•NO, and by extension •NO-donors, have been investigated intensely for over a century as therapeutics (Schmidt & Walter, 1994). •NO modulation not only affects traditional pathways connected to this highly reactive molecule, but it also drastically alters S-nitrosation levels in the cell. GSNOR is unique among the ADH family of enzymes in that it targets GSNO and varies the body's response to endogenously generated •NO carried as GSNO. *ADH5*$^{-/-}$ animal and cell models have provided a unique window into the importance of GSNOR in nearly every tissue type. The up/down regulation of GSNOR in humans has also provided invaluable data to the medical and research communities concerning its role in disease states. There are currently no FDA-approved modulators of GSNOR; however, several drugs are being investigated, and some are in clinical trial. Indeed, our understanding of the dysregulation of GSNOR and its effect on protein S-nitrosation and other glutathione/•NO-mediated events is in its infancy. Further investigations into the role of GSNOR in health and disease are needed to reveal the most effective therapeutic options.

Disclosure statement

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References

Achkor H, Díaz M, Fernández MR, *et al.* (2003). Enhanced formaldehyde detoxification by overexpression of glutathione-dependent formaldehyde dehydrogenase from Arabidopsis. Plant Physiol 132:2248–55.

Adinolfi A, Adinolfi M, Hopkinson DA. (1984). Immunological and biochemical characterization of the human alcohol dehydrogenase chi-ADH isozyme. Ann Hum Genet 48:1–10.

Al-Sa'doni HH, Ferro A. (2005). Current status and future possibilities of nitric oxide-donor drugs: focus on S-nitrosothiols. Mini Rev Med Chem 5:247–54.

Alvarez B, Radi R. (2003). Peroxynitrite reactivity with amino acids and proteins. Amino Acids 25:295–311.

Anand P, Hausladen A, Wang Y-J, *et al.* (2014). Identification of S-nitroso-CoA reductases that regulate protein S-nitrosylation. Proc Natl Acad Sci USA 111:18572–7.

Apel K, Hirt H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–99.

Bateman RL, Rauh D, Tavshanjian B, Shokat KM. (2008). Human carbonyl reductase 1 is an S-nitrosoglutathione reductase. J Biol Chem 283:35756–62.

Battelli F, Stern L. (1910). Die alkoholoxydase in den tiergeweben. Biochem Z 28.

Beckman JS, Koppenol WH. (1996). Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 271:C1424–37.

Beigi F, Gonzalez DR, Minhas KM, *et al*. (2012). Dynamic denitrosylation via S-nitrosoglutathione reductase regulates cardiovascular function. Proc Natl Acad Sci USA 109:4314–9.

Beisswenger TB, Holmquist B, Vallee BL. (1985). chi-ADH is the sole alcohol dehydrogenase isozyme of mammalian brains: implications and inferences. Proc Natl Acad Sci USA 82:8369–73.

Benhar M, Forrester MT, Stamler JS. (2009). Protein denitrosylation: enzymatic mechanisms and cellular functions. Nat Rev Mol Cell Biol 10:721–32.

Blonder JP, Mutka SC, Sun X, *et al*. (2014). Pharmacologic inhibition of S-nitrosoglutathione reductase protects against experimental asthma in BALB/c mice through attenuation of both bronchoconstriction and inflammation. BMC Pulm Med 14:3.

Boleda MD, Saubi N, Farrés J, Parés X. (1993). Physiological substrates for rat alcohol dehydrogenase classes: aldehydes of lipid peroxidation, omega-hydroxyfatty acids, and retinoids. Arch Biochem Biophys 307:85–90.

Bonaventura C, Ferruzzi G, Stevens RD, Tesh S. (1999). Cell biology and metabolism: effects of S-nitrosation on oxygen binding by normal and sickle cell hemoglobin effects of S-nitrosation on oxygen binding by normal and sickle cell hemoglobin. J Biol Chem 274:24742–8.

Bonaventura C, Godette G, Ferruzzi G, *et al*. (2002). Responses of normal and sickle cell hemoglobin to S-nitroscysteine: implications for therapeutic applications of NO in treatment of sickle cell disease. Biophys Chem 98:165–81.

Bowers K, Li Q, Bressler J, *et al*. (2011). Glutathione pathway gene variation and risk of autism spectrum disorders. J Neurodev Disord 3:132–43.

Bradley KK, Buxton IL, Barber JE, *et al*. (1998). Nitric oxide relaxes human myometrium by a cGMP-independent mechanism. Am J Physiol 275:C1668–73.

Bredt DS, Snyder SH. (1992). Nitric oxide, a novel neuronal messenger. Neuron 8:3–11.

Broniowska KA, Diers AR, Hogg N. (2013). S-nitrosoglutathione. Biochim Biophys Acta 1830:3173–81.

Broniowska KA, Hogg N. (2012). The chemical biology of S-nitrosothiols. Antioxid Redox Signal 17:969–80.

Brown-Steinke K, Deronde K, Yemen S, Palmer LA. (2010). Gender differences in s-nitrosoglutathione reductase activity in the lung. PLoS One 5:e14007. doi:10.1371/journal.pone.0014007

Buervenich S, Sydow O, Carmine A, *et al*. (2000). Alcohol dehydrogenase alleles in Parkinson's disease. Mov Disord 15:813–8.

Buxton IL. (2004). Regulation of uterine function: a biochemical conundrum in the regulation of smooth muscle relaxation. Mol Pharmacol 65:1051–9.

Buxton ILO, Kaiser RA, Malmquist NA, Tichenor S. (2001). NO-induced relaxation of labouring and non-labouring human myometrium is not mediated by cyclic GMP. Br J Pharmacol 134:206–14.

Buxton ILO, Milton D, Barnett SD, Tichenor SD. (2010). Agonist-specific compartmentation of cGMP action in myometrium. J Pharmacol Exp Ther 335:256–63.

Cañas A, López-Sánchez LM, Peñarando J, *et al*. (2016). Altered S-nitrosothiol homeostasis provides a survival advantage to breast cancer cells in HER2 tumors and reduces their sensitivity to trastuzumab. Biochim Biophys Acta – Mol Basis Dis 1862:601–10.

Cañestro C, Albalat R, Postlethwait JH. (2010). *Oikopleura dioica* alcohol dehydrogenase class 3 provides new insights into the evolution of retinoic acid synthesis in chordates. Zool Sci 27:128–33.

Carvalho-Filho MA, Ueno M, Hirabara SM, *et al*. (2005). S-Nitrosation of the insulin receptor, insulin receptor substrate 1, and protein kinase B/Akt: a novel mechanism of insulin resistance. Diabetes 54:959–67.

Cederbaum AI. (2013). Alcohol metabolism. Clin Liver Dis 16:667–85.

Chase JR, Poolman MG, Fell DA. (2009). Contribution of NADH increases to ethanol's inhibition of retinol oxidation by human ADH isoforms. Alcohol Clin Exp Res 4:571–80.

Chen Y-J, Ching W-C, Lin Y-P, Chen Y-J. (2013). Methods for detection and characterization of protein S-nitrosylation. Methods 62:138–50.

Choudhry S, Que LG, Yang Z, *et al*. (2010). GSNO reductase and β2-adrenergic receptor gene–gene interaction: bronchodilator responsiveness to albuterol. Pharmacogenet Genom 20:351–8.

Ckless K, Van Der Vliet A, Janssen-Heininger Y. (2007). Oxidative-nitrosative stress and post-translational protein modifications: implications to lung structure–function relations: arginase modulates NF-kappaB activity via a nitric oxide-dependent mechanism. Am J Respir Cell Mol Biol 36:645–53.

Colagiovanni DB, Drolet DW, Langlois-Forget E, *et al*. (2012). A nonclinical safety and pharmacokinetic evaluation of N6022: a first-in-class S-nitrosoglutathione reductase inhibitor for the treatment of asthma. Regul Toxicol Pharmacol 62:115–24.

Corti A, Franzini M, Scataglini I, Pompella A. (2014). Mechanisms and targets of the modulatory action of S-nitrosoglutathione (GSNO) on inflammatory cytokines expression. Arch Biochem Biophys 562:80–91.

Coste K, Labbe A. (2011). Étude de la voie métabolique de la vitamine A dans le poumon fœtal humain. Rev Mal Respir 28:283–9.

Dalle-Donne I, Rossi R, Colombo G, *et al*. (2009). Protein S-glutathionylation: a regulatory device from bacteria to humans. Trends Biochem Sci 34:85–96.

Dalle-Donne I, Rossi R, Colombo R, *et al*. (2006). Biomarkers of oxidative damage in human disease. Clin Chem 52:601–23.

Daniel JF. (1909). Adaptation and immunity of lower organisms to ethyl alcohol. J Exp Zool 6:571–611.

Devarie-Baez NO, Zhang D, Li S, *et al*. (2013). Direct methods for detection of protein S-nitrosylation. Methods 62:171–6.

Duester G, Farrés J, Felder MR, *et al*. (1999). Recommended nomenclature for the vertebrate alcohol dehydrogenase gene family. Biochem Pharmacol 58:389–95.

Edenberg HJ. (2007). The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res Health 30:5–14.

Engeland K, Höög JO, Holmquist B, *et al*. (1993). Mutation of Arg-115 of human class III alcohol dehydrogenase: a binding site required for formaldehyde dehydrogenase activity and fatty acid activation. Proc Natl Acad Sci USA 90:2491–4.

Fauconnier J, Thireau J, Reiken S, *et al*. (2010). Leaky RyR2 trigger ventricular arrhythmias in Duchenne muscular dystrophy. Proc Natl Acad Sci USA 107:1559–64.

Fernández MR, Biosca JA, Parés X. (2003). S-nitrosoglutathione reductase activity of human and yeast glutathione-dependent formaldehyde dehydrogenase and its nuclear and cytoplasmic localisation. Cell Mol Life Sci 60:1013–8.

Ferrini ME, Simons BJ, Bassett DJP, *et al*. (2013). S-nitrosoglutathione reductase inhibition regulates allergen-induced lung inflammation and airway hyperreactivity. PLoS One 8:e70351.

Foglio MH, Duester G. (1996). Characterization of the functional gene encoding mouse class III alcohol dehydrogenase (glutathione-dependent formaldehyde dehydrogenase) and an unexpressed processed pseudogene with an intact open reading frame. Eur J Biochem 237:496–504.

Foster MW, Hess DT, Stamler JS. (2009). Protein S-nitrosylation in health and disease: a current perspective. Trends Mol Med 15:391–404.

Foster MW, McMahon TJ, Stamler JS. (2003). S-nitrosylation in health and disease. Trends Mol Med 9:160–8.

Galter D, Carmine A, Buervenich S, *et al*. (2003). Distribution of class I, III and IV alcohol dehydrogenase mRNAs in the adult rat, mouse and human brain. Eur J Biochem 270:1316–26.

Giri PR, Krug JF, Kozak C, *et al*. (1989). Cloning and comparative mapping of a human class III (x) alcohol dehydrogenase cDNA. Biochem Biophys Res Commun 164:453–60.

Giustarini D, Milzani A, Colombo R, *et al*. (2003). Nitric oxide and S-nitrosothiols in human blood. Clin Chim Acta 330:85–98.

Gonzàlez-Duarte R, Albalat R. (2005). Merging protein, gene and genomic data: the evolution of the MDR-ADH family. Heredity (Edinb) 95:184–97.

Grasemann H, Gaston B, Fang K, *et al*. (1999). Decreased levels of nitrosothiols in the lower airways of patients with cystic fibrosis and normal pulmonary function. J Pediatr 135:770–2.

Grassie ME, Moffat LD, Walsh MP, MacDonald JA. (2011). The myosin phosphatase targeting protein (MYPT) family: a regulated mechanism for achieving substrate specificity of the catalytic subunit of protein phosphatase type 1δ. Arch Biochem Biophys 510:147–59.

Green LS, Chun LE, Patton AK, *et al*. (2012). Mechanism of inhibition for N6022, a first-in-class drug targeting S-nitrosoglutathione reductase. Biochemistry 51:2157–68.

Guerra D, Ballard K, Truebridge I, Vierling E. (2016). S-nitrosation of conserved cysteines modulates activity and stability of *S*-nitrosoglutathione reductase (GSNOR). Biochemistry 55:2452–64.

Gupta KJ, Igamberdiev I. (2015). Reactive oxygen and nitrogen species signaling and communication in plants. In: Signaling and communication in plants. Switzerland: Springer International Publishing.

Guzik TJ, West NEJ, Pillai R, *et al*. (2002). Nitric oxide modulates superoxide release and peroxynitrite formation in human blood vessels. Hypertension 39:1088–94.

Hatzistergos KE, Paulino EC, Dulce RA, *et al*. (2015). *S*-nitrosoglutathione reductase deficiency enhances the proliferative expansion of adult heart progenitors and myocytes post myocardial infarction. J Am Heart Assoc 4:e001974.

Hedberg JJ, Griffiths WJ, Nilsson SJF, Höög JO. (2003). Reduction of S-nitrosoglutathione by human alcohol dehydrogenase 3 is an irreversible reaction as analysed by electrospray mass spectrometry. Eur J Biochem 270: 1249–56.

Hedberg JJ, Hoog JO, Nilsson JA, *et al*. (2000). Expression of alcohol dehydrogenase 3 in tissue and cultured cells from human oral mucosa. Am J Pathol 157:1745–55.

Henderson EM, Gaston B. (2005). SNOR and wheeze: the asthma enzyme? Trends Mol Med 11:481–4.

Hess DT, Matsumoto A, Kim S-O, *et al*. (2005). Protein S-nitrosylation: purview and parameters. Nat Rev Mol Cell Biol 6:150–66.

Holmquist B, Vallee BL. (1991). Human liver class III alcohol and glutathione dependent formaldehyde dehydrogenase are the same enzyme. Biochem Biophys Res Commun 178:1371–7.

Höög JO, Brandt M, Hedberg JJ, Stromberg P. (2001). Mammalian alcohol dehydrogenase of higher classes: analyses of human ADH5 and rat ADH6. Chem Biol Interact 130–132:395–404.

Hou Q, Jiang H, Zhang X, *et al*. (2011). Nitric oxide metabolism controlled by formaldehyde dehydrogenase (FDH, homolog of mammalian GSNOR) plays a crucial role in visual pattern memory in Drosophila. Nitric Oxide – Biol Chem 24:17–24.

Hur MW, Edenberg HJ. (1992). Cloning and characterization of the ADH5 gene encoding human alcohol dehydrogenase 5, formaldehyde dehydrogenase. Gene 121:305–11.

Hurley TD, Edenberg HJ. (2012). Genes encoding enzymes involved in ethanol metabolism. Alcohol Res 34:339–44.

If IH, Wb PA. (2017). Human Protein Atlas [website]. Available from: http://www.proteinatlas.org/ENSG00000197894-ADH5/tissue.

Iyera AKV, Rojansakulb Y, Neelam A. (2011). Nitrosothiol signaling and protein nitrosation in cell death. Biol Bull 221:18–34.

Jaffrey SR, Snyder SH. (2001). The biotin switch method for the detection of S-nitrosylated proteins. Sci STKE 2001:pl1.

Jelski W, Orywal K, Panek B, *et al*. (2009). The activity of class I, II, III and IV of alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) in the wall of abdominal aortic aneurysms. Exp Mol Pathol 87:59–62.

Jelski W, Szmitkowski M. (2008). Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in the cancer diseases. Clin Chim Acta 395:1–5.

Jensen DE, Belka GK, Du Bois GC. (1998). S-Nitrosoglutathione is a substrate for rat alcohol dehydrogenase class III isoenzyme. Biochem J 331:659–68.

Jiang H, Polhemus DJ, Islam KN, *et al*. (2016). Nebivolol acts as a S-nitrosoglutathione reductase inhibitor: a new mechanism of action. J Cardiovasc Pharmacol Ther 21:478–85.

Julià P, Pareś X, Jörnvall H. (1988). Rat liver alcohol dehydrogenase of class III. Primary structure, functional

consequences and relationships to other alcohol dehydrogenases. Eur J Biochem 172:73–83.
Kaiser R, Holmquist B, Hempel J, *et al*. (1988). Class III human liver alcohol dehydrogenase: a novel structural type equidistantly related to the class I and class II enzymes. Biochemistry 27:1132–40.
Kelm M, Schrader J. (1990). Control of coronary vascular tone by nitric oxide. Circ Res 66:1561–75.
Klatt P, Lamas S. (2000). Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. Eur J Biochem 267:4928–44.
Koivusalo M, Baumann M, Uotila L. (1989). Evidence for the identity of glutathione-dependent formaldehyde dehydrogenase and class III alcohol dehydrogenase. FEBS Lett 257:105–9.
Lancaster JR. (2008). Protein cysteine thiol nitrosation: maker or marker of reactive nitrogen species-induced nonerythroid cellular signaling? Nitric Oxide – Biol Chem 19:68–72.
Laniewska-Dunaj M, Jelski W, Orywal K, *et al*. (2013). The activity of class I, II, III and IV of alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) in brain cancer. Neurochem Res 38:1517–21.
Lee SL, Chau GY, Yao CT, *et al*. (2006). Functional assessment of human alcohol dehydrogenase family in ethanol metabolism: significance of first-pass metabolism. Alcohol Clin Exp Res 30:1132–42.
Lee SL, Wang MF, Lee AI, Yin SJ. (2003). The metabolic role of human ADH3 functioning as ethanol dehydrogenase. FEBS Lett 544:143–7.
Leikauf GD. (1992). Mechanisms of aldehyde-induced bronchial reactivity: role of airway epithelium. Res Rep Health Eff Inst 1–35.
Leterrier M, Chaki M, Airaki M, *et al*. (2011). Function of S-nitrosoglutathione reductase (GSNOR) in plant development and under biotic/abiotic stress. Plant Signal Behav 6:789–93.
Leung J, Wei W, Liu L. (2013). S-nitrosoglutathione reductase deficiency increases mutagenesis from alkylation in mouse liver. Carcinogenesis 34:984–9.
Lima B, Lam GKW, Xie L, *et al*. (2009). Endogenous S-nitrosothiols protect against myocardial injury. Proc Natl Acad Sci USA 106:6297–302.
Liu L, Hausladen A, Zeng M, *et al*. (2001). A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans. Nature 410:490–4.
Liu L, Yan Y, Zeng M, *et al*. (2004). Essential roles of S-nitrosothiols in vascular homeostasis and endotoxic shock. Cell 116:617–28.
Liu T, Schroeder HJ, Zhang M, *et al*. (2016). S-nitrosothiols dilate the mesenteric artery more potently than the femoral artery by a cGMP and L-type calcium channel-dependent mechanism. Nitric Oxide 58:20–7.
Luo X, Kranzler HR, Zuo L, Huiping Zhang JG. (2008). ADH7 variation modulates extraversion and conscientiousness in substance dependent subjects. Am J Med Genet B Neuropsychiatr Genet 48:1–6.
Lutwak-Mann C. (1938). Alcohol dehydrogenase of animal tissues. Biochem Lab Cambridge 32:1364–74.
Luzina IG, Lockatell V, Todd NW, *et al*. (2015). Pharmacological in vivo inhibition of S-nitrosoglutathione reductase attenuates bleomycin-induced inflammation and fibrosis. J Pharmacol Exp Ther 355:13–22.
MacMicking J, Xie Q, Nathan C. (1997). Nitric oxide and macrophage function. Annu Rev Immunol 15:323–50.
Martínez-Ruiz A, Lamas S. (2004). S-nitrosylation: a potential new paradigm in signal transduction. Cardiovasc Res 62:43–52.
Methner C, Chouchani ET, Buonincontri G, *et al*. (2014). Mitochondria selective S-nitrosation by mitochondria-targeted S-nitrosothiol protects against post-infarct heart failure in mouse hearts. Eur J Heart Fail 16:712–7.
Molotkov A, Fan X, Deltour L, *et al*. (2002). Stimulation of retinoic acid production and growth by ubiquitously expressed alcohol dehydrogenase Adh3. Proc Natl Acad Sci USA 99:5337–42.
Moncada S, Palmer RM, Higgs EA. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 43:109–42.
Montagna C, Di Giacomo G, Rizza S, *et al*. (2014). S-nitrosoglutathione reductase deficiency-induced S-nitrosylation results in neuromuscular dysfunction. Antioxid Redox Signal 21:570–87.
Moore PE, Ryckman KK, Williams SM, *et al*. (2009). Genetic variants of GSNOR and ADRB2 influence response to albuterol in African-American children with severe asthma. Pediatr Pulmonol 44:649–54.
Moulis JM, Holmquist B, Vallee BL. (1991). Hydrophobic anion activation of human liver chi chi alcohol dehydrogenase. Biochemistry 30:5743–9.
Münzel T, Heitzer T, Harrison DG. (1997). The physiology and pathophysiology of the nitric oxide/superoxide system. Herz 22:158–72.
Murray CI, Uhrigshardt H, O'Meally RN, *et al*. (2012). Identification and quantification of S-nitrosylation by cysteine reactive tandem mass tag switch assay. Mol Cell Proteomics 11:M111.013441.
Musicki B, Lagoda G, Goetz T, *et al*. (2016). Transnitrosylation: a factor in nitric oxide-mediated penile erection. J Sex Med 13:808–14.
Nakamura K, Koga Y, Sakai H, *et al*. (2007). cGMP-dependent relaxation of smooth muscle is coupled with the change in the phosphorylation of myosin phosphatase. Circ Res 101:712–22.
Oscar-Berman M, Marinkovic K. (2003). Alcoholism and the brain: an overview. Alcohol Alcohol Res Health 27:125–33.
Östberg LJ, Persson B, Höög J-O. (2016). Computational studies of human class V alcohol dehydrogenase – the odd sibling. BMC Biochem 17:16.
Pacher P, Beckman JS, Liaudet L. (1995). Nitric oxide and peroxynitrite in health and disease. Physiol Rev 87:315–424.
Palmer LA, deRonde K, Brown-Steinke K, *et al*. (2015). Hypoxia-induced changes in protein s-nitrosylation in female mouse brainstem. Am J Respir Cell Mol Biol 52:37–45.
Pontel LB, Rosado IV, Burgos-Barragan G, *et al*. (2015). Endogenous formaldehyde is a hematopoietic stem cell genotoxin and metabolic carcinogen. Mol Cell 60:177–88.
Prudente A, Favaro WJ, Reis LO, Riccetto CLZ. (2017). Nitric oxide coating polypropylene mesh increases angiogenesis and reduces inflammatory response and apoptosis. Int Urol Nephrol 49:597–605.
Puetz S, Lubomirov LT, Pfitzer G. (2009). Regulation of smooth muscle contraction by small GTPases. Physiology (Bethesda) 24:342–56.

Que LG, Liu L, Yan Y, *et al*. (2005). Protection from experimental asthma by an endogenous bronchodilator. Science 308:1618–21.

Que LG, Yang Z, Stamler JS, *et al*. (2009). S-nitrosoglutathione reductase. Am J Respir Crit Care Med 180:226–31.

Radi R, Beckman JS, Bush KM, Freeman BA. (1991). Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. J Biol Chem 266:4244–50.

Rahman I, MacNee W. (2000). Oxidative stress and regulation of glutathione in lung inflammation. Eur Respir J 16:534–54.

Ramchandani VA, Bosron WF, Li TK. (2001). Research advances in ethanol metabolism. Pathol Biol 49:676–82.

Rastaldo R, Pagliaro P, Cappello S, *et al*. (2007). Nitric oxide and cardiac function. Life Sci 81:779–93.

Ricciardolo FLM, Sterk PJ, Gaston B, Folkerts G. (2004). Nitric oxide in health and disease of the respiratory system. Physiol Rev 84:731–65.

Rizza S, Cirotti C, Montagna C, *et al*. (2015). S-nitrosoglutathione reductase plays opposite roles in SH-SY5Y models of Parkinson's disease and amyotrophic lateral sclerosis. Mediators Inflamm 2015:536238. doi:10.1155/2015/536238

Roux E, Mbikou P, Fajmut A. (2012). Role of protein kinase network in excitation–contraction coupling in smooth muscle cell. In: Da Silva Xavier G, ed. Protein kinases. InTech.

Salisbury D, Bronas U. (2015). Reactive oxygen and nitrogen species. Nurs Res 64:53–66.

Salvador Moncada AH. (1994). The l-arginine–nitric oxide pathway. N Engl J Med 329:2002–12.

Sanghani PC, Bosron WF, Hurley TD. (2002). Human glutathione-dependent formaldehyde dehydrogenase. Structural changes associated with ternary complex formation. Biochemistry 41:15189–94.

Sanghani PC, Davis WI, Fears SL, *et al*. (2009). Kinetic and cellular characterization of novel inhibitors of S-nitrosoglutathione reductase. J Biol Chem 284:24354–62.

Sanghani PC, Davis WI, Zhai L, Robinson H. (2006). Structure–function relationships in human glutathione-dependent formaldehyde dehydrogenase. Role of Glu-67 and Arg-368 in the catalytic mechanism. Biochemistry 45:4819–30.

Sanghani PC, Stone CL, Ray BD, *et al*. (2000). Kinetic mechanism of human glutathione-dependent formaldehyde. Methods 39:10720–9.

Schmidt HHHW, Walter U. (1994). NO at work. Cell 78:919–25.

Sears CE, Ashley EA, Casadei B. (2004). Nitric oxide control of cardiac function: is neuronal nitric oxide synthase a key component? Philos Trans R Soc Lond B Biol Sci 359:1021–44.

Sengupta R, Holmgren A. (2012a). Thioredoxin and thioredoxin reductase in relation to reversible S-nitrosylation. Antioxid Redox Signal 18:259–69.

Sengupta R, Holmgren A. (2012b). The role of thioredoxin in the regulation of cellular processes by S-nitrosylation. Biochim Biophys Acta – Gen Subj 1820:689–700.

Shahani N, Sawa A. (2011). Nitric oxide signaling and nitrosative stress in neurons: role for S-nitrosylation. Antioxid Redox Signal 14:1493–504.

Sharma CP, Fox EA, Holmquist B, *et al*. (1989). cDNA sequence of human class III alcohol dehydrogenase. Biochem Biophys Res Commun 164:631–7.

Shi YF, Wang DL, Wang C, *et al*. (2015). Loss of GSNOR1 function leads to compromised auxin signaling and polar auxin transport. Mol Plant 8:1350–65.

Smith BC, Marletta MA. (2012). Mechanisms of S-nitrosothiol formation and selectivity in nitric oxide signaling. Curr Opin Chem Biol 16:498–506.

Smith M. (1986). Genetics of human alcohol and aldehyde dehydrogenases. Adv Hum Genet 15:249–90.

Snyder AH, McPherson ME, Hunt JF, *et al*. (2002). Acute effects of aerosolized S-nitrosoglutathione in cystic fibrosis. Am J Respir Crit Care Med 165:922–6.

Squadrito GL, Pryor WA. (1998). Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. Free Radic Biol Med 25:392–403.

Staab CA, Ålander J, Brandt M, *et al*. (2008). Reduction of S-nitrosoglutathione by alcohol dehydrogenase 3 is facilitated by substrate alcohols via direct cofactor recycling and leads to GSH-controlled formation of glutathione transferase inhibitors. Biochem J 413:493–504.

Staab CA, Ålander J, Morgenstern R, *et al*. (2009). The Janus face of alcohol dehydrogenase 3. Chem Biol Interact 178:29–35.

Staab CA, Hellgren M, Höög JO. (2008). Dual functions of alcohol dehydrogenase 3: implications with focus on formaldehyde dehydrogenase and S-nitrosoglutathione reductase activities. Cell Mol Life Sci 65:3950–60.

Stamler JS, Simon DI, Osborne JA, *et al*. (1992). S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. Proc Natl Acad Sci USA 89:444–8.

Straub AC, Billaud M, Johnstone SR, *et al*. (2011). Compartmentalized connexin 43 s-nitrosylation/denitrosylation regulates heterocellular communication in the vessel wall. Arterioscler Thromb Vasc Biol 31:399–407.

Sun J, Xin C, Eu JP, *et al*. (2001). Cysteine-3635 is responsible for skeletal muscle ryanodine receptor modulation by NO. Proc Natl Acad Sci USA 98:11158–62.

Sun X, Qiu J, Strong SA, *et al*. (2011b). Discovery of potent and novel S-nitrosoglutathione reductase inhibitors devoid of cytochrome P450 activities. Bioorg Med Chem Lett 21:5849–53.

Sun X, Qiu J, Strong SA, *et al*. (2011a). Structure–activity relationships of pyrrole based S-nitrosoglutathione reductase inhibitors: pyrrole regioisomers and propionic acid replacement. Bioorg Med Chem Lett 21:3671–5.

Sun X, Qiu J, Strong SA, *et al*. (2012). Structure–activity relationship of pyrrole based S-nitrosoglutathione reductase inhibitors: carboxamide modification. Bioorg Med Chem Lett 22:2338–42.

Svensson S, Strömberg P, Sandalova T, Höög J. (2001). Class II alcohol dehydrogenase (ADH2) – adding the structure. Chem Biol Interact 130–132:339–50.

Tamargo J, Caballero R, Gomez R, Delpon E. (2010). Cardiac electrophysiological effects of nitric oxide. Cardiovasc Res 87:593–600.

Tang CH, Seeley EJ, Huang X, *et al*. (2013b). Increased susceptibility to *Klebsiella pneumonia* and mortality in GSNOR-deficient mice. Biochem Biophys Res Commun 442:122–6.

Tang C-H, Wei W, Hanes MA, Liu L. (2013a). Hepatocarcinogenesis driven by GSNOR deficiency is prevented by iNOS inhibition. Cancer Res 73:2897–904.

Tang C-H, Wei W, Liu L. (2012). Regulation of DNA repair by S-nitrosylation. Biochim Biophys Acta 1820:730–5.

Theorell H, Yonetani T, Sjöberg B. (1969). On the effects of some heterocyclic compounds on the enzymic activity of liver alcohol dehydrogenase. Acta Chem Scand 23:255–60.

Thomas DD, Jourd'heuil D. (2012). S-Nitrosation: current concepts and new developments. Antioxid Redox Signal 17:934–6.

Thompson CM, Grafström RC. (2007). Mechanistic considerations for formaldehyde-induced bronchoconstriction involving S-nitrosoglutathione reductase. J Toxicol Environ Health A 71:244–8.

Tomita R, Fujisaki S, Ikeda T, Fukuzawa M. (2002). Role of nitric oxide in the colon of patients with slow-transit constipation. Dis Colon Rectum 45:593–600.

Ulrich C, Quilici DR, Schlauch KA, Buxton ILO. (2013). The human uterine smooth muscle S-nitrosoproteome fingerprint in pregnancy, labor, and preterm labor. AJP Cell Physiol 305:C803–16.

Ulrich C, Quillici DR, Schegg K, *et al*. (2012). Uterine smooth muscle S-nitrosylproteome in pregnancy. Mol Pharmacol 81:143–53.

Valko M, Leibfritz D, Moncol J, *et al*. (2007). Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84.

Wagner FW, Pares X, Holmquist B, Vallee BL. (1984). Physical and enzymatic properties of a class III isozyme of human liver alcohol dehydrogenase: chi-ADH. Biochemistry 23:2193–9.

Wain HM, Wain HM, Bruford EA, *et al*. (2002). Guidelines for human gene nomenclature. Genomics 79:464–70.

Wei W, Li B, Hanes MA, *et al*. (2010). S-nitrosylation from GSNOR deficiency impairs DNA repair and promotes hepatocarcinogenesis. Sci Transl Med 2:19ra13.

Wei W, Yang Z, Tang CH, Liu L. (2011). Targeted deletion of GSNOR in hepatocytes of mice causes nitrosative inactivation of O6-alkylguanine-DNA alkyltransferase and increased sensitivity to genotoxic diethylnitrosamine. Carcinogenesis 32:973–7.

Wink DA, Mitchell JB. (1998). Chemical biology of nitric oxide: insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. Free Radic Biol Med 25:434–56.

Wu H, Romieu I, Rio BE, *et al*. (2007). Genetic variation in S-nitrosoglutathione reductase (GSNOR) and childhood asthma. J Allergy Clin Immunol 120:322–8.

Wu K, Ren R, Su W, *et al*. (2014). A novel suppressive effect of alcohol dehydrogenase 5 in neuronal differentiation. J Biol Chem 289:20193–9.

Xie S-H, Liu A-L, Chen Y-Y, *et al*. (2010). DNA damage and oxidative stress in human liver cell L-02 caused by surface water extracts during drinking water treatment in a waterworks in China. Environ Mol Mutagen 51:229–35.

Xu W, Liu LZ, Loizidou M, *et al*. (2002). The role of nitric oxide in cancer. Cell Res 12:311–20.

Yang Z, Wang Z-E, Doulias P-T, *et al*. (2010). Lymphocyte development requires S-nitrosoglutathione reductase. J Immunol 185:6664–9.

Yang ZN, Bosron WF, Hurley TD. (1997). Structure of human chi chi alcohol dehydrogenase: a glutathione-dependent formaldehyde dehydrogenase. J Mol Biol 265:330–43.

Yun B, Skelly MJ, Yin M, *et al*. (2016). Nitric oxide and S-nitrosoglutathione function additively during plant immunity. New Phytol 211:516–26.

Zaman K, Fraser-Butler M, Bennett D. (2013). Novel s-nitrosothiols have potential therapeutic uses for cystic fibrosis. Curr Pharm Des 19:3509–20.

Zaman K, McPherson M, Vaughan J, *et al*. (2001). S-nitrosoglutathione increases cystic fibrosis transmembrane regulator maturation. Biochem Biophys Res Commun 284:65–70.

Zuo L, Wang K, Zhang XY, *et al*. (2013). Association between common alcohol dehydrogenase gene (ADH) variants and schizophrenia and autism. Hum Genet 132:735–43.