



Gasotransmitters in health and disease: a mitochondria-centered view

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Gasotransmitters fulfill important roles in cellular homeostasis having been linked to various pathologies, including inflammation and cardiovascular diseases. In addition to the known pathways mediating the actions of gasotransmitters, their effects in regulating mitochondrial function are emerging. Given that mitochondria are key organelles in energy production, formation of reactive oxygen species and apoptosis, they are important mediators in preserving health and disease. Preserving or restoring mitochondrial function by gasotransmitters may be beneficial, and mitigate pathogenetic processes. In this review we discuss the actions of gasotransmitters with focus on their role in mitochondrial function and their therapeutic potential.

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Introduction

Gasotransmitters are small, chemically reactive, molecules with short half-lives that played crucial roles in the development of life. Nitric oxide (NO) and carbon monoxide (CO) were the first described and best-known gasotransmitters, with hydrogen sulfide (H₂S) being discovered more recently. Given that gasotransmitters

diffuse freely across cellular membranes, they can potentially regulate a broad range of important cellular functions throughout the body. These include regulating vascular tone [1], neuromodulation [2], paracrine cell signaling [3], and mitochondrial function [4]. Because of their effect on key cellular functions, any disturbance in their availability is linked to a variety of pathological conditions. The mitochondrion is an organelle targeted by gasotransmitters where they modulate mitochondrial function, including adenosine triphosphate (ATP) production, reactive oxygen species (ROS) formation and initiation of apoptotic cascades, which are all important mediators in inflammation and disease.

The present review provides an overview of recent findings on the role of gasotransmitters modulating inflammation, disease pathogenesis, and mitochondrial function. It also explores avenues to target enzyme activity or supply gasotransmitter donors as therapeutic interventions.

Gasotransmitter synthesis and bioavailability

Several enzymes can produce gasotransmitters. NO is formed by the conversion of L-arginine to L-citrulline, an oxidative process regulated by three subtypes of nitric oxide synthases (NOS) with different expression levels in different cells: neuronal (nNOS), endothelial (eNOS) and inducible (iNOS) nitric oxide synthase. Within a cell, iNOS and nNOS are mainly cytosolic, although nuclear localization of nNOS in rat astrocytes has been reported [5]. eNOS is membrane-bound, to facilitate release of NO to the extracellular environment.

CO is synthesized by conversion of heme to biliverdin through heme oxygenase (HO), an enzyme that occurs in three different isoforms: HO-1, HO-2 and HO-3. HO is mainly located in the endoplasmic reticulum (ER), but similar to NOS, HO is also present in the mitochondria [6].

H₂S is derived from cysteine by enzymatic reactions catalyzed by mainly cytosolic cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and cysteine aminotransferase (CAT). However, in line with the mitochondrial NO and CO, CBS and CSE translocate to mitochondria during cellular stress such as hypoxia [7]. Additionally, H₂S is produced directly within mitochondria by 3-mercaptopyruvate sulfur-transferase (3MST) [8]. Summarizing, the production of gasotransmitters is regulated by different

enzymes, of which spatial expression patterns differ between organs and cell types. All gasotransmitters can be produced near or inside mitochondria, which indicates a potentially important role of these molecules in mitochondrial function.

A simplified overview of the synthesis and bioavailability of gasotransmitters is outlined in Figure 1.

Gasotransmitters in physiology and disease

A plethora of physiological effects of gasotransmitters have been documented. For instance, gasotransmitters, both via direct intracellular effects and released in the extracellular space, play an important role in regulation of vascular tone, reduce oxidative stress, and induce angiogenesis [9]. More specifically, CO is involved in regulation of endothelial cell survival and proliferation, protection from ischemia-reperfusion injury (IRI), vasorelaxation and inhibition of pro-inflammatory responses. HO-1 acts as an inflammation-neutralizing factor regulated by nuclear-factor-E2-related factor-2 (Nrf2), as observed in lung inflammation after intestinal IRI [10]. NO regulates numerous intra-cellular and inter-cellular processes such as platelet aggregation, endothelial adhesion of leukocytes and relaxation of smooth muscle cells. Moreover, iNOS activated by nuclear-factor-kappa B (NF-κB) activation and signal-transducer-and-activator-of-transcription-1α (STAT-1α) results in elevated NO levels and represents an important component in the inflammatory response [11]. Excess production of NO, leading to

nitrosative stress, is correlated with the severity of liver disease in mice [12]. In contrast, the anti-inflammatory action of NO is revealed in iNOS-knockout high-fat-diet fed mice that show an increased inflammation leading to liver fibrosis [13]. These data indicate that NO harbors potential to exert both pro-inflammatory and anti-inflammatory functions, most likely in a dose-dependent manner. H₂S has important anti-inflammatory and antioxidant potential, and causes relaxation of blood vessels [14]. H₂S protects endothelial cells from lipopolysaccharide (LPS)-induced inflammation by blocking NF-κB transactivation [15]. In addition, exogenous H₂S treatment decreased inflammation and IRI following intestinal ischemia, whereas eNOS knockout mice were not protected by exogenous H₂S. These data suggest that H₂S shows protective effects in an eNOS-dependent manner [16]. NADPH oxidase (Nox), a mitochondrial source of ROS, is a key-signaling pathway responsible for the increased inflammatory response of macrophages *in vitro* and in septic mice [17,18**], which could be ameliorated by endogenous H₂S.

Reduced bioavailability of gasotransmitters has been observed in vascular pathology [19], aging [20] and aging-related pathologies [21], renal pathology [22] and diabetes [23] (Figure 2). These associations suggest causality between gasotransmitter bioavailability and disease pathogenesis.

The various pathways, in which gasotransmitters are involved in disease pathogenesis and inflammation become of even more interest when looking at mitochondrial dysfunction, for example, in sepsis. Brealey *et al.* demonstrated lowered ATP levels, overproduction of NO, and mitochondrial dysfunction in skeletal muscle biopsies of septic patients [24]. Using H₂S and CO, potentiation of mitochondrial function could preserve tissue function during sepsis [25*]. The authors suggested various therapeutic interventions to increase exogenous and endogenous H₂S production, to specifically inhibit iNOS and to stimulate HO-1 activity, in order to target mitochondrial pathways in sepsis and inflammation.

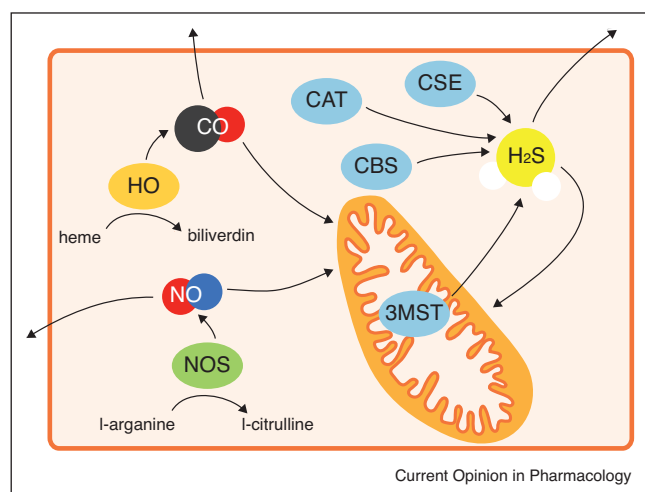
A schematic overview of some of the involved pathways is shown in Figure 2.

Mitochondrial aspects of gasotransmitters

Mitochondria, ‘the powerhouses of the cell’ represent the main source of energy using oxidative phosphorylation, but also modulate important regulatory and signaling processes. In oxidative phosphorylation, mitochondria oxidize substrates via the electron transport chain (ETC) to create a proton gradient, which is used to drive the ATP synthesis. Gasotransmitters regulate this process, supporting normal physiology.

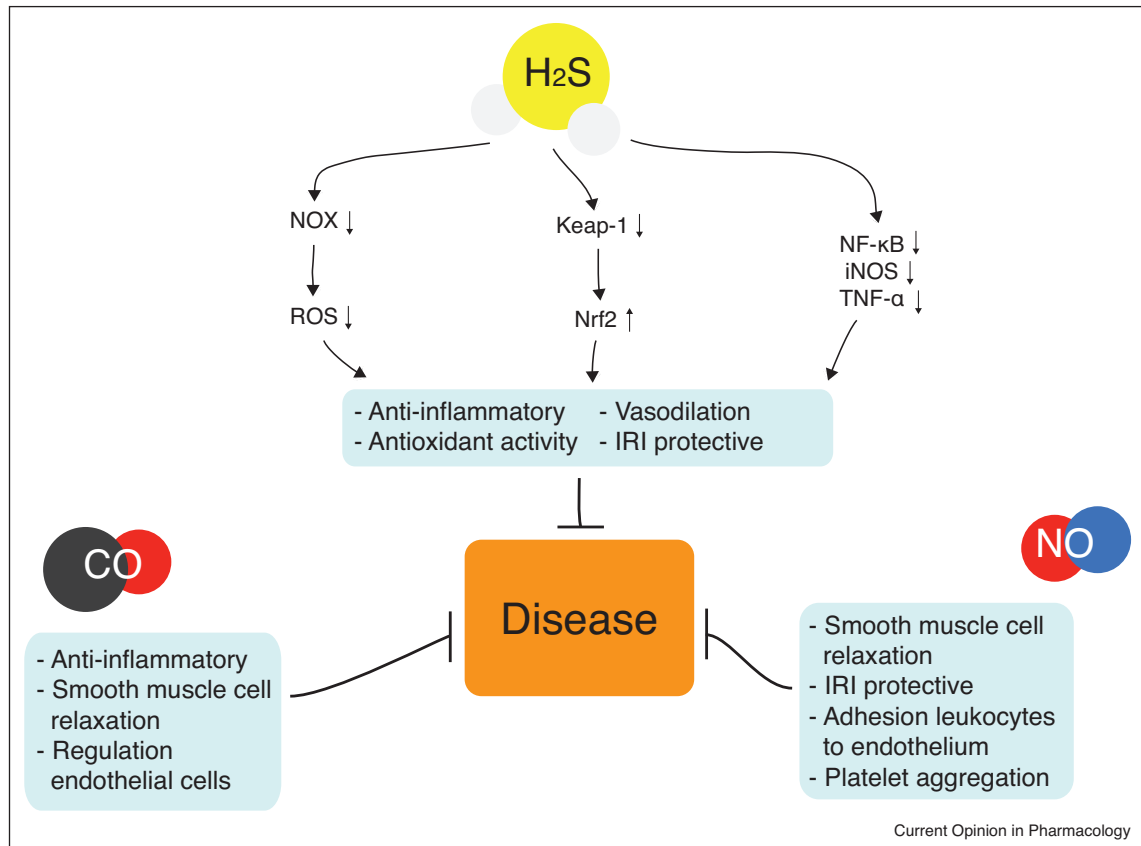
NO, CO, and H₂S all reduce the ETC activity via inhibition of cytochrome c oxidase (COX) in a reversible,

Figure 1



A general overview of the cellular synthesis and bioavailability of gasotransmitters within a cell. 3MST (3-mercaptopyruvate sulfur-transferase), CBS (cystathionine β-synthase), CSE, (cystathionine γ-lyase) and CAT (cysteine aminotransferase) produce H₂S (hydrogen sulfide). HO (heme oxygenase) produces CO (carbon monoxide). NO (Nitric oxide) is produced by NOS (nitric oxide synthase).

Figure 2



A schematic overview of some of the disease-related pathways gasotransmitters are involved in. All three gasotransmitters, H₂S (hydrogen sulfide), CO (carbon monoxide) and NO (nitric oxide) showed mitigating effects in a variety of diseases. IRI = ischemia reperfusion injury.

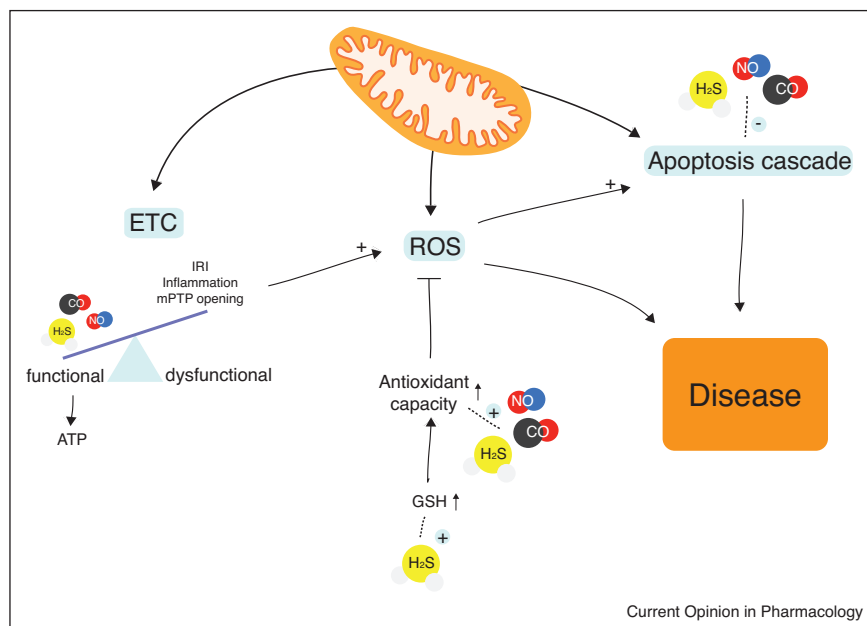
fast-acting and dose-dependent manner [1]. Accordingly, gasotransmitters may preserve normal ETC function. Indeed, administration of NO and CO protected mitochondria, presumably by decreasing ROS production, during hemorrhagic shock [26]. Furthermore, upregulation of HO-1 normalized mitochondrial function and decreased ROS formation in IRI [27]. Also H₂S protects the ETC through different mechanisms [28^{*}]. In line with this, CSE knockout mice are more susceptible to cerebral IRI compared to controls; which could be reversed using exogenous H₂S [29]. Interestingly, in contrast to NO and CO, H₂S can act as hydrogen donor and functions as substrate for mitochondrial respiration [30].

High-dose treatment with CO, NO or H₂S can almost completely inhibit mitochondrial activity, and especially H₂S harbors the potential to suppress metabolism in a safe manner: the induction of a hypometabolic state [31,32^{*}]. This hibernation-like state has is protective to IRI, thereby having therapeutic potential in, for example, organ transplantation [33].

Besides direct effects on mitochondrial function, gasotransmitters play an important role in ROS scavenging. NO is a potent antioxidant by virtue of its fast reaction with hydroxyl radicals, superoxides and lipid peroxides [34]. Exogenous H₂S administration protected cardiac tissue from ROS damage in a myocardial injury rat model [35].

In addition to the direct scavenging potential, gasotransmitters are also important in the activation of scavenging pathways, such as Nrf2 and glutathione (GSH). Kelch-like-ECH-associated-protein-1 (Keap1) serves as a negative regulator of Nrf2, during stress-free physiology, by binding to Nrf2 in the cytoplasm and promoting degradation of Nrf2. Cellular stress provoked by ROS, inactivates Keap1 and therefore stabilizes Nrf2, allowing translocation to the nucleus and activation of its target: the antioxidant-response-element (ARE) [36,37]. H₂S can promote Keap1-dependent Nrf2 stabilization, which facilitates Nrf2 translocation into the nucleus [38]. Indeed, exogenous NaHS administration to a diabetic

Figure 3



A simplified overview of the interactions between gasotransmitters and mitochondria.

ETC = electron transport chain, ROS = reactive oxygen species, GSH = glutathione, IRI = ischemia reperfusion injury.

stressed rat model resulted in increased nuclear Nrf2 levels, activation of superoxide dismutase (SOD) and limited the numbers of apoptotic cells [39]. Besides increasing GSH production, H₂S is thought to redistribute GSH into the mitochondria to directly scavenge mitochondrial-produced superoxides [40]. CO exposure in transplanted rat lungs protected against apoptosis, likely via increased SOD activity and decreased ROS-induced damage [41].

Another important pathway that gasotransmitters are involved in is the opening of the mitochondrial permeability transition pore (mPTP). Full opening of these pores in response to several factors including excessive ROS production and calcium-overload, results in a loss of mitochondrial membrane potential and reduced oxidative phosphorylation, mitochondrial swelling and a burst of ROS, eventually leading to necrosis or apoptosis [42]. Exogenous H₂S inhibits apoptosis via blockade of mPTP formation and cytochrome c (cyt c) release [43]. Apoptosis can be activated by the Bcl2-family, cyt c release and caspase activation. Both NO and CO are known to suppress the Bcl2-family and caspase activation [44,45].

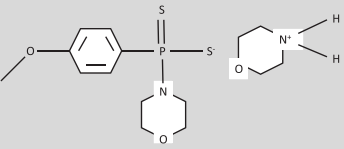
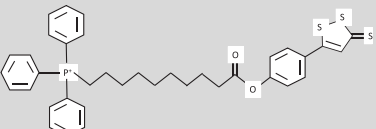

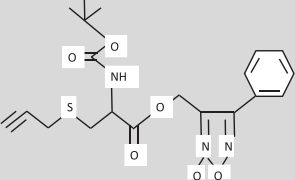
These findings indicate that gasotransmitters have an important role in the cellular energetic state and apoptosis by regulating several mitochondrial-related and ROS-related actions, as outlined in Figure 3.

Treatment perspectives

Exogenous administration of gasotransmitters is an emerging therapeutic option. The oldest and most used donor is the acute NO donor nitroglycerin, causing vasodilation and relieving acute pain during angina pectoris. Another clinically relevant NO donor in current use is sodium nitroprusside (SNP), also playing an important role in vasorelaxation. On the basis of these successes, several NO donors were synthesized, among which combined therapeutics, such as NO-NSAID [46]. Additionally, downstream NO-modulating drugs were tested, for example, the phosphodiesterase 5 (PDE5) inhibitor sildenafil [47]. Sildenafil treatment increased activity of the NO/cGMP pathway and protected from oxidative damage and apoptosis in diabetes [48] and cardiovascular dysfunction [49]. In contrast, recent findings in pregnant women with fetal growth restriction revealed detrimental effects of sildenafil treatment [50]. In line with the functions of CO, carbon monoxide-releasing-molecules (CORMs) have anti-apoptotic, anti-inflammatory, and antioxidant effects [51]. The fast releasing H₂S donors NaHS and Na₂S are widely used in the experimental setting and induce a hypometabolic state [32^{*}]. However, these donors are not suitable for precise and sustained administration. A potential alternative can be found in thiosulfate (STS). STS showed positive effects on hypertension and renal injury [52]. The potential of STS on reducing cardiac ischemia is now being clinically tested.

Table 1

Overview of different exogenous gasotransmitter donors

Compound	Chemistry	Characteristics
GY4137 [58]	$C_{11}H_{16}NO_2PS_2 \cdot C_4H_{10}NO$ 	Slow-releasing H ₂ S donor
AP39 [54**,59]	$C_{37}H_{38}O_2PS_3$ 	Mitochondria-targeted H ₂ S donor
DATS-MSN [53,60]	$C_6H_{10}S_3$ [DATS] 	Trisulfide (DATS) conjugated to a mesoporous silica nanoparticles (MSN) carrier
ZYZ-803 [56,57**]	$C_{20}H_{23}N_3O_6S$ 	Slow-releasing NO/H ₂ S hybrid molecule

Recently, to exploit the protective properties of H₂S, slow-releasing H₂S molecules have been synthesized, including morpholin-4-ium 4-methoxyphenyl (morpholino) phosphinodithioate (GY4137), 10-oxo-10-[4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy]decyltriphenylphosphonium (AP39), and a natural garlic-derived polysulfide compound—diallyl trisulfide (DATS) conjugated to a mesoporous silica nanoparticles (MSN) carrier (DATS-MSN) (Table 1). Whereas GYY4137 is not specifically targeted, AP39 is a mitochondria-targeted H₂S donor, with potent protective effects in an organ transplantation model [54**]. DATS-MSN shows superior anti-apoptotic, antioxidant and anti-inflammatory abilities as compared to NaHS [53]. Also ROS-triggered H₂S donors [55] and slow-releasing NO/H₂S hybrid molecules have been developed (e.g. ZYZ-803) [56] (Table 1), their use showing promising protective effects against heart failure [57**].

Conclusion

Gasotransmitters play a key role in the pathogenesis of various diseases, with a unifying role in preservation of mitochondrial function. H₂S, CO, and NO contribute to maintaining normal mitochondrial function and show a broad variety of potential therapeutic properties: influencing ETC activity, direct scavenging of ROS, activation of scavenging pathways, and attenuation of apoptosis. Accordingly, gasotransmitters are potential efficacious drugs and this insight has led to the synthesis of long-lasting and slow-releasing donors. Although promising results have been obtained in experimental disease

models, these compounds have not been extensively tested in the clinic. This urges the need for more extensive research and new compounds. A mitochondrial targeted combination of H₂S–NO–CO donor is an attractive concept to protect mitochondria from noxious insults; whether this concept is actually feasible remains to be seen in the near future.

Conflict of interest statement

Nothing declared.

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