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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 (H_2S) 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].

H_2S 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, H_2S 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. H_2S has important anti-inflammatory and antioxidant potential, and causes relaxation of blood vessels [14]. H_2S protects endothelial cells from lipopolysaccharide (LPS)-induced inflammation by blocking NF-κB transactivation [15]. In addition, exogenous H_2S treatment decreased inflammation and IRI following intestinal ischemia, whereas eNOS knockout mice were not protected by exogenous H_2S. These data suggest that H_2S 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 H_2S.

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 H_2S and CO, potentiation of mitochondrial function could preserve tissue function during sepsis [25•]. The authors suggested various therapeutic interventions to increase exogenous and endogenous H_2S production, to specifically inhibit iNOS and to stimulate HO-1 activity, in order to target mitochondrial pathways in sepsis and inflammation.

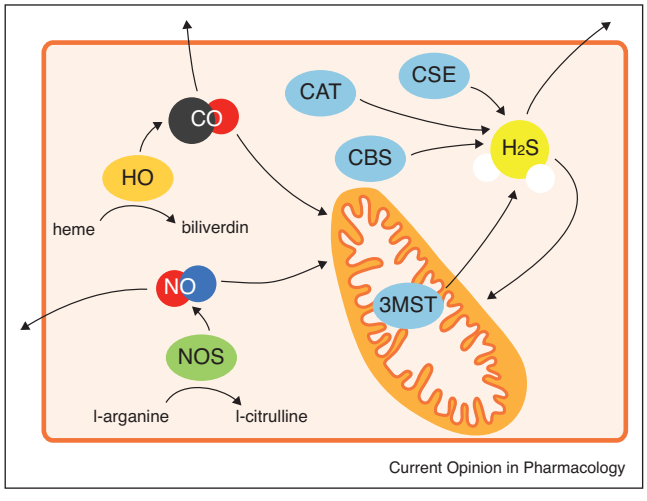
A schematic overview of some of the involved pathways is shown in Figure 2.

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.

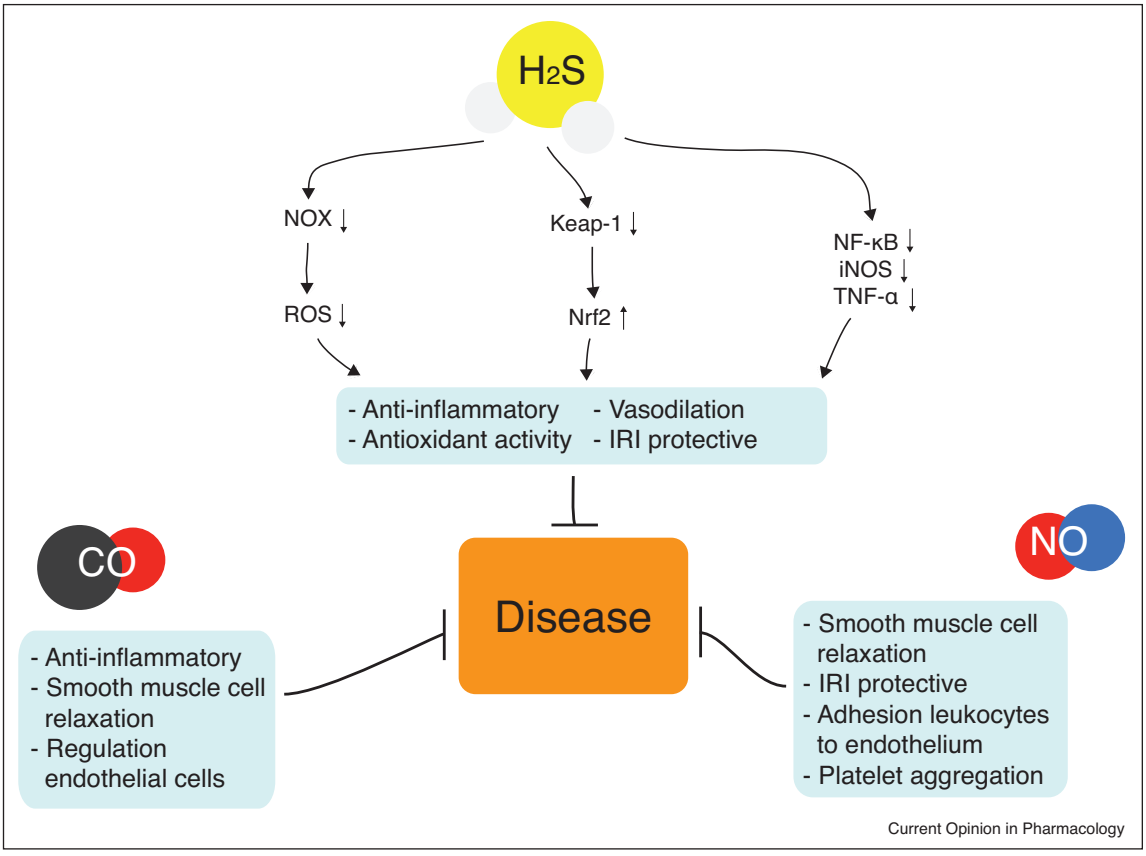
NO, CO, and H_2S all reduce the ETC activity via inhibition of cytochrome c oxidase (COX) in a reversible,

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 H_2S (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, H_2S (hydrogen sulfide), CO (carbon monoxide) and NO (nitric oxide) showed mitigating effects in a variety of diseases.
IRI = ischemia reperfusion injury.

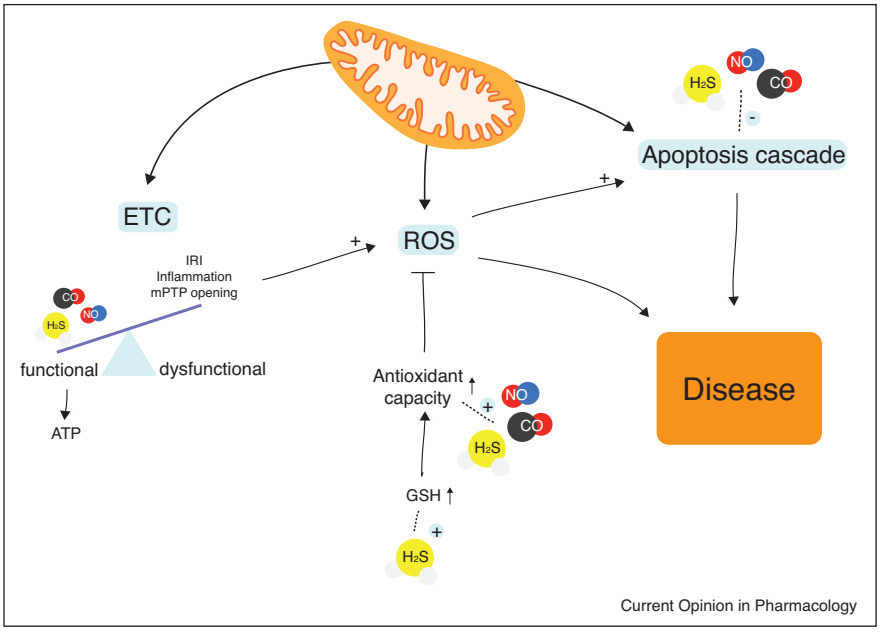
fast-acting and dose-dependent manner [1]. Accordingly, gasotransmitters may preserve normal ETC function. Indeed, administration of NO and CO protected mitochondria, presumably by decreasing ROS production, during hemorrhagic shock [26]. Furthermore, upregulation of HO-1 normalized mitochondrial function and decreased ROS formation in IRI [27]. Also H_2S 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 H_2S [29]. Interestingly, in contrast to NO and CO, H_2S can act as hydrogen donor and functions as substrate for mitochondrial respiration [30].

High-dose treatment with CO, NO or H_2S can almost completely inhibit mitochondrial activity, and especially H_2S 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 H_2S 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]. H_2S 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, H_2S 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 H_2S 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].

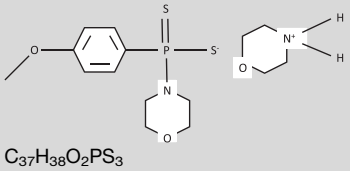
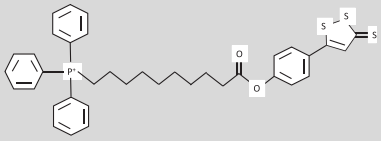
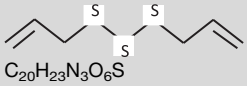
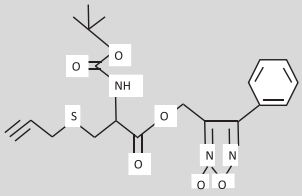
These findings indicate that gasotransmitters have an important role in the cellular energetic state and apoptosis by regulating several mitochondrial-related and ROS-related actions, as outlined in Figure 3.

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 H_2S donors NaHS and Na_2S 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
GYY4137 [58]	$C_{11}H_{16}NO_2PS_2{\cdot}C_4H_{10}NO$ 	Slow-releasing H_2S donor
AP39 [54••,59]	$C_{37}H_{38}O_2PS_3$ 	Mitochondria-targeted H_2S 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_2S hybrid molecule

Recently, to exploit the protective properties of H_2S, slow-releasing H_2S molecules have been synthesized, including morpholin-4-ium 4 methoxyphenyl (morpholino) phosphinodithioate (GYY4137), 10-oxo-10-[4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy]decyl]triphenylphosphonium (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 H_2S 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 H_2S donors [55] and slow-releasing NO/H_2S 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. H_2S, 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 H_2S–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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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Gheibi S, Jeddi S, Kashfi K, Ghasemi A: **Regulation of vascular tone homeostasis by NO and H2S: implications in hypertension**. *Biochem Pharmacol* 2018, **149**:42-59.

2. Shefa U, Kim D, Kim MS, Jeong NY, Jung J: **Roles of gasotransmitters in synaptic plasticity and neuropsychiatric conditions**. *Neural Plast* 2018:1824713. 15 pages.

3. Mustafa AK, Gadalla MM, Snyder SH: **Signaling by gasotransmitters**. *Sci Signal* 2009, **2**:re2.

4. Hartmann C, Nussbaum B, Calzia E, Radermacher P, Wepler M: **Gaseous mediators and mitochondrial function: the future of pharmacologically induced suspended animation?** *Front Physiol* 2017, **8**:691.

5. Zhou L, Zhu DY: **Neuronal nitric oxide synthase: structure, subcellular localization, regulation, and clinical implications**. *Nitric Oxide* 2009, **4**:223-230.

6. Waltz PK, Kautza B, Luciano J, Dyer M, Stolz DB, Loughran P, Neal MD, Sperry JL, Rosengart MR, Zuckerbraun BS: **Heme oxygenase-2 localizes to mitochondria and regulates hypoxic responses in hepatocytes**. *Oxid Med Cell Longev* 2018:2021645. 10 pages.

7. Teng H, Wu B, Zhao K, Yang G, Wu L, Wang R: **Oxygen-sensitive mitochondrial accumulation of cystathionine β-synthase mediated by Lon protease**. *Proc Natl Acad Sci U S A* 2013, **31**:12679-12684.

8. Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K, Kimura H: **3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain**. *Antioxid Redox Signal* 2009, **11**:703-714.

9. Wu D, Hu Q, Zhu D: **An update on hydrogen sulfide and nitric oxide interactions in the cardiovascular system**. *Oxid Med Cell Longev* 2018:4579140. 16 pages.

10. Meng QT, Cao C, Wu Y, Liu HM, Li W, Sun Q, Chen R, Xiao YG, Tang LH, Jiang Y: **Ischemic post-conditioning attenuates acute lung injury induced by intestinal ischemia-reperfusion in mice: role of Nrf2**. *Lab Invest* 2016, **96**:1087-1104.

11. Kleinert H, Pautz A, Linker K, Schwarz PM: **Regulation of the expression of inducible nitric oxide synthase**. *Eur J Pharmacol* 2004, **500**:255-266.

12. Salamone F, Galvano F, Cappello F, Mangiameli A, Barbagallo I, Li Volti G: **Silibinin modulates lipid homeostasis and inhibits nuclear factor kappa B activation in experimental nonalcoholic steatohepatitis**. *Transl Res* 2012, **159**:477-486.

13. Nozaki Y, Fujita K, Wada K, Yoneda M, Kessoku T, Shinohara Y, Imajo K, Ogawa Y, Nakamuta M, Saito S: **Deficiency of iNOS-derived NO accelerates lipid accumulation-independent liver fibrosis in non-alcoholic steatohepatitis mouse model**. *BMC Gastroenterol* 2015, **15**:42.

14. Hedegaard ER, Gouliaev A, Winther AK, Arcanjo DD, Aalling M, Renaltan NS, Wood ME, Whiteman M, Skovgaard N, Simonsen U: **Involvement of potassium channels and calcium-independent mechanisms in hydrogen sulfide-induced relaxation of rat mesenteric small arteries**. *J Pharmacol Exp Ther* 2016, **356**:53-63.

15. Bourque C, Zhang Y, Fu M, Racine M, Greasley A, Pei Y, Wu L, Wang R, Yang G: **H2S protects lipopolysaccharide-induced inflammation by blocking NFκB transactivation in endothelial cells**. *Toxicol Appl Pharmacol* 2018, **338**:20-29.

16. Jensen AR, Drucker NA, Khaneki S, Ferkowicz MJ, Markel TA: **Hydrogen sulfide improves intestinal recovery following ischemia by endothelial nitric oxide-dependent mechanisms**. *Am J Physiol Gastrointest Liver Physiol* 2017, **312**:G450-G456.

17. Liu W, Wu H, Chen L, Wen Y, Kong X, Gao WQ: **Park7 interacts with p47(phox) to direct NADPH oxidase-dependent ROS production and protect against sepsis**. *Cell Res* 2015, **25**: 691-706.

18. •• Wang XL, Pan LL, Long F, Wu WJ, Yan D, Xu P, Liu SY, Qin M, Jia WW, Liu XH, Zu YZ: **Endogenous hydrogen sulfide ameliorates nox4 induced oxidative stress in LPS-stimulated macrophages and mice**. *Cell Physiol Biochem* 2018, **47**:458-474.

Using Nox4 knockdown and CSE knockout mice, the authors demonstrated that CSE/H2S attenuated LPS-induced sepsis against oxidative stress and inflammation damage largely by mediating Nox4.

19. Bibli SI, Hu J, Sigala F, Wittig I, Heidler J, Zukunft S, Tsilimigras DI, Randriamboavonjy V, Wittig J, Kojonazarov B *et al.*: **Cystathionine γ lyase sulfhydrates the RNA binding protein human antigen R to preserve endothelial cell function and delay atherogenesis**. *Circulation* 2019, **139**:101-114.

20. Perridon BW, Leuvenink HG, Hillebrands JL, van Goor H, Bos EM: **The role of hydrogen sulfide in aging and age-related pathologies**. *Aging (Albany NY)* 2016, **8**:2264-2289.

21. Shefa U, Yeo SG, Kim MS, Song IO, Jung J, Jeong NY, Huh Y: **Role of gasotransmitters in oxidative stresses, neuroinflammation, and neuronal repair**. *BioMed Res Int* 2017, **2017**:1689341.

22. Koning AM, Frenay AS, Leuvenink HGD, van Goor H: **Hydrogen sulfide in renal physiology, disease and transplantation – the smell of renal protection**. *Nitric Oxide* 2015, **46**:37-49.

23. van den Born JC, Hammes HP, Greffrath W, van Goor H, Hillebrands JL: **Gasotransmitters in vascular complications of diabetes**. *Diabetes* 2016, **65**:331-345.

24. Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, Davies NA, Cooper CE, Singer M: **Association between mitochondrial dysfunction and severity and outcome of septic shock**. *Lancet* 2002, **360**:219-223.

25. • Reitsema VA, Star BS, de Jager VD, van Meurs M, Henning RH, Bouma HR: **Metabolic resuscitation strategies to prevent organ dysfunction in sepsis**. *Antioxid Redox Signal* 2019, **31**:134-152.

This article reviews the role and potential treatment with gasotransmitters in sepsis. The authors suggested various future perspectives on therapeutic interventions to increase exogenous and endogenous H2S production.

26. Haugaa H, Gómez H, Maberry DR, Holder A, Ogundele O, Quintero AM, Escobar D, Tønnessen TI, Airgood H, Dezfulian C *et al.*: **Effects of inhalation of low-dose nitrite or carbon monoxide on post-reperfusion mitochondrial function and tissue injury in hemorrhagic shock swine**. *Crit Care* 2015, **19**:184.

27. Chen D, Jin Z, Zhang J, Jiang L, Chen K, He X, Song Y, Ke J, Wang Y: **HO-1 protects against hypoxia/reoxygenation-induced mitochondrial dysfunction in H9c2 cardiomyocytes**. *PLoS One* 2016, **11**:e0153587.

28. • Wetzel MD, Wenke JC: **Mechanisms by which hydrogen sulfide attenuates muscle function following ischemia-reperfusion injury: effects on Akt signaling, mitochondrial function, and apoptosis**. *J Transl Med* 2019, **17**:33.

This article reviews the mechanisms of H_2S and IRI, specifically focusing on mitochondrial function.

29. Wen JY, Wang M, Li YN, Jiang HH, Sun XJ, Chen ZW: **Vascular protection of hydrogen sulfide on cerebral ischemia/reperfusion injury in rats**. *Front Neurol* 2018, **9**:779.

30. Helmy N, Prip-Buus C, Vons C, Lenoir V, Abou-Hamdan A, Guedouari-Bounihi H, Lombès A, Bouillaud F: **Oxidation of hydrogen sulfide by human liver mitochondria**. *Nitric Oxide* 2014, **41**:105-112.

31. Blackstone E, Morrison M, Roth MB: **H2S induces a suspended animation-like state in mice**. *Science* 2005, **308**:518.

32. • Dugbartey GJ, Bouma HR, Saha MN, Lobb I, Henning RH, Sener A: **A hibernation-like state for transplantable organs: is hydrogen sulfide therapy the future of organ preservation?** *Antioxid Redox Signal* 2018, **28**:1503-1515.

This article discusses mammalian hibernation as a natural model of cold organ preservation, including recent developments on protective effects and mechanisms of exogenous and endogenous H_2S in preclinical models of transplant IRI.

33. Lobb I, Davison M, Carter D, Liu W, Haig A, Gunaratnam L, Sener A: **Hydrogen sulfide treatment mitigates renal allograft ischemia-reperfusion injury during cold storage and improves early transplant kidney function and survival following allogeneic renal transplantation**. *J Urol* 2015, **194**:1806-1815.

34. Wink DA, Miranda KM, Espey MG, Pluta RM, Hewett SJ, Colton C, Vitek M, Feelisch M, Grisham MB: **Mechanisms of the antioxidant effects of nitric oxide**. *Antioxid Redox Signal* 2001, **3**:203-213.

35. Geng B, Chang L, Pan C, Qi Y, Zhao J, Pang Y, Du J, Tang C: **Endogenous hydrogen sulfide regulation of myocardial injury induced by isoproterenol**. *Biochem Biophys Res Commun* 2004, **318**:756-763.

36. Suzuki T, Yamamoto M: **Molecular basis of the Keap1-Nrf2 system**. *Free Radic Biol Med* 2015, **88**:93-100.

37. Raghunath A, Sundarraj K, Nagarajan R, Arfuso F, Bian J, Kumar AP, Sethi G, Perumal E: **Antioxidant response elements: discovery, classes, regulation and potential applications**. *Redox Biol* 2018, **17**:297-314.

38. Guo C, Liang F, Shah Masood W, Yan X: **Hydrogen sulfide protected gastric epithelial cell from ischemia/reperfusion injury by Keap1 s-sulfhydration, MAPK dependent anti-apoptosis and NF-κB dependent anti-inflammation pathway**. *Eur J Pharmacol* 2014, **725**:70-78.

39. Liu J, Wu J, Sun A, Sun Y, Yu X, Liu N, Dong S, Yang F, Zhang L, Zhong X *et al.*: **Hydrogen sulfide decreases high glucose/palmitate-induced autophagy in endothelial cells by the Nrf2-ROS-AMPK signaling pathway**. *Cell Biosci* 2016, **6**:33.

40. Kimura Y, Goto Y, Kimura H: **Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria**. *Antioxid Redox Signal* 2010, **12**:1-13.

41. Song R, Kubo M, Morse D, Zhou Z, Zhang X, Dauber JH, Fabisiak J, Alber SM, Watkins SC, Zuckerbraun BS *et al.*: **Carbon monoxide induces cytoprotection in rat orthotopic lung transplantation via anti-inflammatory and anti-apoptotic effects**. *Am J Pathol* 2003, **163**:231-242.

42. Rottenberg H, Hoek JB: **The path from mitochondrial ROS to aging runs through the mitochondrial permeability transition pore**. *Aging Cell* 2017, **16**:943-955.

43. Li H, Zhang C, Sun W, Li L, Wu B, Bai S, Li H, Zhong X, Wang R, Wu L *et al.*: **Exogenous hydrogen sulfide restores cardioprotection of ischemic post-conditioning via inhibition of mPTP opening in the aging cardiomyocytes**. *Cell Biosci* 2015, **5**:43.

44. Olson SY, Garbán HJ: **Regulation of apoptosis-related genes by nitric oxide in cancer**. *Nitric Oxide* 2008, **19**:170-176.

45. Wang X, Wang Y, Kim HP, Nakahira K, Ryter SW, Choi AM: **Carbon monoxide protects against hyperoxia-induced endothelial cell apoptosis by inhibiting reactive oxygen species formation**. *J Biol Chem* 2007, **282**:1718-1726.

46. Miller MR, Megson IL: **Recent developments in nitric oxide donor drugs**. *Br J Pharmacol* 2007, **151**:305-321.

47. Francis SH, Busch JL, Corbin JD, Sibley D: **cGMP-dependent protein kinases and cGMP phosphodiesterases in nitric oxide and cGMP action**. *Pharmacol Rev* 2010, **62**:525-563.

48. Ebrahimi F, Shafaroodi H, Asadi S, Nezami BG, Ghasemi M, Rahimpour S, Hashemi M, Doostar Y, Dehpour AR: **Sildenafil decreased cardiac cell apoptosis in diabetic mice: reduction of oxidative stress as a possible mechanism**. *Physiol Pharmacol* 2009, **87**:556-564.

49. Itani N, Skeffington KL, Beck C, Giussani DA: **Sildenafil therapy for fetal cardiovascular dysfunction during hypoxic development: studies in the chick embryo**. *J Physiol* 2017, **595**:1563-1573.

50. Groom KM, Ganzevoort W, Alfirevic Z, Lim K, Papageorghiou AT: **Clinicians should stop prescribing sildenafil for fetal growth restriction (FGR): comment from the STRIDER consortium**. *Ultrasound Obstet Gynecol* 2018, **52**:295-296.

51. Kim HH, Choi S: **Therapeutic aspects of carbon monoxide in cardiovascular disease**. *Int J Mol Sci* 2018, **19**:2381.

52. Snijder PM, Frenay AR, Koning AM, Bachtler M, Pasch A, Kwakernaak AJ, van den Berg E, Bos EM, Hillebrands JL, Navis G: **Sodium thiosulfate attenuates angiotensin II-induced hypertension, proteinuria and renal damage**. *Nitric Oxide* 2014, **42**:87-98.

53. Sun X, Wang W, Dai J, Jin S, Huang J, Guo C, Wang C, Pang L, Wang Y: **A Long-term and slow-releasing hydrogen sulfide donor protects against myocardial ischemia/reperfusion injury**. *Sci Rep* 2017, **7**:3541.

54. •• Juriasingani S, Akbari M, Chan JY, Whiteman M, Sener A: **H2S supplementation: a novel method for successful organ preservation at subnormothermic temperatures**. *Nitric Oxide* 2018, **81**:57-66.
The authors showed a cytoprotective effect of the mitochondrial targeted H_2S donor AP39 in a clinical representative model of kidney transplantation.

55. Zhao Y, Pluth MD: **Hydrogen sulfide donors activated by reactive oxygen species**. *Angew Chem Int Ed Engl* 2016, **55**:14638-14642.

56. Hu Q, Wu D, Ma F, Yang S, Tan B, Xin H, Gu X, Chen X, Chen S, Mao Y *et al.*: **Novel angiogenic activity and molecular mechanisms of ZYZ-803, a slow-releasing hydrogen sulfide-nitric oxide hybrid molecule**. *Antioxid Redox Signal* 2016, **25**:498-514.

57. •• Wu D, Hu Q, Xiong Y, Zhu D, Mao Y, Zhu YZ: **Novel H2S-NO hybrid molecule (ZYZ-803) promoted synergistic effects against heart failure**. *Redox Biol* 2018, **15**:243-252.
Using a H_2S–NO hybrid molecule, this article shows that H_2S and NO cooperatively protects against heart failure.

58. Li L, Whiteman M, Guan YY, Neo KL, Cheng Y, Lee SW, Zhao Y, Baskar R, Tan C, Moore PK: **Characterization of a novel, water-soluble hydrogen sulfide–releasing molecule (GYY4137)**. *Circulation* 2008, **117**:2351-2360.

59. Szczesny B, Módis K, Yanagi K, Coletta C, Le Trionnaire S, Perry A, Wood ME, Whiteman M, Szabo C: **AP39, a novel mitochondria-targeted hydrogen sulfide donor, stimulates cellular bioenergetics, exerts cytoprotective effects and protects against the loss of mitochondrial DNA integrity in oxidatively stressed endothelial cells in vitro**. *Nitric Oxide* 2014, **41**:120-130.

60. Wang W, Sun X, Zhang H, Yang C, Liu Y, Yang W, Guo C, Wang C: **Controlled release hydrogen sulfide delivery system based on mesoporous silica nanoparticles protects graft endothelium from ischemia-reperfusion injury**. *Int J Nanomed* 2016, **11**:3255-3263.