Hydrogen sulphide and the hyperdynamic circulation in cirrhosis: a hypothesis

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Abstract
Cirrhosis is associated with the development of a hyperdynamic circulation, which is secondary to the presence of systemic vasodilatation. Several mechanisms have been postulated to be involved in the development of systemic vasodilatation including increased synthesis of nitric oxide, hyperglucagonemia, increased carbon monoxide synthesis, and the activation of \( K_{\text{ATP}} \) channels in vascular smooth muscle cells in the systemic and splanchnic arterial circulation. Hydrogen sulphide (H\(_2\)S) has recently been identified as a novel gaseous transmitter that induces vasodilatation through activation of \( K_{\text{ATP}} \) channels in vascular smooth muscle cells. In this brief review we comment on what is known about H\(_2\)S, vascular and neurological function, and postulate its role in the pathogenesis of the vascular abnormalities in cirrhosis.
Introduction
Endogenous gaseous transmitters such as nitric oxide (NO) and carbon monoxide (CO) constitute a unique class of mediators, which play an important role in cell physiology. The high membrane-permeability of these gases enables their rapid transfer across the cell membrane where they bind directly to the heme group of guanylate cyclase or cytochrome oxidase resulting in cell signalling in a receptor-independent manner. A number of other biologically active gases such as nitrous oxide (N₂O), ammonia (NH₃) and hydrogen sulphide (H₂S) may also participate in the regulation of cell function. Among them, recent reports have proposed H₂S as a novel endogenous transmitter with potential roles in both physiology and disease.

Formation and metabolism of H₂S
H₂S is produced endogenously from de-sulphydration of cysteine (or cystine) by three different enzymes (1,2). The reaction is catalysed either by cystathionine-γ−lyase (sometimes termed cystathionase), cystathionine-β-synthase or 3-mercapto-sulphurtransferase (Figure 1). The first two enzymes are cytosolic heme proteins, and the latter is a zinc-dependent protein, which is present in both the cytoplasm as well as mitochondria (2). Cystathionase is currently the only identified H₂S-generating enzyme present in the vasculature (3), whereas cystathionine-β-synthase is the only H₂S-generating system found in the nervous system (4). However, all three enzymes are present in the liver and kidney with cystathionine-β-synthase being most prominent in the liver (5).

H₂S is permeable to plasma membranes as its solubility in lipophilic solvents is 5-fold greater than in water (6). It can be hydrolyzed to hydrosulphide and sulphide ions in the following sequential reactions:

$$\text{H}_2\text{S} \rightleftharpoons \text{H}^+ + \text{HS}^- \rightleftharpoons 2\text{H}^+ + \text{S}^{2-}$$

However, even in an aqueous solution, about one-third of H₂S remains un-dissociated at pH 7.4 (6). The cellular concentrations of H₂S are reported to be in the micro-molar range (50-160 µM reported in brain, and 45 µM in plasma) (4,7) with a short half-life due to its rapid reaction with heme groups or disulphide-containing proteins, or its oxidation to thiosulfate (S₂O₃) and sulphate (2,6). These relatively high concentrations, together with its short half life suggest that the generation or flux of H₂S is high. The amounts of urinary thiosulphate as well as sulphaemoglobin (S-Hb) in the erythrocytes are currently believed to be among the best markers of H₂S formation in vivo (2,6), although these do have limitations, and recent studies have suggested that fluxes of hydrogen sulphide can be measured using polarographic techniques (8).

Physiological actions of H₂S and underlying mechanisms
The first and most important evidence for the physiological role of H₂S was obtained in 1989 when endogenous sulphide levels in rat brain tissues (1.6 µg/g) (9) and in normal human post-mortem brainstem (0.7 µg/g) were reported (10). The study by Awata et al. in 1995 (11) provided the enzymatic mechanisms for this endogenous H₂S in rat brain, in which the activities of cystathionine-β-synthase and cystathionine-γ−lyase in six different brain regions were measured, with the activity of cystathionine-β−synthase being >30-fold greater than that of cystathionine-γ−lyase. Further evidence for a physiological role of H₂S was reported by Abe and Kimura in 1996, who suggested that it may act as a neuro-modulator since physiological concentrations of H₂S enhance glutamate-mediated transmission via N-methyl-D-aspartate (NMDA) receptors which promote neuronal long-term potentiation (4). Since these initial publications, there has been an explosion of interest in the biochemistry, physiology and pharmacology of hydrogen sulphide and which is rapidly emerging as a new biological mediator.
At present, the functional role of endogenous H$_2$S in the cardiovascular system is still the subject of much on-going research and so far there have been no human studies on the physiological role of endogenously produced H$_2$S in cardiovascular system. However, the expression of cystathionine-$\gamma$-lyase mRNA and endogenous production of H$_2$S have been demonstrated in the aorta (3,7), mesenteric artery (12), portal vein (3) as well as cardiac tissue (13) in rats. Hosoki et al. have demonstrated that H$_2$S could be produced in the portal vein, mesenteric artery, pulmonary artery and thoracic aorta (3). H$_2$S is only generated from vascular smooth muscle cells since no H$_2$S-generating enzyme systems are expressed in the endothelial layer (7). This is in contrast to nitric oxide and carbon monoxide, which can be produced from both endothelial and vascular smooth muscle cells. Moreover, unlike nitric oxide or carbon monoxide, H$_2$S relaxed vascular tissues independent of the activation of cGMP pathway (8). Whereas the vasorelaxation induced by NO is virtually abolished by ODQ, a specific inhibitor of soluble guanylyl cyclase, the H$_2$S-induced vasorelaxation is not inhibited by ODQ (7). The vasorelaxant activity of H$_2$S is mimicked by ATP-sensitive K+ channels (K$_{ATP}$) openers, and antagonized by glibenclamide (a K$_{ATP}$ channel blocker) (12). In a series of studies Wang and colleagues have shown that H$_2$S induces vasorelaxation through activation of ATP-sensitive K$^+$ channels (K$_{ATP}$) in vascular smooth muscles in vitro and in vivo (7,12,13). H$_2$S may also exert effects on adjacent cell types. Thus, H$_2$S released from vascular smooth muscle cells may stimulate endothelial cells of small peripheral resistant arteries to release endothelium derived hyperpolarizing factor (EDHF), which further hyperpolarizes vascular smooth muscle cells, and potentiates vascular relaxation (12,13). In vitro studies have also shown that H$_2$S exerts a negative inotropic effect on cardiac function, primarily through the activation of K$_{ATP}$ channels (14). A summary of the major physiological effects of H$_2$S is presented at figure 2.

**Interaction of H$_2$S with nitric oxide and carbon monoxide**

Nitric oxide can regulate the endogenous production of H$_2$S in vascular tissues by either increasing cystathionine-$\gamma$-lyase gene expression; this is evident by the fact that incubating the cultured vascular smooth muscle cells with an NO donor significantly increased the transcriptional level of cystathionine-$\gamma$-lyase (7). On the other hand, H$_2$S, even at a very low concentration, can enhance the relaxation of smooth muscle induced by nitric oxide by ~10-fold (3). This effect is independent of free thiol groups, since both cysteine and glutathione do not have such an effect (3). Hydrogen sulphide has also recently been shown to up-regulate carbon monoxide synthesis through induction of heme oxygenase (15). Altered synthesis of hydrogen sulphide may also affect the pulmonary circulation (15). Thus, Qingyou et al. have shown that administration of sodium hydrosulphide (a donor of H$_2$S) causes a decrease in pulmonary artery pressure in rats with hypoxic pulmonary hypertension, and the administration of an inhibitor of cystathionine-$\gamma$-lyase, led to an increase of pulmonary artery pressure and a decrease in carbon monoxide synthesis (15). This suggests that there is a dynamic interplay between not only the hydrogen sulphide and nitric oxide pathways, but also between the hydrogen sulphide and carbon monoxide system.

**Potassium channels and the control of vascular function in cirrhosis**

Hypotension, low systemic vascular resistance and a reduced responsiveness to vasoconstrictors are all features of the hyperdynamic circulation in cirrhosis. These changes have been attributed to increased synthesis of NO, CO, anandamide and CGRP (calcitonin gene-related polypeptide)(16-19); however the precise mechanisms underlying the cardiovascular changes in cirrhotic subjects are not completely understood. In 1994 Moreau et al showed that there is activation of K$_{ATP}$ channels in vascular smooth muscle cells in rats with
cirrhosis, and that this is partly responsible for the development of systemic vasodilatation in this animal model (20,21). In arterial smooth muscle cells, plasma-lemmal K<sub>ATP</sub> channels play an important role in arterial vasodilatation by modulating the membrane potential (22). In cirrhosis, activation of K<sub>ATP</sub> leads to membrane hyperpolarization, which results in closure of L-type Ca<sup>2+</sup> channel and subsequent decrease in Ca<sup>2+</sup> entry and vasorelaxation (20,21). One potential mechanism of K<sub>ATP</sub> channel activation involves prostaglandins such as prostacyclin, since K<sub>ATP</sub> activation can be partially inhibited by cyclo-oxygenase inhibitors (20). However, the observation that H<sub>2</sub>S can cause K<sub>ATP</sub> activation in a variety of experimental systems, lends support to the idea that H<sub>2</sub>S may be involved in K<sub>ATP</sub> channel activation in cirrhosis.

**Hydrogen Sulphide and the hyperdynamic circulation**

In this paper, we suggest that H<sub>2</sub>S may contribute to the pathogenesis of vascular dysfunction in cirrhosis (figure 3). This hypothesis is based on the following evidence.

1. Plasma H<sub>2</sub>S concentrations increase in rats with endotoxemia (23). Endotoxemia is a common feature of cirrhosis (24) and high concentrations of circulating endotoxins are observed in cirrhotic patients with no clinical evidence of infection, and this may be due to impaired clearance of gut bacteria in cirrhotic liver (24,25). Studies are emerging which increasingly link the development of extra-hepatic complications of cirrhosis (e.g. hyperdynamic circulation, cirrhotic cardiomyopathy and hepatic encephalopathy) to the advent of endotoxemia or sepsis in cirrhosis (26-28). Since endotoxin can induce the synthesis of H<sub>2</sub>S, this may have two consequences. Firstly there may be increased H<sub>2</sub>S synthesis leading to increased K<sub>ATP</sub> activation in vascular smooth muscle cells and a resulting systemic vasodilatation. Secondly, increased H<sub>2</sub>S formation may lead to altered cardiac function as it has been shown that H<sub>2</sub>S exerts a negative inotropic effect on cardiac function, primarily through the activation of K<sub>ATP</sub> channels (14).

2. Increased synthesis of nitric oxide is well recognised in cirrhosis and portal hypertension (16), and may lead to increased expression of cystathionine-γ-lyase, the main H<sub>2</sub>S-producing enzyme in vascular smooth muscle cells (7). Thus, increased NO synthesis may enhance the formation of H<sub>2</sub>S in cirrhosis, thus leading indirectly to activation of K<sub>ATP</sub> channels.

3. Increased activity of serum cystathionine-γ-lyase has been demonstrated in rats with liver injury due to carbon tetrachloride (29). Whether this is applicable to other forms of liver injury is unknown, but increased cystathionine-γ-lyase activity would be expected to increase H<sub>2</sub>S formation in this model (23).

In conclusion, we propose a mechanism by which endotoxemia either alone, or in combination with increased NO synthesis leads to up-regulation of cystathionine-γ-lyase activity and H<sub>2</sub>S synthesis. Increase synthesis of H<sub>2</sub>S leads to activation of K<sub>ATP</sub> channels and systemic vasodilatation (figure 3). Studies in the future will determine the validity of this hypothesis in man.
REFERENCES


Figures

**Figure 1.** There are 3 enzymatic pathways involved in the synthesis of H$_2$S from cysteine in mammals. Of these only cystathionine-$\gamma$-lyase is found in the vasculature. All three enzymes are present in the liver and kidney, with most activity residing in cystathionine-$\beta$-synthase.

**Figure 2.** Major physiological actions of endogenously produced hydrogen sulphide (H$_2$S). Activation of ATP-sensitive K$^+$ channels ($K_{\text{ATP}}$) is a common mechanism of H$_2$S physiological effects which induces vasorelaxation and neuronal hyperpolarization in cardiovascular system and nervous system respectively. Hydrogen sulphide can also promote glutamate-mediated transmission via NMDA receptors which enhance neuronal long-term potentiation.

**Figure 3.** Postulated role of H$_2$S in the development of a hyperdynamic circulation in cirrhosis. Endotoxemia leads to increased nitric oxide (NO) synthesis and up-regulation of the enzyme responsible for H$_2$S production (Cystathionine-$\gamma$-lyase). H$_2$S causes activation of $K_{\text{ATP}}$ channels, which causes vasodilatation in liver disease. Increased nitric oxide (NO) synthesis may also cause vasodilatation directly.
Homocysteine → Cystathionine

Cystathionine

L-Cysteine

Pyruvate + NH₃ + H₂S → 3-mercaptopyruvate → Pyruvate + H₂S

L-Serine + H₂S → 3-mercapto-sulfurtransferase

Cystathionine-β-synthase → Cystathionine-γ-lyase

Cystathionine-β-synthase → Cystathionine-γ-lyase
L-Cysteine

Cystathionine-β-synthase

H₂S

Cystathionine-γ-lyase

Nervous system
Hyperpolarization (activation of K<sub>ATP</sub> channels)
Promotion of neuronal long-term potentiation

Cardiovascular system
Vaso-relaxation (activation of K<sub>ATP</sub> channels)
Negative inotropic effect on cardiac function
Cirrhosis

NO overproduction \rightarrow Endotoxemia

\downarrow \downarrow

Up-regulation of Cystathionine γ lyase

\downarrow

H$_2$S

\downarrow

K$_{ATP}$ activation in vascular smooth muscles

\downarrow

Vasorelaxation
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