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Hydrogen sulphide and the hyperdynamic circulation in cirrhosis: a hypothesis

M R Ebrahimkhani, A R Mani, K Moore

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, hyperglucagonaemia, increased carbon monoxide synthesis, and activation of K\textsubscript{ATP} channels in vascular smooth muscle cells in the systemic and splanchnic arterial circulation. Hydrogen sulphide (H\textsubscript{2}S) has recently been identified as a novel gaseous transmitter that induces vasodilatation through activation of K\textsubscript{ATP} channels in vascular smooth muscle cells. In this brief review, we comment on what is known about H\textsubscript{2}S, vascular and neurological function, and postulate its role in the pathogenesis of the vascular abnormalities in cirrhosis.

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 haeme 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, ammonia, and hydrogen sulphide (H\textsubscript{2}S) may also participate in the regulation of cell function. Among them, recent reports have proposed H\textsubscript{2}S as a novel endogenous transmitter with potential roles in both physiology and disease.

FORMATION AND METABOLISM OF H\textsubscript{2}S

H\textsubscript{2}S is produced endogenously from desulphhydration of cysteine (or cystine) by three different enzymes. The reaction is catalysed by cystathionine-γ-lyase (sometimes termed cystathionase), cystathionine-β-synthase, or 3-mercaptop-sulphurtransferase (fig 1). The first two enzymes are cytosolic haeme proteins, and the latter is a zinc dependent protein which is present in both the cytoplasm as well as mitochondria. Cystathionase is currently the only identified H\textsubscript{2}S generating enzyme present in the vasculature whereas cystathionine-β-synthase is the only H\textsubscript{2}S generating enzyme found in the nervous system. However, all three enzymes are present in the liver and kidney, with cystathionine-β-synthase being most prominent in the liver. H\textsubscript{2}S is permeable to plasma membranes as its solubility in lipophilic solvents is fivefold greater than in water. It can be hydrolysed to hydro sulphide and sulphide ions in the following sequential reactions:

\[
\text{H}_2\text{S} \leftrightarrow \text{H}^+ + \text{HS}^- \leftrightarrow 2\text{H}^+ + \text{S}^{2-}
\]

However, even in an aqueous solution, approximately one third of H\textsubscript{2}S remains undissociated at pH 7.4. Cellular concentrations of H\textsubscript{2}S are reported to be in the micromolar range (50–160 \textmu M reported in the brain and 45 \textmu M in plasma) with a short half life due to its rapid reaction with haeme groups or disulphide containing proteins, or its oxidation to thiosulphate (S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-}) and sulphate. These relatively high concentrations, together with its short half life, suggest that generation or flux of H\textsubscript{2}S is high. The amounts of urinary thiosulphate as well as sulphhemoglobin in erythrocytes are currently believed to be among the best markers of H\textsubscript{2}S formation in vivo, although these do have limitations, and recent studies have suggested that fluxes of H\textsubscript{2}S can be measured using polarographic techniques.

PHYSIOLOGICAL ACTIONS OF H\textsubscript{2}S AND UNDERLYING MECHANISMS

The first and most important evidence for a physiological role of H\textsubscript{2}S was obtained in 1989 when endogenous sulphide levels in rat brain tissues (1.6 \textmu g/g) and in normal human post mortem brainstem (0.7 \textmu g/g) were reported. The study by Awata et al in 1995 provided the enzymatic mechanisms for this endogenous H\textsubscript{2}S 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 that of cystathionine-γ-lyase.

“There has been an explosion of interest in the biochemistry, physiology, and pharmacology of H\textsubscript{2}S, which is rapidly emerging as a new biological mediator”

Further evidence for a physiological role of H\textsubscript{2}S was reported by Abe and Kimura in 1996, who suggested that it may act as a neuromodulator as physiological concentrations of H\textsubscript{2}S enhance glutamate mediated transmission via glutamate receptors.

Abbreviations: H\textsubscript{2}S, hydrogen sulphide; NO, nitric oxide; CO, carbon monoxide; NMDA, N-methyl-D-aspartate
Cystathionine-β-synthase

Cystathionine-γ-lyase

L-Cysteine

Pyruvate + NH₃ + H₂S

L-Serine + H₂S

3-Mercaptopyruvate

Pyruvate + H₂S

3-mercapto-sulfurtransferase

Figure 1  There are three enzymatic pathways involved in the synthesis of hydrogen sulphide (H₂S) from cysteine in mammals. Of these only cystathionine-γ-lyase is found in the vasculature. All three enzymes are present in the liver and kidney, with most activity residing in cystathionine-β-synthase.

N-methyl-D-aspartate (NMDA) receptors which promote neuronal long term potentiation. Since these initial publications there has been an explosion of interest in the biochemistry, physiology, and pharmacology of H₂S, which is rapidly emerging as a new biological mediator.

At present, the functional role of endogenous H₂S in the cardiovascular system is still the subject of much ongoing research and so far there have been no human studies on the physiological role of endogenously produced H₂S in the cardiovascular system. However, expression of cystathionine-γ-lyase mRNA and endogenous production of H₂S have been demonstrated in the aorta, mesenteric artery, portal vein, as well as cardiac tissue in rats. Hosoki et al have demonstrated that H₂S could be produced in the portal vein, mesenteric artery, pulmonary artery, and thoracic aorta. H₂S is only generated from vascular smooth muscle cells as no H₂S generating enzyme systems are expressed in the endothelial layer. This is in contrast with NO and CO which can be produced from both endothelial and vascular smooth muscle cells. Moreover, unlike NO or CO, H₂S released vascular tissues independent of activation of the cGMP pathway.

“H₂S induces vasorelaxation through activation of ATP sensitive K⁺ channels in vascular smooth muscles in vitro and in vivo”

Whereas vasorelaxation induced by NO is virtually abolished by ODQ, a specific inhibitor of soluble guanylyl cyclase, H₂S induced vasorelaxation is not inhibited by ODQ. The vasorelaxant activity of H₂S is mimicked by ATP sensitive K⁺ channel (K_ATP) openers, and antagonised by glibenclamide (a K_ATP channel blocker). In a series of studies, Wang and colleagues have shown that H₂S induces vasorelaxation through activation of ATP sensitive K⁺ channels (K_ATP) in vascular smooth muscles in vitro and in vivo. H₂S may also exert effects on adjacent cell types. Thus H₂S released from vascular smooth muscle cells may stimulate endothelial cells of small peripheral resistant arteries to release endothelium derived hyperpolarising factor which further hyperpolarises vascular smooth muscle cells and potentiates vascular relaxation. In vitro studies have also shown that H₂S exerts a negative inotropic effect on cardiac function, primarily through activation of K_ATP channels. A summary of the major physiological effects of H₂S is presented at fig 2.

INTERACTION OF H₂S WITH NO AND CO

NO can regulate endogenous production of H₂S in vascular tissues by increasing cystathionine-γ-lyase gene expression; this is evident by the fact that incubating cultured vascular smooth muscle cells with an NO donor significantly increased the transcriptional level of cystathionine-γ-lyase. On the other hand, H₂S, even at a very low concentration, can enhance relaxation of smooth muscle induced by NO by approximately 10-fold. This effect is independent of free thiol groups as both cysteine and glutathione do not have such an effect.

“There is a dynamic interplay between not only the H₂S and NO pathways but also between the H₂S and CO systems”

H₂S has also recently been shown to upregulate CO synthesis through induction of haeme oxygenase. Altered synthesis of H₂S may also affect the pulmonary circulation. Thus Qingyou et al have shown that administration of sodium hydrosulphide (a donor of H₂S) causes a decrease in pulmonary artery pressure in rats with hypoxic pulmonary hypertension, and administration of an inhibitor of cystathionine-γ-lyase led to an increase in pulmonary artery pressure and a decrease in CO synthesis. This suggests that there is a

Figure 2  Major physiological actions of endogenously produced hydrogen sulphide (H₂S). Activation of ATP sensitive K⁺ channels (K_ATP) is a common mechanism of H₂S physiological effects, which induce vasorelaxation and neuronal hyperpolarisation in the cardiovascular system and nervous system, respectively. H₂S can also promote glutamate mediated transmission via N-methyl-D-aspartate receptors which enhance neuronal long term potentiation.
dynamic interplay between not only the H$_2$S and NO pathways but also between the H$_2$S and CO systems.

**POTASSIUM CHANNELS AND CONTROL OF VASCULAR FUNCTION IN CIRRHOSIS**

Hypotension, low systemic vascular resistance, and 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 calcitonin gene related polypeptide; however, the precise mechanisms underlying the cardiovascular changes in cirrhotic subjects are not completely understood. In 1994, Moreau et al showed that there was activation of K$_{ATP}$ channels in vascular smooth muscle cells in rats with cirrhosis, and that this was partly responsible for the development of systemic vasodilatation in this animal model. In arterial smooth muscle cells, plasmaemal K$_{ATP}$ channels play an important role in arterial vasodilatation by modulating membrane potential. In cirrhosis, activation of K$_{ATP}$ leads to membrane hyperpolarisation which results in closure of the L-type Ca$^{2+}$ channel and subsequent decrease in Ca$^{2+}$ entry and vasorelaxation. One potential mechanism of K$_{ATP}$ channel activation involves prostaglandins such as prostacyclin as K$_{ATP}$ activation can be partially inhibited by cyclooxygenase inhibitors. However, the observation that H$_2$S can cause K$_{ATP}$ activation in a variety of experimental systems lends support to the idea that H$_2$S may be involved in K$_{ATP}$ channel activation in cirrhosis.

**H$_2$S AND THE HYPERDYNAMIC CIRCULATION**

In this paper, we suggest that H$_2$S may contribute to the pathogenesis of vascular dysfunction in cirrhosis (fig 3). This hypothesis is based on the following evidence.

(1) Plasma H$_2$S concentrations increase in rats with endotoxaemia. Endotoxaemia is a common feature of cirrhosis 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. Studies are emerging which increasingly link the development of extrahepatic complications of cirrhosis (for example, hyperdynamic circulation, cirrhotic cardiomyopathy, and hepatic encephalopathy) to the advent of endotoxaemia or sepsis in cirrhosis. As endotoxin can induce the synthesis of H$_2$S, this may have two consequences. Firstly, there may be increased H$_2$S synthesis leading to increased K$_{ATP}$ activation in vascular smooth muscle cells and a resulting systemic vasodilatation. Secondly, increased H$_2$S formation may lead to altered cardiac function as it has been shown that H$_2$S exerts a negative inotropic effect on cardiac function, primarily through activation of K$_{ATP}$ channels.

(2) Increased synthesis of NO is well recognised in cirrhosis and portal hypertension, and may lead to increased expression of cystathionine-$\gamma$-lyase, the main H$_2$S producing enzyme in vascular smooth muscle cells. Thus increased NO synthesis may enhance the formation of H$_2$S in cirrhosis, thus leading indirectly to activation of K$_{ATP}$ channels.

(3) Increased activity of serum cystathionine-$\gamma$-lyase has been demonstrated in rats with liver injury due to carbon tetrachloride. Whether this is applicable to other forms of liver injury is unknown but increased cystathionine-$\gamma$-lyase activity would be expected to increase H$_2$S formation in this model.

In conclusion, we propose a mechanism by which endotoxemia, either alone or in combination with increased NO synthesis, leads to upregulation of cystathionine-$\gamma$-lyase activity and H$_2$S synthesis. Increased synthesis of H$_2$S leads to activation of K$_{ATP}$ channels and systemic vasodilatation (fig 3). Studies in the future will determine the validity of this hypothesis in humans.

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Conflict of interest: None declared.

**REFERENCES**


An unusual cause of diarrhoea

Clinical presentation
A 70 year old Caucasian female presented with a six month history of intermittent diarrhoea, abdominal pain, and weight loss of 3 stone. Routine blood tests and liver ultrasound were normal.

Question
What abnormalities do the computed tomography scan (fig 1) and histology slide (fig 2) demonstrate? What was the cause of her diarrhoea?
See page 1713 for answer

This case is submitted by:
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