Hydrogen sulphide and the hyperdynamic circulation in cirrhosis: a hypothesis

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LEADING ARTICLE

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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_{ATP} channels in vascular smooth muscle cells in the systemic and splanchnic arterial circulation. Hydrogen sulphide (H_2S) has recently been identified as a novel gaseous transmitter that induces vasodilatation through activation of K_{ATP} channels in vascular smooth muscle cells. In this brief review, we comment on what is known about H_2S, 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_2S) may also participate in the regulation of cell function. Among them, recent reports have proposed H_2S as a novel endogenous transmitter with potential roles in both physiology and disease.

See end of article for authors' affiliations

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FORMATION AND METABOLISM OF H_2S

H_2S is produced endogenously from desulphydration of cysteine (or cystine) by three different enzymes.[1 2] The reaction is catalysed by cystathionine-γ-lyase (sometimes termed cystathionase), cystathionine-β-synthase, or 3-mercapto-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.[2] Cystathionase is currently the only identified H_2S generating enzyme present in the vasculature[3] whereas cystathionine-β-synthase is the only H_2S 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]

H_2S is permeable to plasma membranes as its solubility in lipophilic solvents is fivefold greater than in water.[6] It can be hydrolysed to hydrosulphide and sulphide ions in the following sequential reactions:

$$H_2S \leftrightarrow H^+ + HS^- \leftrightarrow 2H^+ + S^{2-}$$

However, even in an aqueous solution, approximately one third of H_2S remains undissociated at pH 7.4.[6] Cellular concentrations of H_2S are reported to be in the micromolar range (50–160 μM reported in the brain and 45 μM in plasma)[4 7] with a short half life due to its rapid reaction with haeme groups or disulphide containing proteins, or its oxidation to thiosulphate (S_2O_3) and sulphate.[2 6] These relatively high concentrations, together with its short half life, suggest that generation or flux of H_2S is high. The amounts of urinary thiosulphate as well as sulphaemoglobin in erythrocytes are currently believed to be among the best markers of H_2S formation in vivo,[2 6] although these do have limitations, and recent studies have suggested that fluxes of H_2S can be measured using polarographic techniques.[8]

PHYSIOLOGICAL ACTIONS OF H_2S AND UNDERLYING MECHANISMS

The first and most important evidence for a physiological role of H_2S 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 H_2S 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_2S, which is rapidly emerging as a new biological mediator"

Further evidence for a physiological role of H_2S was reported by Abe and Kimura in 1996, who suggested that it may act as a neuromodulator as physiological concentrations of H_2S enhance glutamate mediated transmission via

Abbreviations: H_2S, hydrogen sulphide; NO, nitric oxide; CO, carbon monoxide; NMDA, N-methyl-D-aspartate

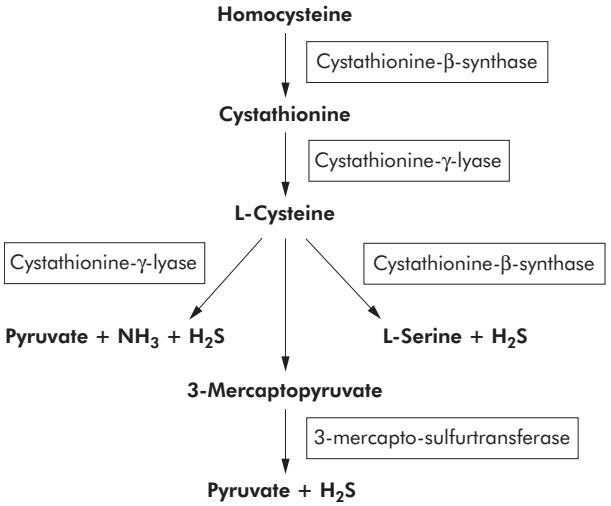

Figure 1 There are three enzymatic pathways involved in the synthesis of hydrogen sulphide (H_2S) from cysteine in mammals. Of these only cystathionine-γ-lyase is found in the vasculature. All three enzymes are present in the liver and kidney, with most activity residing in cystathionine-β-synthase.

N-methyl-D-aspartate (NMDA) receptors which promote neuronal long term potentiation.[4] Since these initial publications there has been an explosion of interest in the biochemistry, physiology, and pharmacology of H_2S, which is rapidly emerging as a new biological mediator.

At present, the functional role of endogenous H_2S 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 H_2S in the cardiovascular system. However, expression of cystathionine-γ-lyase mRNA and endogenous production of H_2S 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_2S could be produced in the portal vein, mesenteric artery, pulmonary artery, and thoracic aorta.[3] H_2S is only generated from vascular smooth muscle cells as no H_2S generating enzyme systems are expressed in the endothelial layer.[7] 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, H_2S relaxed vascular tissues independent of activation of the cGMP pathway.[8]

> "H_2S 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, H_2S induced vasorelaxation is not inhibited by ODQ.[7] The vasorelaxant activity of H_2S is mimicked by ATP sensitive K^+ channel (K_{ATP}) openers, and antagonised by glibenclamide (a K_{ATP} channel blocker).[12] In a series of studies, Wang and colleagues have shown that H_2S induces vasorelaxation through activation of ATP sensitive K^+ channels (K_{ATP}) in vascular smooth muscles in vitro and in vivo.[7 12 13] H_2S may also exert effects on adjacent cell types. Thus H_2S 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.[12 13] In vitro studies have also shown that H_2S exerts a negative inotropic effect on cardiac function, primarily through activation of K_{ATP} channels.[14] A summary of the major physiological effects of H_2S is presented at fig 2.

INTERACTION OF H_2S WITH NO AND CO

NO can regulate endogenous production of H_2S 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.[7] On the other hand, H_2S, even at a very low concentration, can enhance relaxation of smooth muscle induced by NO by approximately 10-fold.[3] This effect is independent of free thiol groups as both cysteine and glutathione do not have such an effect.[3]

> "There is a dynamic interplay between not only the H_2S and NO pathways but also between the H_2S and CO systems"

H_2S has also recently been shown to upregulate CO synthesis through induction of haeme oxygenase.[15] Altered synthesis of H_2S may also affect the pulmonary circulation.[15] Thus Qingyou *et al* have shown that administration of sodium hydrosulphide (a donor of H_2S) 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.[15] This suggests that there is a

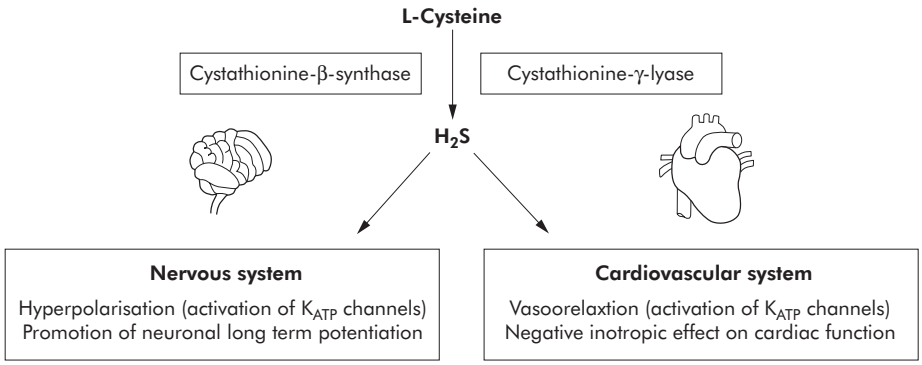

Figure 2 Major physiological actions of endogenously produced hydrogen sulphide (H_2S). Activation of ATP sensitive K^+ channels (K_{ATP}) is a common mechanism of H_2S physiological effects, which induce vasorelaxation and neuronal hyperpolarisation in the cardiovascular system and nervous system, respectively. H_2S can also promote glutamate medicated transmission via N-methyl-D-aspartate receptors which enhance neuronal long term potentiation.

dynamic interplay between not only the H_2S and NO pathways but also between the H_2S 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[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 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.[20 21] In arterial smooth muscle cells, plasmalemmal K_{ATP} channels play an important role in arterial vasodilatation by modulating membrane potential.[22] 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.[20 21] One potential mechanism of K_{ATP} channel activation involves prostaglandins such as prostacyclin as K_{ATP} activation can be partially inhibited by cyclooxygenase inhibitors.[20] However, the observation that H_2S can cause K_{ATP} activation in a variety of experimental systems lends support to the idea that H_2S may be involved in K_{ATP} channel activation in cirrhosis.

H_2S AND THE HYPERDYNAMIC CIRCULATION

In this paper, we suggest that H_2S may contribute to the pathogenesis of vascular dysfunction in cirrhosis (fig 3). This hypothesis is based on the following evidence.

(1) Plasma H_2S concentrations increase in rats with endotoxaemia.[23] Endotoxaemia 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 extrahepatic complications of cirrhosis (for example, hyperdynamic circulation, cirrhotic cardiomyopathy, and hepatic encephalopathy) to the advent of endotoxaemia or sepsis in cirrhosis.[26–28] As endotoxin can induce the synthesis of H_2S, this may have two consequences. Firstly, there may be increased H_2S synthesis leading to increased K_{ATP} activation in vascular smooth muscle cells and a resulting systemic vasodilatation. Secondly, increased H_2S formation may lead to altered cardiac function as it has been shown that H_2S exerts a negative inotropic effect on cardiac function, primarily through activation of K_{ATP} channels.[14]

(2) Increased synthesis of NO is well recognised in cirrhosis and portal hypertension,[16] and may lead to increased expression of cystathionine-γ-lyase, the main H_2S producing enzyme in vascular smooth muscle cells.[7] Thus increased NO synthesis may enhance the formation of H_2S in cirrhosis, thus leading indirectly to activation of K_{ATP} 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_2S formation in this model.[23]

In conclusion, we propose a mechanism by which endotoxaemia, either alone or in combination with increased NO synthesis, leads to upregulation of cystathionine-γ-lyase activity and H_2S synthesis. Increased synthesis of H_2S 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.

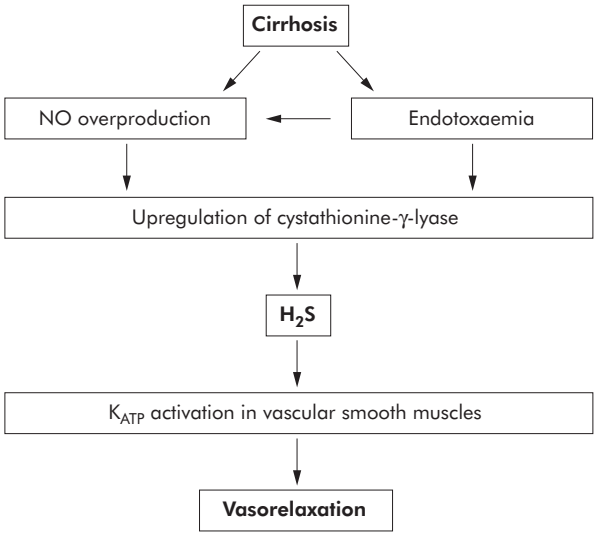

Figure 3 Postulated role of hydrogen sulphide (H_2S) in the development of a hyperdynamic circulation in cirrhosis. Endotoxaemia leads to increased nitric oxide (NO) synthesis and upregulation of the enzyme responsible for H_2S production (cystathionine-γ-lyase). H_2S causes activation of K_{ATP} channels which causes vasodilatation in liver disease. Increased NO synthesis may also cause vasodilatation directly.

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Conflict of interest: None declared.

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EDITOR'S QUIZ: GI SNAPSHOT

Robin Spiller, *Editor*

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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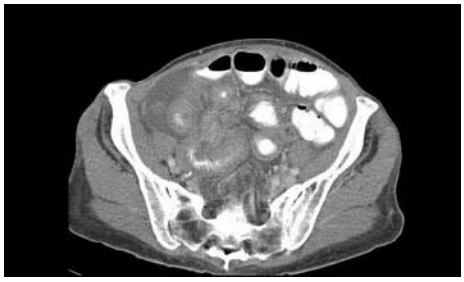

Figure 1 Computed tomography scan of the abdomen.