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REVIEW ARTICLE

Luminal sulfide and large intestine mucosa: friend or foe?

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Abstract Hydrogen sulfide (H_2S) is present in the lumen of the human large intestine at millimolar concentrations. However, the concentration of free (unbound) sulfide is in the micromolar range due to a large capacity of fecal components to bind the sulfide. H_2S can be produced by the intestinal microbiota from alimentary and endogenous sulfur-containing compounds including amino acids. At excessive concentration, H_2S is known to severely inhibit cytochrome *c* oxidase, the terminal oxidase of the mitochondrial electron transport chain, and thus mitochondrial oxygen (O_2) consumption. However, the concept that sulfide is simply a metabolic troublemaker toward colonic epithelial cells has been challenged by the discovery that micromolar concentration of H_2S is able to increase the cell respiration and to energize mitochondria allowing these cells to detoxify and to recover energy from luminal sulfide. The main product of H_2S metabolism by the colonic mucosa is thiosulfate. The enzymatic activities involved in sulfide oxidation by the colonic epithelial cells appear to be sulfide quinone oxidoreductase considered as the first and rate-limiting step followed presumably by the action of sulfur dioxygenase and rhodanese. From clinical studies with human volunteers and experimental works with rodents, it appears that H_2S can exert mostly pro- but also anti-inflammatory effects on the colonic mucosa. From the available data, it is tempting to propose that imbalance between the luminal concentration of free sulfide and the capacity of colonic epithelial cells to metabolize this compound will result in an impairment of the colonic epithelial cell O_2 consumption with consequences on the process of mucosal inflammation. In addition, endogenously produced sulfide is emerging as a prosecretory neuromodulator and as a relaxant agent toward the intestinal contractibility. Lastly, sulfide has been recently described as an agent involved in nociception in the large intestine although, depending on the experimental design, both pro- and anti-nociceptive effects have been reported.

Keywords Sulfide · Large intestine · Colon · Detoxification · Energy metabolism

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Introduction

Alimentary and endogenous protein digestion followed by amino acid and oligopeptide absorption in the small intestine is an efficient process. Indeed, the protein digestibility for most proteins is equal or even higher than 90% (Bos et al. 2005; Baglieri et al. 1994; Gausseres et al. 1996; Gaudichon et al. 1999). However, significant amounts of even highly digestible proteins escape digestion and enter the large intestine through the ileocecal junction (Evenepoel et al. 1999; Gaudichon et al. 2002). Indeed, nitrogenous material (mainly in the form of proteins and peptides), which represents between 6 and 18 g per day is transferred from the small intestine to the cecum in humans (Chacko and Cummings 1988; Silvester and Cummings 1995; Smiddy et al. 1960; Kramer 1966; Gibson et al. 1976). The fate of

these compounds is to be degraded through proteolysis in the lumen of the large intestine by the colonic flora followed by the production of numerous metabolites including hydrogen sulfide (Blachier et al. 2007). Hydrogen sulfide (H_2S) has been recognized originally as a toxic gas known to disturb lung and brain functions (Nicholson et al. 1998; Grieshaber and Völkel 1998). In fact, human responses to H_2S are, depending on doses, irritation of the respiratory tract, pulmonary edema, respiratory paralysis, neural paralysis, cardiac arrhythmias, and death (Reiffenstein et al. 1992).

However, beyond this identity of a toxic gas and an environmental hazard, H_2S contained in the large intestine luminal content and produced by the microbiota from alimentary and endogenous sulfur-containing compounds has emerged as a compound with both beneficial and deleterious effects on the large intestine mucosa, depending on the doses tested and on the experimental situation chosen. In addition, H_2S is now considered as an endogenously produced signaling molecule by which its production is associated with important physiological and physiopathological functions including inhibition of insulin secretion, effects on smooth muscle cells and vasoconstrictor response, effects on mononuclear cell infiltration and leucocyte adhesion, and effects on haemodynamic parameters (see the review by Szabo 2007). Recently, several neuromodulator functions of H_2S on the large intestine have been reported and will be presented in this review.

The main goal of this article is to present an overview of the different parameters which will change H_2S from a friend to a foe toward the large intestine mucosa according to (a) the metabolic capacity of the intestine microbiota for H_2S production, (b) the amounts of alimentary and endogenous substrates used by the microbiota for sulfide production, (c) the effects of luminal sulfide on the large intestine mucosa depending on the capacity of colonocytes to detoxify this compound, and (d) the role of endogenously produced sulfide in neuromodulation and nociception.

Production of sulfide by the large intestine microbiota

General characteristics of bacterial sulfide production

In prokaryotes, sulfide can be produced from sulfur-containing organic compound degradation and assimilatory or dissimilatory sulfate/sulfur reduction (Rabus et al. 2006). Some bacteria, including anaerobic ones, are then able to derive energy from the carbon chains of peptides and amino acids. Those bacterial sulfur-containing amino-acid-degrading activities produce sulfide (Smith and Marcfarlane 1997; Awano et al. 2005). Bacterial assimilatory sulfate reduction generates reduced sulfur for biosynthesis processes (i.e., synthesis of sulfur-containing amino acids) and does not result in the excretion of sulfide. Therefore, this latter bacterial metabolism cannot be considered significant for sulfide release whatever the ecosystem be. In dissimilatory reduction, sulfur compounds are involved in respiration processes and are used as electron acceptors instead of oxygen for energy conservation during anaerobic growth. This phenomenon is coupled to the oxidation of hydrogen or organic substrates which are acting as electron donors (Rabus et al. 2006; Hedderich et al. 1999). All these metabolic pathways are concomitant with bacterial growth and may contribute to the production of H_2S within the intestinal tract. However, it is worth noting that diet, transit time, and other parameters (e.g., pH, redox potential) are important determinants of bacterial metabolism. Consequently, the major part of the bacterial sulfide production takes place within the large intestine. Proteins and peptides entering the colon are first hydrolyzed to peptides and free amino acids by intestinal bacteria that produce a wide variety of proteolytic and peptidolytic enzymes (Macfarlane and Macfarlane 2003a). Peptides and free amino acids are predominantly incorporated and/or degraded by bacteria since the large intestine lacks significant capacity for their uptake. Peptide and amino acid degradation generate a wide range of gases and metabolites among which sulfide; but also other compounds including hydrogen, carbon dioxide, and short-chain fatty acids (SCFAs). These metabolites also result from carbohydrate fermentation and they can be utilized in dissimilatory sulfate reduction in the colon providing that oxidized sulfur species are present in the luminal content. It is worth noting that the presence of bacteria utilizing H_2S as electron donor (lithotrophic bacteria) has never been reported.

Bacterial metabolic pathways for sulfide production

Bacteria that degrade sulfur-containing amino acids possess specific desulfhydrases. For instance, cysteine desulfhydrase converts cysteine to pyruvate, H_2S, and ammonia according to the following reaction:

$$HSCH_2CH(NH_2)COOH + H_2O \rightarrow CH_3COCOOH + H_2S + NH_3$$

This type of enzymatic activity is exhibited by some anaerobic bacteria like *Escherichia coli*, *Salmonella enterica*, *Clostridia*, and *Enterobacter aerogenes* that are commonly found in the large intestine (Awano et al. 2005; Kumagai et al. 1975). An in vitro study has shown that slurries of human fecal bacteria ferment cysteine and methionine. In these experiments, the pyruvate formed at the expense of cysteine was fermented or transaminated to yield alanine. Methionine was thought to be converted to α-ketobutyrate, ammonia, and possibly methanethiol (Smith and Marcfarlane 1997; Kadota and Ishida 1972).

Bacterial sulfate reducers may utilize hydrogen or a wide variety of simple organic compounds such as carboxylic acids, alcohols, polar aromatic compounds, and amino acids as electron donors. Their oxidation may be either incomplete leading to acetate excretion or complete leading to carbon dioxide production. Many sulfate-reducing bacteria can grow by utilizing sulfite or thiosulfate as alternate electron acceptors. A few of them are able to use cystine or oxidized glutathione as electron acceptors (Rabus et al. 2006; Schauder and Kröger 1993). Another pathway to generate H_2S is metabolism of sulfite by sulfite reductase. This iron flavoprotein is present in a wide variety of microbial genera and species of the γ-subclass of Proteobacteria (like *E. coli*, *Salmonella*, *Enterobacter*, *Klebsiella*), of the Firmicutes (like *Bacillus* and *Staphylococcus*), of the Actinobacteria (like *Corynebacterium* and *Rhodococcus*), and of the Bacteroidetes. This enzyme which was first studied in *E. coli* is able to balance the generation or the consumption of H_2S according to the needs in redox potential (Siegel et al. 1973). The reaction is:

$$\text{Sulfite} + \text{NADPH} + 3H^+ \leftrightarrow H_2S + \text{NADP}^+ + 3H_2O.$$

Cysteine synthase (CSase) is the enzyme responsible for the formation of cysteine from *O*-acetyl-serine and H_2S with the concomitant release of acetic acid. In bacteria such as *E. coli*, two forms of that enzyme are known: genes cysK and cysM. In fact, this activity is widespread among microbial world and is undoubtedly also present within the intestinal microbiota. At least one gene coding one isoform of this enzyme was identified in 908 bacteria and 67 archaea. Not less than 19 structures have been resolved (Tai et al. 2001; Clauss et al. 2005).

All those reversible pathways as well as concentration of H_2S in the luminal content are mostly dependent on the composition and activity of the microbial flora as well as its metabolic requirements. Moreover, minor specific pathways are operative and are very species-specific. For instance, *Proteus morganii*, with its homocystein-desulfurase, is able to catalyse the following reactions (Kallio 1951):

$$\text{L-homocysteine} + H_2O \rightarrow H_2S + NH_3 + \text{2-oxobutanoate}$$
$$\text{L-homocysteine} \rightarrow H_2S + \text{2-ammoniobut-2-enoate}$$

The following reaction is spontaneous:

$$\text{2 - ammoniobut - 2 - enoate} + H_2O \rightarrow \text{2 - oxobutanoate} + NH_3$$

Taxonomic groups

Bacteria that degrade sulfur-containing amino acids do not belong to a specific taxonomic group. To date, they were surprisingly poorly studied in the digestive ecosystem. These metabolic capacities were considered mainly by biotechnologists whose aim was to obtain overproduction of sulfur-containing amino acids or by dentists who are concerned by halitosis. Nevertheless, the fermentation of amino acids by the colonic bacteria was investigated and the desulfhydration capability is undoubtedly found within the bacterial group composed of *Enterococci*, *Enterobacteria*, *Clostridia*, and species belonging to the genera *Peptostreptococcus*, *Fusobacterium*, and *Eubacterium* (Smith and Marcfarlane 1997).

A wide variety of sulfate-reducing microorganisms have been isolated from diverse ecosystems. The bacteria belong to three main branches: (a) the δ-subclass of Proteobacteria with more than 25 genera, (b) the Gram-positive bacteria with the genera *Desulfotomaculum* and *Desulfosporosinus*, and (c) branches formed by *Thermodesulfobacterium* and *Thermodesulfovibrio*. Archaeal sulfate-reducers have also been isolated. They are strict anaerobes even if a few species tolerate oxygen for limited periods of time. Archae, *Thermodesulfobacterium* and *Thermodesulfovibrio*, are thermophilic organisms and presumably cannot be found within the large intestine. Other groups include psychrophilic, mesophilic, and thermophilic species and they exhibit a wide morphological diversity (cocci, rods, curved forms, sarcina-like cell aggregates, and multicellular gliding filaments) (Rabus et al. 2006; Schauder and Kröger 1993; Hedderich et al. 1999). Most of the published works dealing with colonic sulfate-reducing bacteria only take into account the enumeration or the quantification of these species with molecular tools. Very few studies have been devoted to the assessment of the diversity of bacterial species. Studies based on cultivation methods concluded to the predominance of the genus *Desulfovibrio*. Despite this apparent lack of diversity, the different strains of *Desulfovibrio* isolated from the colon were metabolically versatile (Willis et al. 1997). A study based on molecular characteristics shows the predominance of *Desulfovibrio piger*, followed by *Desulfovibrio fairfieldensis* and *Desulfovibrio desulfuricans* (Loubinoux et al. 2002). Scanlan et al. (2009) performed a study of the diversity of *Desulfovibrio* sp which confirmed the predominance of *D. piger* in the large intestine of humans, and *Bilophila wadsworthia* was the second most recovered isolate. This latter study highlighted the presence of unknown and uncultured *Desulfovibrio* species.

Carriage and dynamics of sulfide-producing bacterial populations: what consequences on luminal environment?

Little is known about the carriage of bacteria degrading sulfur-containing amino acids. The impact of diets enriched with such amino acids has not been determined despite the fact that they potentially lead to high concentrations of H_2S and mercaptans that are toxic to mammalian cells (Smith

and Marcfarlane 1997; Kadota and Ishida 1972). Moreover, longer colonic transit time affect the catabolism of carbohydrates and proteins leading to carbohydrate depletion and synthesis of putrefactive metabolites (Macfarlane and Macfarlane 2003a).

Recently, Stewart et al. (2006) determined the carriage rates of sulfato-reducing bacteria in 12 adults and 40 children. The bacteria were detected in 15% of the children and 58% of the adults. The authors noted that two of the methanogenic adults had, at the same time, high levels of sulfato-reducing-bacteria. Methanogenic archae and sulfato-reducing bacteria compete for hydrogen oxidation in the colon, and thus they are generally described as mutually exclusive populations. Nevertheless, the literature on this topic suggests that the two populations can coexist in the colon of a single host; one of the populations outcompeting the other. Sulfate availability is generally cited as the regulator factor of the competitive relationship. Several studies suggest that other modulating factors likely exist (Gibson et al. 1988a, b; Pochart et al. 1992; Christl et al. 1992; Strocchi et al. 1994; Deplancke et al. 2003). Intestinal transit rate together with sulfate intake has been proved to alter sulfate and hydrogen metabolism (Lewis and Cochrane 2007). Dietary sulfate and faster intestinal transit increase fecal sulfate reduction rate and sulfide concentration. To date, the relative contribution of dietary and genetic factors has not been considered. In addition to the potential production of H_2S, inorganic anions like sulfate affect the route of carbohydrate fermentation. They act as electron sinks that divert hydrogen from other intestinal bacteria metabolism. The main consequences are an increased acetate production and a decreased butyrate and lactate formation (Macfarlane and Macfarlane 2003b).

Alimentary and endogenous substrates for sulfide production and binding of luminal sulfide by fecal components

The H_2S concentrations in the luminal content of the human large intestine have been reported to be between 1.0 and 2.4 mmol/L (Macfarlane et al. 1992) (see Table 1). The sulfide concentration in the feces recovered from humans ranged from 0.22 to 3.38 mmol/kg (Magee et al. 2000). Florin (1991) found a somewhat lower H_2S concentration in human feces (i.e., 0.17 mmol/kg feces). Jorgensen and Mortensen (2001) found a concentration of sulfide in human feces in the range of what was measured by Magee et al. (2000) averaging 0.74 mmol/L. Interestingly, there was a correlation between the level of meat intake (and presumably protein in meat) and the level of fecal excretion of sulfide. In the experimental rodent models, the sulfide concentration in the luminal content has also been measured. The total sulfide concentration in the rat cecal content (i.e., 1.5 mmol/kg), as determined by Levitt et al. (2002), was found to be not vastly different when compared with what is found in the human large intestine content. In mice, Deplancke et al. (2003) found that H_2S concentrations in the large intestine luminal content was lower representing between 0.2 and 1.1 mmol/kg.

However, it has to be emphasized that probably a large fraction of sulfide is in bound form in the colonic luminal content. This can be presumed from experiments with feces recovered from human volunteers, which indicates that 8% of total sulfide is in its free (unbound) form (Jorgensen and Mortensen 2001), representing a free concentration equal to 60 µmol/L. In rats, it was determined that approximately 1% of total sulfide in feces is in its free form (Levitt et al. 2002) averaging 2.7 µmol/kg (Table 1). Although it was determined that there is a large capacity of fecal components to bind H_2S (Jorgensen and Mortensen 2001), the compounds responsible for this binding remain largely unknown. In the rat model, it has been determined that zinc acetate reduced cecal H_2S by fivefold indicating potential capacity of this divalent cation for sulfide binding (Suarez et al. 1998). In batch cultures seeded with fecal flora, bismuth, iron, and zinc (but not magnesium) were able to diminish free H_2S concentration (Mitsui et al. 2003).

H_2S can be produced by the large intestine microbiota from numerous substrates (see Fig. 1.): sulfur-containing amino acids (methionine and cyst(e)ine), inorganic sulfate,

Table 1 Fecal and luminal concentrations of sulfide in the large intestine of human volunteers and experimental animals

Biological material	Total sulfide concentration	Free (unbound) sulfide concentration	References
Human large intestine luminal content	1.0–2.4 mmol/L	ND	Macfarlane et al. (1992)
Human fecal content	0.22–3.38 mmol/kg	ND	Magee et al. (2000)
Human fecal content	0.17 mmol/kg	ND	Florin (1991)
Human fecal content	0.74 mmol/L	60 µmol/L (8% of total concentration)	Jorgensen and Mortensen (2001)
Rat luminal cecal content	1.5 mmol/kg	2.7 µmol/kg (0.2% of total concentration)	Levitt et al. (2002)
Mouse large intestine luminal content	0.2–1.1 mmol/kg	ND	Deplancke et al. (2003)

ND not determined

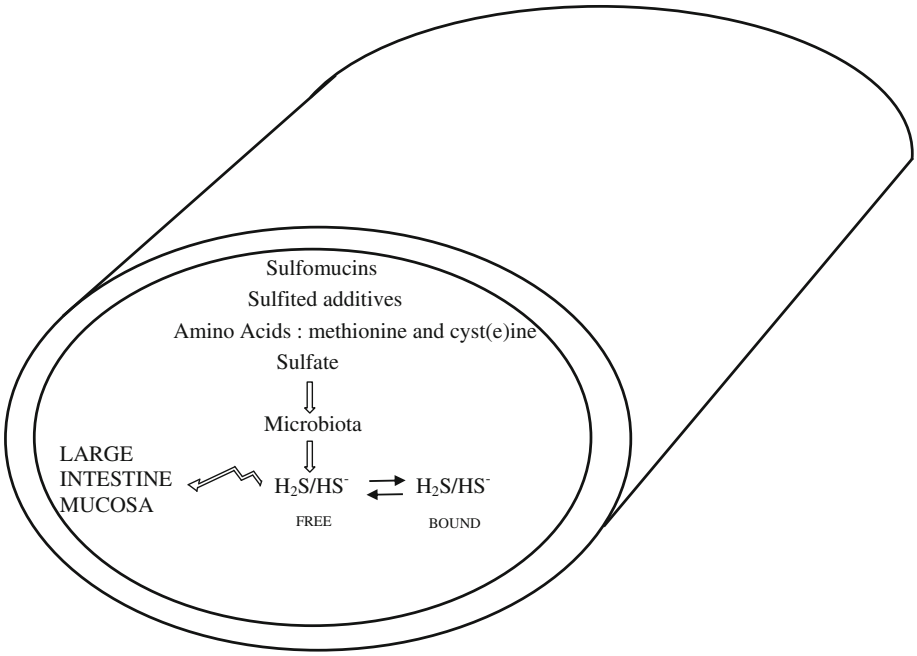

Fig. 1 Production of sulfide by the intestinal microbiota from the luminal substrates in the lumen of large intestine

and sulfited additives (Florin et al. 1991; Gibson et al. 1988a, b). Inorganic sulfur in the form of sulfite, sulfur dioxide, bisulfate and metabisulfite is currently used for preservation of food and beverage (Wedzicha 1984). Sulfur-containing amino acids, which are fermented by the microbiota, can originate from both alimentary and endogenous proteins which have escaped digestion in the small intestine (Evenepoel et al. 1999; Gaudichon et al. 2002). A western diet contains between 1.5 and 16.0 mmol/day of inorganic sulfate and about 3.8 mmol/day of protein-derived sulfate (Lewis and Cochrane 2007). Drinking water, especially those from private wells, may contain up to 20 mmol/L of sulfate (Deplancke et al. 2003). In addition to these different sources of substrates, sulfated polysaccharides such as sulfomucins are endogenous substrates for H_2S production (Willis et al. 1996). Human colonic mucins are known to be more highly sulfated than small intestine mucins (Liau and Horowitz 1976) and the thickness of both the firm and loosely adherent layers is higher in the large than in the small intestine (Allen and Flemström 2005) raising the view that large intestine mucus can represent a significant precursor for H_2S production. Endogenous secretion of sulfate in the upper gastrointestinal tract has been estimated to be between 1.5 and 2.6 mmol/day (Willis et al. 1996). In contrast to conventional animals, germ-free mice accumulate large amounts of mucins in the gut, in particular, in the vastly distended cecum (Drassar and Hill 1974). Interestingly, it has been shown that in vitro mixed culture of human fecal bacteria grown in a three-stage continuous culture system (that mimics the gastrointestinal tract), when fed with porcin gastric mucins in addition to a mixture of polysaccharides and proteins, allows extensive mucin degradation. This phenomenon is evidenced by the stimulation of SCFA production and the rise of sulfate reduction and a concomitant complete inhibition of methanogenesis (Gibson et al. 1988a). In vitro experiments in a single-stage chemostat show that the complete degradation of mucins requires consortia of bacteria. In coculture, *Bacteroides fragilis* releases sulfate from mucins in sufficient amount to support the growth of a sulfate-reducing bacteria (Willis et al. 1996).

Although sulfate was first believed to be poorly absorbed by the human gastrointestinal tract (Wilson 1962), it appears that a net absorption of sulfate is measurable and that the amount of dietary sulfate as well as the small intestine absorption capacity are the main factors affecting the amount of sulfate reaching the colon (Florin et al. 1991). The ability of the small intestine to absorb sulfate is about 16 mmol/day in normal subjects but represents not more than 5 mmol/day in the ileostomists (Florin et al. 1991). It is also possible that secretion of sulfate by the colonic mucosa represents a significant contributor for luminal sulfate content. In human volunteers, the ingestion of sulfate supplement was found to increase the fecal sulfide production rate (Lewis and Cochrane 2007). From these data, it appears that current concentrations of sulfate in the large intestine are adequate to support growth of sulfate-reducing bacteria.

From experimental work using the rat model, it has been determined that the H_2S production in the lumen of the large intestine depends on the dietary conditions since fasting is associated with a marked reduction of cecal H_2S concentrations, and since the ingestion of the nonabsorbable sulfur

compound carrageenan results in a sharp increase of the concentration of this compound (Suarez et al. 1998).

Effects of sulfide on large intestine mucosa

Effects of sulfide on colonic epithelial cell oxidative metabolism

Because of its lipid solubility, H_2S penetrates biological membranes (Reiffenstein et al. 1992). In an aqueous solution, H_2S dissociate yielding hydrosulfide anion and sulfide ion with pKa values being equal to 7.04 and 11.96, respectively. At physiological pH (i.e., pH 7.4), approximately one-third of H_2S remains undissociated (Wang 2002), whereas remaining two-third is hydrosulfide anion (HS^-) at equilibrium with H_2S. Also, H_2S is known to severely inhibit cytochrome oxidase activity; an effect characterized by a binding constant similar to the one measured with cyanide (i.e., 0.2 µmol/L) using purified cytochrome *c* oxidase (Petersen 1977; Hill et al. 1984). Cytochrome *c* oxidase is the terminal oxidase of the mitochondrial electron transport chain which catalyses the reduction of the dioxygen (O_2) to water and harnesses the free energy of the reaction to phosphorylate ADP to ATP (Yoshikawa 1999). Using homogenate of the colonic epithelial cells HT-29, it was determined that IC_{50} of the sodium salt of H_2S-NaHS for cytochrome *c* oxidase activity is equal to 0.32 µmol/L (Leschelle et al. 2005). Accordingly, micromolar concentrations of NaHS, in the range of 30–60 µmol/L, are able to severely decrease HT-29 cellular oxygen consumption. However, this inhibitory effect was found to be largely reversible (Leschelle et al. 2005). H_2S has been reported to be able to inhibit butyrate oxidation in isolated human colonocytes (Roediger et al. 1993a). Using millimolar concentrations (1.25 and 2.5 mmol/L) of sulfide, Jorgensen and Mortensen (2001) found marked inhibition of butyrate oxidation by rat colonocytes. In this latter study, and as expected, the authors found that bound sulfide has little effect on butyrate oxidation by colonocytes. In another work, Roediger et al. (1993b) found a weak inhibition of butyrate oxidation in rat colonocytes using 100 µmol/L NaHS; an effect that was hypothesized to be due to the inhibition of butyryl-CoA dehydrogenase which participates in mitochondrial beta-oxidation (Moore et al. 1997; Shaw and Engel 1987; Babidge et al. 1998). However, since NaHS is also able to affect acetate and L-glutamine oxidation which are not related to the beta-oxidation (Leschelle et al. 2005), this indicates that butyryl-CoA deshydrogenase is not the exclusive point of impact of NaHS on colonic epithelial cell oxidative metabolism. It is worth noting that the concentration of free sulfide necessary for inhibition of butyrate oxidation in isolated rat colonocytes represents at least 30-fold the measured concentration of free sulfide in the large intestine luminal content of the rat; raising the view that in vivo, cytochrome *c* oxidase inhibition is a more likely target of sulfide that enzymes involved in SCFA and other substrate oxidation.

Pre-treatment of the human colonic epithelial cells HT-29 with 1 mmol/L NaHS for 2 h induced neither cell necrosis nor apoptosis, but induced a marked decrease of the cell proliferative capacity (Leschelle et al. 2005). Also, NaHS pre-treatment provoked a more than fourfold increase of the net capacity of HT-29 cells to produce lactate in the glycolytic pathway. Since the adenine nucleotides such as ATP, ADP, and AMP were found unchanged in sulfide-treated cells when compared to untreated cells, despite the inhibitory effect of NaHS on cell oxygen consumption (Leschelle et al. 2005), it was proposed that reduced cell mitosis [thus reduced energy consumption (Buttgereit and Brand 1995)] and increased glycolysis (thus increased anaerobic energy production) represent an adaptation of HT-29 cells toward the effect of sulfide on colonic epithelial cell.

However, the concept that sulfide is simply a metabolic troublemaker toward colonic epithelial cells has been challenged recently (see "Detoxifying metabolism of sulfide by large intestine epithelial cells").

Effects of endogenous sulfide as a neuromodulator in the large intestine

Schicho et al. (2006) reported that in humans, more than 90% of submucous and myenteric neurons are colabeled for cystathionine gamma-lyase (CSE) and cystathionine beta-synthase (CBS). These pyridoxal phosphate-dependent enzymatic activities, which are involved in the *trans*-sulfuration of cysteine (Stipanuk 2004), are responsible for the intracellular production of H_2S (Szabo 2007) (see Fig. 2). Furthermore, Schicho et al. (2006) found that the sodium salt of H_2S at millimolar concentrations increased chloride secretion in a colonic submucosa/mucosa preparation. This effect was mimicked by the utilization of cysteine instead of NaHS. Overall, these results suggest that H_2S represent a novel prosecretory neuromodulator in the colonic mucosa.

Interestingly, it was discovered that NaHS at micromolar concentrations is able to exert relaxant effect in a preparation of isolated ileum and to reduce the contractile response of isolated ileum to acetylcholine (Teague et al. 2002) leading the authors to propose that H_2S is involved in controlling the ileum contractility.

Using immunohistochemistry and RT-qPCR, Linden et al. (2008) showed that the mouse colon express the H_2S-forming enzymes CSE and CBS, reinforcing the idea that

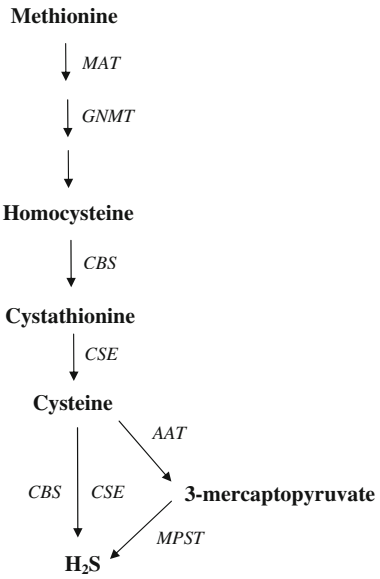

Fig. 2 Endogenous H_2S production. *MAT* methionine adenosyltransferase, *GNMT* glycine *N*-methyltransferase, *CBS* cystathionine beta-synthase, *CSE* cystathionine gamma-lyase, *AAT* aspartate aminotransferase, and *MPST* 3-mercaptopyruvate sulfur transferase

sulfide is an endogenously formed signal molecule in the colon. CSE and CBS are highly expressed in the colonic mucosa, but only CSE appears to be expressed in the external muscle layers including the myenteric plexus.

In isolated segments of mouse colon, sodium H_2S causes an inhibition of spontaneous motor complexes (Gallego et al. 2008). This agent was also able to inhibit the spontaneous motility in strips from human and rat colons. All these effects appear to be critically dependent on the K^+ channels (Gallego et al. 2008).

Sulfide and nociception

Sulfide has recently been implicated in the nociception in the large intestine but controversial results have been obtained in this research area. In 2006, Distrutti et al. (2006b) tested a derivative of mesalamine i.e., 5-amino-2-hydroxybenzoic acid 4-(5-thioxo-5H-(1,2)dithiol-3yl)-phenyl ester as an H_2S-releasing agent for modulating nociception to colorectal distension in healthy and post-colitic rats. They found that this compound, through a mechanism involving the ATP-dependent K^+ channels inhibits the hypersensitivity induced by colorectal distension in both physiological and pathological situations. In the same model of colorectal distension in rats, Distrutti et al. (2006a) found that intraperitoneal injection of 60 µmol/kg NaHS was also efficient for the attenuation of nociception.

These results are in contrast with the one obtained by Matsunami et al. (2009) using the mice model. These authors reported that after intracolonic administration of 0.5–5 nmol/mouse NaHS, visceral pain-like behavior and abdominal hyperalgesia could be recorded. Using the experimental rat model of irritable bowel syndrome in which chronic visceral hyperalgesia is provoked using colonic injection of 0.5% acetic acid, it was found that the expression of CBS in colonic dorsal root ganglion and H_2S signaling is likely to play a role in the development of chronic visceral hyperalgesia (Xu et al. 2009). In fact, CBS immunoreactivity was found in a vast majority of the dorsal root ganglion neurons, and CBS expression was increased after acetic acid treatment. In addition, perfusion of 250 µmol/L NaHS in the dorsal root ganglion neurons leads to an increase in the number of action potentials in 68% of the neurons.

The controversial results obtained by these authors can likely be explained by the different experimental protocols used, by the different doses of active agents used as well as the different route of administration (i.e., intraperitoneal and intracolonic). In that regard, intraluminal injection of sulfide in the colon appears to be more related to the physiological effect of this agent. Additional experiments are needed in order to decipher the respective role of endogenously formed and intraluminal H_2S for the effect of this compound on algesia originating from the large intestine.

Detoxifying metabolism of sulfide by large intestine epithelial cells

Since sulfide is a potentially deleterious compound for the intestinal epithelial cells when present in excess, the identification of the enzymatic systems responsible for sulfide detoxification is of major interest. Thiol *S*-methyltransferase is found to be present with a relatively high activity in the rat cecal and colonic mucosa (Weisiger et al. 1980). This enzyme catalyses the methylation of H_2S in the presence of *S*-adenosylmethionine. However, conversion of sulfide to thiosulfate appears to represent the main process for sulfide detoxication in colonic epithelial cells. In fact, analysis of cecal venous blood obtained after intracecal instillation of radioactive H_2S in rats reveals that virtually all absorbed H_2S is oxidized to thiosulfate (Levitt et al. 1999). This metabolic capacity appears to be a specialized function of the large intestine mucosa since the other tissues tested were much less efficient for such a process (Furne et al. 2001). Another enzyme, namely rhodanese, which is expressed in the submucosa and crypts of the colon has been proposed to be involved in the process of sulfide detoxication in the large intestine (Picton et al. 2002). This enzyme requires the presence of cyanide for catalytic activity (Picton et al. 2002); and this latter property has raised questions about its physiological

implication in the course of sulfide detoxification. Although it was initially proposed that rhodanese activity catalyses the reaction of sulfide and cyanide to form thiocyanate (Picton et al. 2002), it appears from recent data that rhodanese is not involved in the first step of H_2S metabolism, but most likely in a more distal step of sulfide detoxification (Wilson et al. 2008). From experimental work performed with rat liver, it appears that the membrane-bound mitochondrial enzyme sulfide quinone reductase (SQR) is the mitochondrial enzymatic activity involved in the first step of sulfide detoxication (Hildebrand and Grieshaber 2008) allowing conversion of H_2S to persulfide. Then, a putative sulfur dioxygenase located within the mitochondrial matrix would oxidize persulfide to sulfite with molecular oxygen consumption. Much interestingly, the demonstration that mice invalidated for the mitochondrial dioxygenase ETHE1 dies between the fifth and the sixth week after birth due to sulfide toxicity clearly demonstrates that this enzymatic activity is of paramount importance for sulfide detoxification (Tiranti et al. 2009). The enzyme rhodanese with sulfur transferase activity is likely acting in third position in the liver cell mitochondrial matrix allowing conversion of sulfite to the metabolic end product thiosulfate (Hildebrand and Grieshaber 2008). In the colonic mucosa, Wilson et al. (2008) have proposed that sulfide oxidase, as the first and rate-limiting step, catalyses the conversion of H_2S to thiosulfate; thiosulfate being then converted to thiocyanate by the rhodanese activity. However, from previous in vivo work (Levitt et al. 1999), it appears that few thiosulfates are converted into thiocyanate by the large intestine mucosa. A tentative schematic view of the mitochondrial enzymatic system involved in H_2S detoxification in colonic epithelial cells is presented in Fig. 3. According to this scheme, SQR would represent the first enzyme involved in sulfide utilization by colonocytes. These latter enzymes extract electrons from sulfide and forward them to the quinone pool in mitochondria. These electrons are then transferred to the complexes III and IV in the mitochondrial respiratory chain and then to oxygen to form water. In terms of electron transfer, the SQR activity is then similar to that of complex II (i.e., succinate dehydrogenase). If a saturating concentration of succinate (i.e., millimolar) is provided to isolated mitochondria, it leads to fast electron transfer and energization of the mitochondrial inner membrane allowing phosphorylation of ADP into ATP. The use of same millimolar concentrations of sulfide leads to immediate poisoning of the mitochondrial respiration. However, if low micromolar concentrations of sulfide are provided with an infusion pump to permeabilized colonic epithelial cells, it is possible to match sulfide delivery to sulfide oxidation rate in these cells. Using this latter procedure, it has been shown that sulfide oxidation capacity by colonic epithelial cells is not vastly different when compared with succinate oxidation capacity in these cells (Goubern et al. 2007) and leads to mitochondrial energization. Consequently, if colonocytes are facing a continuous production of luminal sulfide by the microbiota which is lower than their oxidative capacity, they will detoxify sulfide and will use it as a mineral energy substrate. If this oxidation of sulfide takes place near its maximal rate, this will represent the coverage of a significant part of the colonocyte energy needs. In other words, oxidation of sulfide thus represents a likely way for colonic epithelial cells to detoxify and recover energy from a luminal inorganic substrate as long as the cellular metabolic capacity is not defeated. Interestingly, sulfide metabolism represents the primary energetic source in ecosystems where no sunlight is available and can be considered as a prokaryotic metabolism older than photosynthesis. It is therefore tempting to propose that sulfide oxidation in colonocytes represent a very ancestral function. Recent data (Lagoutte, Mimoun, Andriamihaja, Chaumontet, Blachier and Bouillaud; unpublished data 2009) have measured the complex interactions between sulfide and other substrate oxidations. Therefore, these observations are relevant to the trophic interactions between the microbiota and the gut mucosa but require further experimental works. It also raises the view that the balance between the sulfide production by the intestinal microbiota and the colonocyte oxidative capacity would determine the nature of sulfide i.e., a friend or a foe.

Sulfide and large intestine inflammation and cancer

Sulfide and large intestine inflammation

Some clinical and experimental data suggest that sulfide in excess may be implicated in the etiology and/or in the risk of relapse of ulcerative colitis. Ulcerative colitis is a colon-localized inflammatory bowel disease characterized by broad epithelial cell damage, crypt abscesses and

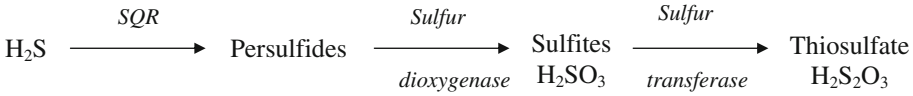

Fig. 3 Tentative schematic view of the mitochondrial enzymatic system involved in H_2S detoxication/utilization in colonic epithelial cells. *SQR* sulfide quinone oxidoreductase

accumulation of neutrophils but is of still unknown aetiology. Ulcerative colitis depends on complex interactions between genetic and environmental factors in susceptible hosts (Ahmad et al. 2001). The putative role of H_2S in ulcerative colitis is originating from several lines of evidence. First, it has been documented that H_2S in excess has a number of adverse effects that could play a role in the pathogenesis of the disease (reviewed in Pitcher and Pitcher 1996). Second, fecal sulfide concentration and production were found to be elevated in patients with ulcerative colitis (Pitcher et al. 2000; Levine et al. 1998). However, it should be noted that other authors found no difference in fecal sulfide concentration between ulcerative colitis patients and control subjects (Moore et al. 1998). Third, in some experimental colitis animal models, it is possible to induce a pathological state similar to the one observed in ulcerative colitis using undigestible sulfates in the form of dextran sulfate sodium (Gaudio et al. 1999; Tamaru et al. 1993; Leung et al. 2000) and sulfate-containing carraghenan (Watt and Marcus 1973). It has been reported that ulcerative colitis patients who take 5-aminosalicylic acid-containing drugs were characterized by lower fecal sulfide levels than those not taking these drugs (Edmond et al. 2003), which indicates that inhibition of sulfide production may contribute to the therapeutic effect of these agents in ulcerative colitis.

Recently, and in contrast with the putative role of H_2S in ulcerative colitis, Wallace et al. (2009) reported that intracolonic administration of NaHS (30 μmol/kg) reduced the severity of colitis induced by intracolonic administration of trinitrobenzene sulfonic acid. In the meantime, they observed a reduction of the expression of the proinflammatory cytokine tumor necrosis factor alpha. In addition, inhibition of H_2S synthesis in healthy rats was found to result in colonic inflammation leading the authors to propose that endogenous and exogenous H_2S promote resolution of colitis in rats in this experimental model. However, it is worth noting that the effect of H_2S is probably depending on the experimental model of colitis used. Indeed, using the dextran sulfate-induced colitis in rats, Furne et al. (2000) observed that feeding rats with bismuth subsalicylate, a compound that bind sulfide, reduced the fecal release of H_2S in dextran sulfate-treated rats to values below that of controls without modification of the histological aspect of colitis raising the view that excessive sulfide production does not play a major role in this experimental model of colitis.

A very significant clinical study was performed with ulcerative colitis patients in remission who were followed up for 1 year to determine the effect of habitual diet on the relapse (Jowett et al. 2004). In this study, it was found that high consumption of meat, protein as well as high sulfur or sulfate intakes was associated with an increased likelihood of relapse for patients. Even if, as noted by the authors, further studies are required to determine if the sulfur compounds within the foods mediate the risk of relapse in patients, the data presented strongly suggest that the bacterial synthesis of sulfide from alimentary substrates in the lumen of the large intestine plays a role in such a relapse process. It is also possible that the capacity of colonic epithelial cells for H_2S detoxification is altered in ulcerative colitis patients, but a role of such a defect remains a working hypothesis that needs to be urgently tested.

Some experimental facts suggest that the capacity of colonocytes to metabolize H_2S is an important feature for their resistance toward an excessive concentration of free luminal sulfide. In the experimental model of dextran sulfate sodium-evoked colitis in mice, Taniguchi et al. (2009) found that rhodanese expression and activity was decreased in the colonic tissues in parallel with the development of colitis. Roediger et al. (1996) reported that *S*-adenosylmethionine (a cosubstrate for the methylation of H_2S) at a concentration of 5 mmol/L is able to reduce the inhibitory effect of sulfide upon butyrate oxidation in human colectomy specimens. Although methylation of sulfide appears to play a minor role for detoxification of excessive luminal sulfide, this metabolic pathway may play a role in limiting sulfide toxicity toward colonocytes. Two other enzymatic activities, i.e., rhodanese and thiol methyltransferase believed to play a role in sulfide detoxication, were measured in rectal mucosa obtained from patients with ulcerative colitis and control individuals (Picton et al. 2007). No difference was found for both enzymatic activities between the two groups of individuals. However, the activity of the first and rate-limiting enzyme for H_2S metabolism, i.e., sulfide quinone oxidoreductase in colonic epithelial cells, was not measured in this study.

Sulfide and large intestine cancer

The data which would allow implying prolonged excessive concentration of sulfide in the luminal content of the large intestine and/or defective expression of detoxifying catalytic activities in large intestine epithelium in the colon carcinogenesis are scarce. Attene-Ramos et al. (2006) reported that Na_2S at a concentration of 500 μmol/L (and above) is able to provoke genomic DNA damage in HT-29-Cl.16E colonic cells using an assay in which DNA repair is inhibited. Furthermore, using naked nuclei from Chinese hamster ovary cells (CHO cells) treated with Na_2S, it was found that a concentration as low as 1 μmol/L was able to damage genomic DNA with single and double breaks or lesions that can be converted to strand breaks (Attene-Ramos et al. 2007); such a phenomenon appears to involve free radical production. Although this study was interesting and provocative, it has to be kept in mind that the

experimental design chosen was far away from the physiological conditions which prevails in the large intestine.

Ramasamy et al. (2006) reported that the immunoreactivities of thiosulfate sulfurtransferase (TST) and mercaptopyruvate sulfurtransferase (MST), two isoenzymes of rhodanese, in the luminal mucosal surface are markedly reduced in advanced colon cancer. Furthermore, they found that TST activity and expression in the human colon adenocarcinoma HT-29 cells can be increased by differentiating agents like butyrate and histone deacetylase inhibitor and increased by sulfide itself. From these results, the authors concluded that dysregulation of TST activity in the colonic mucosa could represent a factor involved in colorectal cancer. New experiments are needed to establish possible causal relationship between TST expression in the colonic mucosa and the colorectal carcinogenesis.

The incubation of mucosal biopsies from the sigmoid rectum obtained from ten patients with normal mucosa, polyps, or inflammatory bowel diseases in the presence of 1 mmol/L NaHS resulted in mucosal hyperproliferation with an expansion of the proliferative zone to the upper crypt (Christl et al. 1996), suggesting that sulfide can affect epithelial cell homeostasis. Accordingly, Deplancke and Gaskins (2003) reported that exposure of nontransformed intestinal epithelial IEC-18 cells with millimolar concentration of NaHS resulted in an inhibition of mitochondrial respiratory activity and an increase of the proliferative cell fraction leading the authors to propose that sulfide may initiate epithelial cell dysregulation and to speculate that these events may contribute to colorectal carcinogenesis.

Lastly, by comparing the fecal amounts of H_2S between volunteers who had previously undergone surgery for sigmoid colon cancer and who later developed new epithelial colon neoplasia and individuals whose large bowel was entirely normal (as verified by total colonoscopy), Kanazawa et al. (1996) found increased fecal excretion of sulfide in the sigmoid cancer group although the possible causal relationship between the two parameters would require new investigations.

Conclusion

From the available data regarding the effects of H_2S on the large intestine mucosa, it appears that there is little doubt that this compound resulting from the microbiota metabolic activity can interfere with the colonic epithelial cell metabolism. Both beneficial and deleterious effects of luminal sulfide are possible toward this intestinal epithelium which represents a frontier between the luminal content and the "milieu intérieur". Considering sulfide as a friend or a foe depends on numerous parameters including (a) sulfide concentration inside the colonic lumen and thus substrate availability from exogenous (alimentary) and endogenous origins and metabolic capacity for the microbiota to produce H_2S, (b) percentages of sulfide in free and bound forms, (c) capacities of colonic epithelial cells to detoxicate and to use sulfide as an energy source and availability of anaerobic metabolic pathway (i.e., glycolysis) for energy production when mitochondrial oxygen consumption is impaired, and (d) capacity of colonic epithelial cells to rapidly adapt to an excess in luminal sulfide production. Although there have been an improvement in the recent years in our understanding on how H_2S is metabolized in colonocytes, there is still a need for a consensus regarding the metabolic pathways involved, even if sulfide quinone oxidoreductase, sulfur dioxygenase, and rhodanese are likely to play an important role in the detoxication process when sulfide is present in excess. Also, the intraluminal compounds responsible for sulfide binding need further works for identification and characterization since they are likely to represent one major parameter in determining the free sulfide luminal concentration. From the overall available data, it is tempting to propose, as a working hypothesis, that any imbalance between concentration of free sulfide in the large intestine mucosa and the capacity of epithelial cells to metabolize, it will result in a loss of normal oxidative cell capacity and maybe in the process of normal epithelial cell renewal with possible consequences on the process of mucosal inflammation and/or relapse risk.

Recent works have also raised the idea that endogenously produced H_2S is likely to play a role in the neuromodulation of chloride secretion, in the control of intestinal contractibility, and on the nociception from the large intestine. About this latter effect, it is not clear from the experimental evidence if H_2S is acting as a pro- or anti-nociceptive agent toward the large intestine.

The capacity of mammalian cells to use a very simple sulfur-containing gas molecule is most probably a reminiscence of a very ancestral function as indicated by metabolic studies with bacteria and marine animals living in a rich sulfur-containing environment (Griesbeck et al. 2002; Grieshaber and Völkel 1998).

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