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Neuromodulatory effects and targets of the SCFAs and gasotransmitters produced by the human symbiotic microbiota

Alexander V. Oleskin[1] and Boris A. Shenderov[2]*

[1]General Ecology Department, Biology Faculty, Lomonosov Moscow State University, Moscow, Russia;
[2]Moscow Research Institute of Epidemiology and Microbiology after G.N. Gabrichevsky, Moscow, Russia

The symbiotic gut microbiota plays an important role in the development and homeostasis of the host organism. Its physiological, biochemical, behavioral, and communicative effects are mediated by multiple low molecular weight compounds. Recent data on small molecules produced by gut microbiota in mammalian organisms demonstrate the paramount importance of these biologically active molecules in terms of biology and medicine. Many of these molecules are pleiotropic mediators exerting effects on various tissues and organs. This review is focused on the functional roles of gaseous molecules that perform neuromediator and/or endocrine functions. The molecular mechanisms that underlie the effects of microbial fermentation-derived gaseous metabolites are not well understood. It is possible that these metabolites produce their effects via immunological, biochemical, and neuroendocrine mechanisms that involve endogenous and microbial modulators and transmitters; of considerable importance are also changes in epigenetic transcriptional factors, protein post-translational modification, lipid and mitochondrial metabolism, redox signaling, and ion channel/gap junction/transporter regulation. Recent findings have revealed that interactivity among such modulators/transmitters is a prerequisite for the ongoing dialog between microbial cells and host cells, including neurons. Using simple reliable methods for the detection and measurement of short-chain fatty acids (SCFAs) and small gaseous molecules in eukaryotic tissues and prokaryotic cells, selective inhibitors of enzymes that participate in their synthesis, as well as safe chemical and microbial donors of pleiotropic mediators and modulators of host intestinal microbial ecology, should enable us to apply these chemicals as novel therapeutics and medical research tools.

Keywords: *short-chain fatty acids*; *gasotransmitters*; *nitric oxide*; *carbon monoxide*; *hydrogen sulfide*; *ammonia*; *microbiome*; *neurotransmitter*; *neuromediator*

*Correspondence to: Boris A. Shenderov, Moscow Research Institute of Epidemiology and Microbiology after G.N. Gabrichevsky, Moscow, Russia, Email: shenderov@yandex.ru; aoleskin@rambler.ru

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The symbiotic microbiota of mammals constitutes a peculiar 'microbial organ' (1–4) that is directly or indirectly involved in a variety of metabolic, communicative, and behavioral processes in the host organism (4, 5). Using various animal models and also human subjects, researchers have demonstrated that intestinal symbiotic microbiota influences the host's food and sexual partner choice; recognition of olfactory signals; stress responses; and numerous other psychological, behavioral, and social activities (3, 6–8). The functional role of intestinal microorganisms in the ongoing host-microbiota dialog is based on the production of numerous low molecular weight compounds (amino acids, biogenic amines, volatile fatty acids, and so on) that behave as effectors, enzyme cofactors, and signal molecules (5, 9). The biologically active substances (BASs) are subdivided into autocrine (targeting the BAS-synthesizing cell), paracrine (targeting adjacent cells), and endocrine (functioning as hormones that globally mediate processes in remote tissues) agents. Microbiota-produced low molecular weight compounds are metaphorically denoted as the 'words' of the language used for microbiota-host communication (9). Specialized endocrine glands and the nervous system only synthesize a limited number of signals (hormones and neurotransmitters), whereas the human microbiota produces hundreds of BASs (4, 5). Microbial BASs exert their neurochemical effects via neuroendocrine, immune, metabolic, and epigenetic pathways (5, 7, 10–12). Taking into account our current knowledge concerning the role of the gastrointestinal (GI) microbiota in terms of the health and

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psyche of human individuals, it has been suggested to change the popular term 'gut-brain axis' to the term 'microbiota-gut-brain axis' (1–3, 7, 11, 13).

In recent decades, a wide spectrum of microecological agents have been employed in order to optimize the intestinal microbial consortium. Of particular importance are *probiotics* defined as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' (14, 15). The main goal of administering probiotics is to optimize the qualitative and quantitative composition of the symbiotic microbiota, increase its stability, and maintain its physiological, metabolic, and communicative activities at a level that conforms with the consumer's living standard (9, 14). A special subgroup of probiotics is called *psychobiotics.* They are 'live organisms that, when ingested in adequate amounts, produce a health benefit in patients suffering from psychiatric illness (16)'. Psychobiotics are exemplified by *Lactobacillus rhamnosus* JB-1 and *Bifidobacterium longum* NCC3001 strains, whose administration to mice suppressed their anxiety-like behavior. A similar effect was produced by *L. helveticus* R0052 and *B. longum* R0175 in rats. The manifestation of depression, similar to the behavior of young rats upon weaning, was mitigated by the oral administration of *Bacteroides infantis* 35624 (17). Candidate strains to be used for producing psychobiotics should satisfy, apart from safety criteria, the following requirements. They should produce sufficient amounts of BASs that should be functionally analogous and structurally homologous or identical to nutritional or endogenous hormones and neurotransmitters (or, alternatively, their precursors and cofactors), such as dopamine, norepinephrine, L-DOPA, serotonin, melatonin, kynurenine, tryptophan, acetylcholine, histamine, aspartic acid, glutamic acid, taurine, glycine, acetate, butyrate, propionate, glutamate and γ-aminobutyrate (GABA), opiates (endorphins, enkephalins, and dynorphins), substances P and Y, ghrelin, leptin, and others (3–5, 7, 12, 13, 16, 18–22).

Recently, much attention has been paid to simple gaseous substances, such as nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H_2S), hydrogen, methane, and ammonia. Apart from host tissues exemplified by the blood vessel endothelium, such substances may be of microbial origin. They have been shown to behave as mediators and regulators of intra- and intercellular communication in the mammal organism. The qualitative and quantitative composition of gaseous BASs varies depending on the organ, tissue, and even the area of the GI tract involved, as well as on the individual features of tested human subjects (23–28). This review focuses on the analysis of recent data concerning the targets and mechanisms of the action of short-chain fatty acids (SCFAs) and gaseous substances (NO, CO, H_2S, and NH_3) of microbial origin in terms of host behavior regulation and the pathogenesis of a number of neurophysiological and psychiatric disorders.

Short-chain fatty acids

Prior to discussing typical gaseous mediators, SCFAs are to be briefly considered. Shortly after discovering SCFAs, they were often referred to as volatile fatty acids. The main reason for coining this term was their specific odor, not their volatility *per se.* SCFAs are synthesized by host cells and various microorganisms that predominantly inhabit the intestines (*Archaea*, *Bacteroides*, *Bifidobacterium*, *Butyrivibrio*, *Clostridium*, *Collinsella*, *Coprococcus*, *Desulfovibrio*, *Eubacterium*, *Lactobacillus*, *Prevotella*, *Propionibacterium*, *Roseburia*, and others). Representatives of these groups of microorganisms are equipped with a large armamentarium of glycoside hydrolases. This enables them to degrade complex polysaccharide and protein molecules of animal, plant, and microbial origins. The resulting smaller molecules are anaerobically metabolized to form terminal products, including SCFAs and a number of gases (H_2, CH_4, CO_2, H_2S, and NH_3) (29). SCFAs produce multifarious effects on physiological, metabolic, regulatory, and behavioral processes in the mammal host organism that take place both within the GI tract and beyond it. One of the causes of neurophysiological disorders is the disruption of SCFAs formation and metabolism, primarily because SCFAs are actively involved in providing the organism (including its nervous cells) with energy (4, 5, 29, 30). In addition, the activity of the sympathetic nervous system is subject to regulation by SCFAs (e.g. by propionate) via their interaction with G-protein-coupled receptors (GPRs), such as the GPR41 and GPR43 receptors of the ganglia of the enteric nervous system. The activation of these receptors in sympathomimetic neurons involves signaling pathways, for example, the Gβγ–PLCβ–MAPK pathway that controls the organism's energy budget and maintains metabolic homeostasis. Changing the amount of SCFAs and ketone bodies formed from them in the liver, as well as their ratio, causes alterations in energy metabolism and the activity of the sympathetic nervous system (5, 12, 31).

Apart from providing the cells of the central nervous system with energy, SCFAs, such as propionate and butyrate, exert an influence on the intracellular potassium level, which implies the involvement of SCFAs in the operation of cell signaling systems. In particular, these SCFAs regulate the expression of the gene coding for tryptophan hydroxylase, the key enzyme of the serotonin biosynthesis pathway, and, therefore, produce an effect on brain neurochemistry (4). SCFAs decrease the activity of chromosome histone deacetylases (HDACs), thereby facilitating the access of repair enzymes to the DNA. This promotes the improvement of the health state of patients with the excessive activity of these enzymes that is characteristic of Parkinson's disease, depression, and

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schizophrenia. Inhibiting HDAC activity ameliorates the state of patients suffering from malignant tumors of the nervous system. SCFAs as HDAC inhibitors exert a beneficial influence on model animals with cerebral trauma, dementia, autoimmune encephalitis, and depression-like symptoms (4, 5, 12).

In GI endocrine cells, SCFAs induce the synthesis of neuroactive compounds, including histamine, serotonin, 5-aminovaleric acid, γ-aminobutyric acid, β-alanine, leptin, peptide YY (32, 33), glucagon-like peptide hormone-1 (GLP-1) (29), catecholamines, and other hormones and neurotransmitters (34). It is important that SFCAs may behave as neurotoxins when applied at high concentrations. For instance, introducing propionate into the ventricles of the brain causes autism-like behavioral changes in rats (34). Children with autistic spectrum problems are characterized by elevated concentrations of SCFAs, especially of propionate, in the intestines. This is presumably due to the abnormally high activity of *Clostridia* and the presence of an unusual microbial species, *Sutterella wadsworthensis*, which seems to be typical of autism. The behavioral problems of autistic people may result from propionate's capacity to modulate the expression of many autism-related genes, predominantly those associated with mitochondrial processes (35). Studies with mice with autism-like disorders revealed that administering a probiotic based upon a special target-oriented *Bacteroides fragilis* strain ameliorates the microbial ecology of the intestines, decreases their permeability, and mitigates anxiety-related stereotyped behavior (12). Further research will enable us to develop novel microecological approaches for improving autism symptoms under clinical conditions (36).

Gasotransmitters

Gases formed in the animal/human organism, including those produced via microbial fermentation in the GI tract, have received much attention from researchers and clinicians, starting in the 1970s. NO, CO, and H_2S are among the most ancient gas molecules that can perform neuromediator functions. Presumably, some other gases (H_2, CH_4, NH_3, CO_2, and others) also exhibit neuromediator activities. Both the host tissue-dependent and microbial synthesis of gases with proven neuromediator functions is carried out by specific enzymes. For example, the synthesis of NO from arginine is catalyzed by NO synthases (NOSs) while CO synthesis by heme oxygenases (HOs) causes heme degradation. H_2S is predominantly synthesized from L-cysteine, and this reaction is catalyzed by at least three different enzymes (27, 37, 38). To reiterate, the composition and amount of gases synthesized in the human organism vary depending on the individual and GI area involved. Gasotransmitters produce their effects on the cells that synthesize them, on adjacent cells, and on even remote tissues/organs. Gaseous signal molecules do not bind to specific receptors on cell membranes and do not accumulate in synaptic vesicles; upon their synthesis, they are usually released from the synthesizing cells (28, 39, 40). Gases can sufficiently easily penetrate into the cells of the nervous, vascular, and immune systems, as well as into those of other systems. They interact with intracellular enzymes and ion channels. Many host- or microbiota-produced gases are capable of post-translational modification of various proteins, which results in functional alterations. Some of these alterations associated with oxidative stress, mitochondria imbalance, and other cell disorders cause damage to biological macromolecules and even cell death.

Nevertheless, there is much evidence that the interaction of some gases with some peptides does not result in cell death (41). Such processes are referred to as redox signaling (42). All the aforementioned molecules are among the smallest bioactive molecules that perform universal functions related to the life-sustaining activities of both multicellular organisms and bacteria.

The GI tract of adult humans contains about 20 ml of various gaseous products. The volume of intestinal gases that is produced per day varies between 400 and 1,200 ml. Nitrogen, oxygen, hydrogen, methane, carbon dioxide, and H_2S account for 20–90%, 3.9–10%, 20.9–50%, 7.2–10%, 9–30%, and 0.00028% of the total volume, respectively. In addition, ammonia, carbon monoxide, nitrous oxide, acetaldehyde,[1] and sulfur dioxide accumulate in the GI tract. These gaseous substances enter the GI tract with air and food; in addition, they are formed by various eukaryotic and prokaryotic cells via enzymatic or non-enzymatic processes. Most gas molecules are removed from the intestines; they can also be absorbed and delivered to the bloodstream; subsequently, they are released via the respiratory system. H_2 and CH_4 are only formed by microbial fermentation in the GI tract; after entering the bloodstream, they reach the lungs and are exhaled. Their quantity varies to a large extent, depending on a human individual's diet.

Nitric oxide

NO is a small short-lived signal molecule that can modify the functions of diverse proteins both directly and indirectly, via post-translational modification caused by their binding to thiol groups and other amino acid sites (43). In the human organism, NO is formed via both enzymatic and non-enzymatic reactions (44). There are three isoforms of endogenic NOSs in the animal organism (three NOS enzymes), which are NOS1 (neuronal NOS, nNOS), NOS2 (inducible NOS, iNOS), and NOS3 (endothelial NOS, eNOS). Inducible NOS is activated under

[1]Classifying acetaldehyde as a gas is somewhat arbitrary because this compound is in the liquid state at atmospheric pressure up to the boiling point of 20.2°C.

the influence of microbial metabolites and inflammatory cytokines released in response to infection and tissue damage (27, 28). These enzymes and some bacterial NOSs produce NO from L-arginine in a process involving oxygen and NADH and resulting in L-citrulline formation (26, 27, 40, 45, 46). Bacterial NOSs (bNOSs) also catalyze NO synthesis from arginine both *in vitro* and *in vivo*. They are present in various bacterial species (streptomycetes, bacilli, and so on) (45, 47), including human/animal pathogenic and symbiotic microorganisms that inhabit the intestines (44), the oral cavity (40, 48), and the vagina (49). The classical L-arginine-NO pathway coexists with the alternative nitrate-nitrite-NO pathway (46, 50–53). The alternative pathway is characteristic of intestinal bacteria that obtain nitrate and nitrite from digested food (44). Apart from synthesizing their own NO, intestinal bacteria, including probiotic strains (such as lactobacteria, bifidobacteria, and *Escherichia coli* Nissle 1917), can stimulate NO formation by host epithelial cells (40, 48, 54–56). The probiotic strain-synthesized NO is rapidly degraded by *E. coli* and *Staphylococcus aureus* both *in vitro* and in the intestines of test animals (44, 57). The main targets of NO and related compounds are proteins that contain iron (guanylate cyclase, NOS enzymes, hemoglobin, and enzymes involved in the citric acid cycle and protein and DNA synthesis) and SH groups. NO also targets reactive oxygen species (ROS) (28, 50, 58). In mammals, NO is involved in regulating impulse transfer across synaptic clefts, regional blood flow, intestine peristalsis, and water and electrolyte transport. NO influences the functioning of the immune and cardiovascular systems and regulates energy metabolism (40, 44–46, 50, 52, 59, 60). The regulatory influence on the vital functions of the organism is exerted by pico- or nanomolar NO concentrations. At these concentrations, NO behaves as a neuromediator and is implicated in learning and cognition activities. Mice with a defective nNOS are characterized by elevated locomotive activity, virility that is retained for a long time, high fertility, and long-term depression (LTD). Male mice lacking neuronal isoform (NOS-1-/- or nNOS-/-)-encoding genes are more aggressive than wild-type males (61). nNOS-containing mice were more resistant to experimental stroke caused by ligaturing the middle cerebral artery.

In an analogy to eukaryotes, NO performs communicative and antioxidant functions in bacteria and is involved in biofilm formation/dispersal regulation and in expression of genes that are required for iron utilization (28, 45). NO can also protect bacteria from antibiotics. NO-dependent antibiotic resistance implicates chemical modification of antibiotics or attenuation of antibiotic-induced oxidative stress (28, 60) by stimulating bacterial catalase activity (45). Bacteria can also inactivate NO itself by converting it into nitrates (62) or, alternatively, by forming *S*-nitrosothiols (63). Microbial NO produces diverse effects on eukaryotic organisms. In the example of the worm *Caenorhabditis elegans*, it was demonstrated that *B. subtilis*- and *E. coli*-synthesized NO behaves as a transcription activator. It induces processes in the worm's enterocytes that enhance its heat resistance and prolong the lifespan (64). A similar mechanism may operate in higher animals. NO also affects the functions of ionotrophic glutamate receptors (iGluRs) and acid-sensitive ion channels (ASICs) that are present in various areas of the central nervous system and in other mammalian tissues. Dysfunctional ion channels pose the threat of neurological disorders. NO can modify iGluRs and ASICs either directly, by *S*-nitrosylation of cysteine, or indirectly, via cGMP protein kinase G (PKG)-dependent phosphorylation (38).

Recently, the traditional opinion that nitrate and nitrite contained in food can cause stomach cancer has been called into question (65). Moreover, the application of drugs or dietary strategies for modifying NO metabolism in order to exploit the therapeutic potential of nitrates as NO sources has been increasingly discussed in the literature (46, 66). It should be taken into account that, despite the predominantly regulatory role of nanomolar NO concentrations, NO applied at high (micro- to millimolar) concentrations generates toxic compounds, such as NO_2, N_2O_3, and especially ONOO- (peroxynitrite) that impair thiol groups of organic molecules and react with protein tyrosyl groups and DNA nitrogenous bases in various mammal cells and microbial symbionts (28, 50, 58, 67).

Carbon monoxide

Carbon monoxide has long been considered as the most widespread air pollutant and a 'silent killer' because of its high affinity for reduced iron in hemoglobin that transports oxygen to the tissues of the animal/human organism. Endogenous CO was discovered in the human organism in 1950. Various plants and animals, including humans, have been revealed to synthesize CO as an intermediate product formed during heme degradation by heme oxygenases. Currently, three kinds of heme oxygenases (HO-1 to 3) are known; the inducible (HO-1) and the constitutive (HO-2) enzymes are of paramount importance in terms of endogenous CO synthesis. HO-1 activity is induced by various stressors and widely spread in liver, kidney, and spleen cells as well as in aged erythrocytes. HO-2 is located in neurons, other brain cells, and the endothelial layer of blood vessels; the enzyme is activated by Ca^{2+}-calmodulin, glucocorticoids, and opiates (37, 43). CO is also formed by bacteria, including pathogens, plant and animal symbionts, and soil and marine species that contain heme oxygenases (28, 68). Some bacteria contain the specific *coo* operon that codes for CO dehydrogenase. This enzyme is responsible for the anaerobic metabolism of CO, which is the sole carbon

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source in a number of bacteria, for example, *Rhodospirillum rubrum* (69). CO is a sufficiently stable molecule that easily enters cells because it readily crosses the cell membrane; inside a cell, it exerts its biological effects, including anti-apoptotic, anti-proliferative, anti-inflammatory, cytoprotective, and other activities, both at the CO generation site and at a sufficiently large distance from it (43). CO also regulates ion channels/transporters in various subtypes of epithelial cells (26, 27).

Recently, convincing evidence has been presented that CO possesses all typical properties of a gasotransmitter/gasomodulator with a broad biological action spectrum, when applied at physiological concentrations (28, 70). The protective influence of CO on the central nervous system was investigated in model systems. CO inhalation (up to 250 ppm) protects test animals against I/R brain injury and ischemic stroke (71). The same CO concentration was revealed to prevent neurological damage (neuronal apoptosis) in a pig model of deep hypothermic circulatory arrest. Pretreatment of primary cultures of cerebral granular neurons with CO (250 ppm) prevented apoptosis caused by oxidative stress or exogenous glutamate. The CO-dependent protection implicates activation of guanylate cyclase and, subsequently, mitochondrial ATP-sensitive K+ channels. This is accompanied by an increase in intracellular ROS level and stimulation of NO formation (72). In studies with hippocampal neurons, it was established that the key early stage of their apoptosis is activation of membrane K^+ channels, which promotes the extrusion of potassium ions from the cytoplasm and initiates apoptosis. CO can prevent apoptosis by directly inhibiting these channels (43, 72, 73). Collagenase injection results in hemorrhagic changes in the brain tissue of rats. CO pretreatment of the tissue prevented these changes. The neuroprotective effect of CO in this system was attributed to its capacity to stimulate concomitant formation in neurons of two kinds of protective compounds, ROS and NO.

Nonetheless, studies with human neuroblastome cells (SH-SY5Y) revealed that long-term treatment with a CO donor (CO-RM-2) induced symptoms of cell injury caused by lowering antioxidant activity or inhibiting NO formation. The capacity of CO at a concentration of 1,000 or 3,000 ppm (action time 40 min) to disrupt Ca^{2+}-dependent signaling pathways in SH-SY5 cells and in the homogenate of the total brain tissue of rats appears to be due to its modulating effect on the target protein, plasma lemma Ca^{2+} ATPase (PMCA) (74). Intracellular H_2O_2 production in the brain tissue is increased at high CO concentrations, which is accompanied by the enhanced production of hydroxyl radicals and a decrease in the ratio between the reductive and oxidative processes in mitochondria (72). Hence, the action time and concentration of CO at the target site determine whether its effect is beneficial or detrimental, in an analogy to the majority of drugs. Currently, CO is envisaged as a physiological signal molecule regulating the functions of membrane channel proteins and transporters (73). It has been established that the antimicrobial and anti-inflammatory effects of CO and CO-releasing molecules (CO-RMs) (e.g. metal carbonyl CO-RM-3, $RU(CO)_3Cl$, and glycinate) implicate the opening of K+/Na+ channels in eukaryotic and bacterial cells, which decreases the proton motive force and disrupts ion transport for a short time. The mechanisms of protection of the nervous and cardiovascular systems in the presence of CO-RMs have not been completely elucidated yet. There is evidence that CO-RM2 behaves as an inhibitor of voltage-activated potassium channels; and mitochondria represent the main target of CO. This does not rule out an additional effect of CO-RMs, the stimulation of ROS production in mitochondria. It was established that the CO released at low CO-RM2 concentrations can produce a cardioprotective effect, due to its antioxidant properties. Recently, increasing attention has been paid to the use of heme oxygenases, CO inhalation, and CO-RMs for treating various infection and inflammation processes as well as cardiovascular and, potentially, neurophysiological problems (28, 70, 72, 73, 75, 76).

Hydrogen sulfide

Hydrogen sulfide is a very water-soluble gas. At a concentration of 1 ppm, it can be recognized because of its rotten egg odor; 4 ppm H_2S causes a headache; at still higher concentrations (500 ppm and above), H_2S can produce a lethal effect (37, 39). The equilibrium ratio between its three forms (H_2S, HS^-, and S^{2-}) varies depending on medium pH. In the organism, this gas easily enters the cells via passive transfer across the membranes. Acute intoxication is due to H_2S binding to the iron of cytochrome *c* oxidase, which inactivates the enzyme and abolishes oxidative phosphorylation in mitochondria (39). Despite its toxic effect, H_2S has recently been established to play a vital role in bacteria, plants, and invertebrate and vertebrate animals, including mammals. Predominantly, the synthesis of this gas involves three enzymes: cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE or CTH), and 3-mercaptopyruvate sulfur transferase (3-MST) (43). H_2S-synthesizing enzymes are expressed, to a different extent, in the cardiovascular, nervous, immune, urinary, respiratory, and GI systems (77).

The microbiota of the large intestine, which includes more than 26 genera, is one of the key factors implicated in the metabolism of S-containing compounds and endogenous generation of H_2S in the human organism. A red meat-enriched diet stimulates H_2S synthesis by supplying the large intestine with a significant amount of sulfated proteins. The human large intestine contains considerable H_2S amounts that predominantly result from H_2S formation from inorganic (e.g. sulfates and sulfites) and organic

(methionine, cysteine, taurine, sulfate-containing polysaccharides, and lipids) compounds (78, 79). Sulfur-containing organic compounds, including those present in garlic, onion, and other food stuffs, supply the organism with its H_2S pool (78). Sources of microbial H_2S include, for example, *E. coli* strains that possess two enzymes (L-cysteine transaminase and 3-MST), which catalyze its formation. The bacterial production of H_2S can also involve CBS and CSE or CTH (80). Some representatives of intestinal bacteria (*Prevotella*, *Bacteroides*, *Helicobacter*, *Peptococcus*, and *Akkermansia*) produce glycosyl sulfatases or similar enzymes that promote production of sulfates from sulfomucins (78). Sulfate-reducing bacteria compete with methanogenic microorganisms for H_2 molecules both *in vitro* and *in vivo*. In the human large intestine, *Desulfovibrio vulgaris* is predominantly responsible for H_2S generation by reducing various sulfur-containing compounds, including sulfates and S-containing organic substances, e.g., cysteine. The bacteria of the large intestine ferment cysteine, yielding H_2S, ammonia, and pyruvate. If the oxygen content is low (under microaerophilic conditions), H_2S at millimolar concentrations can serve as an electron donor and an energy source. If food contains a limited amount of cysteine, endogenous and microbial CSE activity and, therefore, H_2S production are increased; conversely, enriching food in cysteine or the chemical/genetical suppression of CSE activity results in a decrease in H_2S production (81). Within the lower part of the mammal GI tract, H_2S behaves either as a potential toxin or as a signal molecule, depending on its concentration (28, 82). At high (millimolar) concentrations, H_2S is a highly toxic compound that causes a whole spectrum of pathological processes (78), including those brought about by inhibiting mitochondrial functions; it also produces genotoxic effects by damaging the DNA (78, 83). In contrast, when applied at low (micromolar) concentrations, H_2S serves as an inorganic electron donor for mitochondria. H_2S regulates a number of physiological processes, such as the inflammatory response, apoptosis, cell proliferation, neuronal impulse transfer, and smooth muscle tone (83). The effects of H_2S applied at physiological concentration are mainly focused on the cardiovascular and the nervous systems (77).

The varied regulatory effects of H_2S are due to its capacity for modifying proteins via reducing disulfide (S = S) bonds or attaching a sulfur atom to a thiol group (− SH). As a result, − SH is converted into a hydropersulfide residue (− SSH). These important post-translational processes change the conformation and functional activity of proteins responsible for transmembrane ion transport or enzymes involved in protein phosphorylation/dephosphorylation and synthesis of secondary metabolites and cofactors (39).

Intestinal H_2S of microbial origin is predominantly degraded by intestinal epithelial enzymes (39). Both free and sulfate-conjugated H_2S is excreted from the organism, predominantly by the kidneys (38). The physiological effects of H_2S are due to the influence of this molecule on various molecular targets in diverse tissues, including heme-containing proteins, ion channels, and signal proteins. In the presence of glutathione, cysteine, or dihydrolipoic acid, H_2S is released from the lysate of cultured neurons and astrocytes at pH 8.0–8.4. When excited, neurons take up sodium ions and excrete potassium ions, which results in increasing the intracellular potassium concentration and depolarizing the membranes of adjacent astrocytes. Depolarization causes activation of Na^+/HCO^{-3} co-transporters in the astrocytes. The influx of HCO^{-3} brings about cell cytoplasm alkalization.

The main H_2S targets include ATP-sensitive potassium channels as well as calcium and chloride channels. There is sufficient evidence that the (neuro)modulatory effect of H_2S on cell functions and physiological processes is due to its interaction with several cell transporter systems. It was revealed that H_2S enhances the activity of transporter systems by facilitating the release of antioxidants that are required for protecting the systems against exogenous toxic substance-caused damage. The H_2S-transporter interactivity plays a major role in maintaining the redox potential of nervous cells. This is an additional mechanism of the neuroprotective and neuromodulatory activities of this gaseous substance. Of special note is the impact of H_2S on various types of K^+ channels that are essential for the transfer of ions in epithelial cells. H_2S is likely to indirectly affect Na^+ transfer by acting on the proteins of K+ channels and transporter molecules (26, 27).

The influence of H_2S, a biological signal molecule, on neuronal activity in the hippocampus, cerebellum, cortex, and brain stem has been researched during the course of more than 15 years. It was established that H_2S is an active neuromodulator and neuroprotector in various brain cells (31, 37, 39, 84). At physiological concentrations, H_2S functions as a synaptic activity modulator. CBS, which is present in the cells of various brain areas, is responsible for the generation of H_2S. It activates transmembrane ATP-associated channels (in neurons both inside and outside the brain) via modulating glutamate-dependent *N*-methyl-D-aspartate receptors. This gaseous modulator also regulates the activity of serotonergic neurons and induces the release of corticotrophin-releasing hormone (39, 84). Two different forms of sulfur, acid-labile and bound sulfur, are stored in brain cells. Acid-labile sulfur is incorporated in the iron-sulfur centers of mitochondrial enzymes involved in oxidative phosphorylation. Significant amounts of bound sulfur are present in the cytoplasm of brain neurons and astrocytes. Release of H_2S from the lysate of cultures neurons and astrocytes

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proceeds at pH 8.0–8.4. The excitation of nervous cells results in sodium influx into and potassium efflux from the cells. A drastic increase in ambient potassium concentration results in the depolarization of the membranes of adjacent astrocytes. To abolish membrane depolarization, the proteins responsible for $Na+/HCO^{-3}$ transfer are activated. Taking up HCO^{-3} results in the alkalinization of the intracellular content of astrocytes, which causes H_2S release from sulfur-binding compounds. Unbound H_2S (9.2 μM) is retained for a longer time in the brain tissue than in liver and heart cells (84). In astrocytes, H_2S also influences the intracellular level of calcium that plays a major role in intercellular communication. The intracellular calcium level rapidly increases upon the addition of H_2S; subsequently, it slowly decreases. These effects of H_2S and various H_2S donors were revealed in astrocyte cultures and in the glia of hippocampal sections (84). H_2S was established to exert an influence on the operation of the peripheral nervous system, which involves modulating pain perception and the transfer of pain signals to the relevant brain areas (39).

To reiterate, H_2S serves as a neuroprotector. The neurotoxic effect of glutamate on brain tissue cultures is partly due to inhibiting the entry of cystine into the cells. H_2S can mitigate the toxic effect by reversibly inhibiting cystine transfer by glutamate and, therefore, stimulating cystine influx into the cells (31, 33). There is a supplementary pathway of synthesizing H_2S from D-cysteine, which involves 3-MST and D-amine oxidase (33). In contrast to the pathway of H_2S synthesis from L-cysteine, the D-cysteine-dependent pathway predominantly functions in the cerebellum and the kidneys. Studies with the primary cultures of cerebellar neurons revealed that the cerebellar tissue does not sustain hydrogen peroxide-induced oxidative stress if D-cysteine is available (33). The discovery of the D-cysteine-dependent pathway of synthesizing H_2S provides foundations for a new therapeutic technique that is based on delivering this gas to target tissues (85). H_2S and *S*-adenosyl-methionine impede the increase in the glucocorticoid concentration in blood plasma under stress. This gas also strongly influences cell proliferation and apoptosis. It was established that low H_2S concentrations are capable of neutralizing reactive oxygen and nitrogen species (superoxide radical, hydrogen peroxide, peroxynitrite, hypochlorite, and so on) and of reversibly inhibiting the mitochondrial respiratory chain. It is the antioxidant effect of H_2S that is responsible for its neuro- and cardioprotective activities (39).

Currently, it is widely accepted that H_2S, like NO and CO, is an important neurotransmitter. The modulatory effect of H_2S on cell functions and physiological processes is due to its interaction with several transporter systems. It has been established that H_2S enhances their activity by releasing antioxidants that provide protection from exogenous toxic factors. The H_2S-transporter systems interactivity plays a major role in maintaining the redox potential of nervous cells; this is an additional mechanism of the neuroprotective and modulatory activity of this gaseous agent. An influence of H_2S on human behavior was suggested for the first time in studies with human subjects with seizures, psychiatric disorders, or abnormal electroencephalograms; most of the subjects lacked the enzymes (CBS) that are involved in H_2S synthesis. Subsequently, it was revealed that patients with Down syndrome, in contrast, are characterized by abnormally high concentrations of these enzymes in the brain tissue (86). Further studies demonstrated H_2S involvement in a number of neurogenerative diseases. The H_2S content in the brain tissue was decreased by over 50% in Alzheimer patients, and this deficiency is apparently due to a drastic (70%) decrease in the concentration of *S*-adenosyl-methionine that activates CBS. H_2S is likely to be involved in the pathogenesis of Parkinson disease. H_2S prevented nervous cell damage and apoptosis in a model system in which this disease was caused by administering the toxin rotenone to test animals (39). There is evidence that H_2S functions as a signal molecule in the visual system of mammals. H_2S synthesis-catalyzing enzymes (CBS and CSE) were detected in various kinds of eye cells, and H_2S was found to regulate sympathetic and glutamatergic neurotransmission during the signal transduction processes in this system. Further data on the regulatory influence of H_2S on ion channels and transporters will contribute to our understanding of the role of H_2S in relation to the risk of development of ocular neuropathies (87).

Even though the use of gaseous H_2S for therapeutic purposes is hardly feasible, we can apply chemical compounds that release H_2S in the human organism either rapidly (NaHS) or slowly (GYY 4137). This gives grounds for the suggestion that H_2S should be used for medical purposes (88). It is important that while the one-time use of NaHS provided protection for neurons from oxidative stress, the repeated administration of this substance produced a toxic effect on these cells (84). Nonetheless, H_2S treatment is considered an efficient therapeutic technique in a number of diseases (e.g. lung cystic fibrosis and kidney problems in patients with hereditary hypertension) that are characterized by enhanced $Na+$ influx into cells (81). In all likelihood, the employment of chemical donors or microbial producers of H_2S (as an ion channel/transporter modulator) for medical purposes will hold much promise as a potential pharmacological approach to the treatment of a number of neurodegenerative diseases.

Ammonia

Ammonia is one of the end products of degradation of proteins, peptides, urea, and various amino acids. In the human organism, NH_3 is predominantly formed by the intestinal microbiota and the cells of the GI tract, kidneys,

the liver, and muscles. At least 4–10 g of NH_3 are daily synthesized in the intestines of adult human individuals. Among aerobes, Gram-negative intestinal bacteria of the genera *Proteus*, *Klebsiella*, and *Pseudomonas* as well as *E. coli* are the most active NH_3 producers; active NH_3-producing anaerobes include the genera *Clostridia*, *Ruminococcus*, *Bacteroides*, and some lacto- and bifidobacteria. Peptidococci, ruminococci, coprococci, bifidobacteria, lactobacilli, clostridia, bacteroides, and some streptococci and enterococci exhibit significant urease activity. In healthy human individuals, up to 7 g of urea are degraded daily by microbial ureases (amounting to 50% of the total pool of this compound) (89, 90) and by those of fungi (*Candida albicans*) (91).

The involvement of microorganisms in ammonia metabolism is consistent with the fact that the intestines of germ-free animals contain 20–30% less urea than their conventional counterparts. After administering carbon-labeled urea to these animals, the air they exhaled contained no labeled carbon dioxide. Intestinal urease-producing microorganisms form ammonia that is transferred via the portal vein to the liver, where it is reincorporated into urea. GI microorganisms also incorporate NH_3 into amino acids are synthesized *de novo* using CO_2 or acetic, propionic, and other organic acids as carbon sources. Unless utilized in biochemical processes in the large intestine, microbially produced NH_3 rapidly passes through mucous membranes and spreads within the organism. For the most part, intestinal unbound NH_3 reaches the liver via the portal vein; in the liver, it is virtually completely converted into urea and glutamine via a series of reactions (the urea cycle). The unbound NH_3 content in the blood of healthy adults is approximately 35 μM (ca. 0.67 mg/L). Unbound NH_3 circulates in the organism; it is excreted with urine and, to a lesser extent, with feces. Approximately, 2–3 μg of ammonia are excreted per day with urine. Ammonia-derived metabolites are also excreted with urine or, alternatively, used for synthesizing amino acids and other biological molecules. Endogenous NH_3 formed in the brain and in peripheral tissues is not transferred to the liver; instead, it is transformed in these tissues into glutamine and alanine (89, 90).

A genetic disruption of the biosynthesis of urea cycle enzymes, liver and kidney dysfunction, excessive NH_3 formation in skeletal muscles caused by physical exertion or other kinds of stress, or an imbalance in the intestinal ecological system result in increasing NH_3 content in the organism to a toxic level (hyperammonemia). For instance, liver cirrhosis is associated with the formation of a direct bypass between the portal vein system and the bloodstream; this prevents the detoxification of harmful GI tract-produced compounds, including NH_3, that reach the bloodstream. Increased NH_3 concentrations penetrate into the brain tissue, which is a major factor of pathogenesis of hepatic encephalopathy (HE). A mild form of HE occurs in 80% of patients with liver cirrhosis. It manifests itself in fatigue, ache, muscle weakness, loss of appetite, nausea, vomiting, diarrhea, pain in the back, sides, or the abdomen, and motor and cognitive disturbances.

In the early 1970s, it was established that the genetic disruption of the urea cycle results in pathological changes in the brain of newborns. It was suggested that GABA is implicated in causing ammonia-induced toxicity in the nervous system. An increase in ammonia concentration in the brain tissue results in stimulating GABA-induced chloride channels in neurons and astrocytes. Evidence was presented that liver dysfunction-induced hyperammonemia is accompanied by changes in cell energy metabolism and formation of excessive glutamine amounts in astrocytes. An increase in the glutamine concentration in astrocytes disrupts neurotransmission processes. It is astrocytes that incorporate ammonia in glutamine molecules after it crosses the blood-brain barrier (BBB). Hyperammonemia is also responsible for osmotic stress in the brain, which results in redistribution of cerebrospinal fluid and causes low-grade swelling in astrocytes and edema in the white matter as well as an increase in intracranial pressure (92–95). Ammonia also inhibits energy production in mitochondria, which is attributed to ammonia's capacity to suppress ketoglutarate dehydrogenase activity and stimulate glycolysis (94).

An increase in ammonia concentration in the arteries affects the expression of a number of genes that code for neuroglial proteins. These proteins regulate cell growth, mitochondrial functions, and transfer of neuroactive amino acids (95). When applied at supraphysiological concentrations, NH_3 induces rapid release of glutamate from neurons. In humans, this results in the development of irritability, aggression, hyperexcitability, and even movement disorders. Hyperammonemia also promotes removal of GABA from neurons, which frequently manifests itself in somnolence or lethargy; these symptoms may ultimately cause the development of a comatose state (95, 96). Apart from the direct neurotoxic effects, high NH_3 concentrations increase the permeability of the BBB; modulate the serotonergic and dopaminergic systems of the brain; cause the accumulation of abnormal neurotransmitters, such as octopamine, in the brain; and result in glucose intolerance and increased urinary output calcium and phosphate concentrations (12, 95).

Systemic inflammation that constantly accompanies acute and chronic liver dysfunction represents an important risk factor in terms of encephalopathic complications. Hyperammonemia and the attendant neuroinflammatory response to liver cirrhosis cause microglia activation and monocyte recruitment; the enhanced synthesis of proinflammatory cytokines (TNF, IL-1β, and IL-6); the accumulation of ammonia, lactate, and manganese; and an increased permeability of the BBB. Encephalopathy may result from the synergistic effect of ammonia and cytokines

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(93, 95). Astrocytes lose the capacity to adequately regulate their own volume, glutamine accumulation is increased, and a cascade of signaling processes is triggered. This results in enhanced Ca^{2+} accumulation and an increased formation of reactive oxygen and nitrogen species, which is due to the activation of NADPH oxidase and NOS (93). Some patients suffering from hyperammonemia and the attendant GI dysfunction (constipation or diarrhea) exhibit psychotic symptoms and movement disorders that resemble those typical of genuine autism spectrum disorders (35, 91, 96). The important role of microbial NH_3 with respect to the brain functions and life expectancy of HE patients is consistent with the fact that the patients' state is markedly improved after administering the antibiotic rifamycin, the prebiotic lactulose, and probiotics to them (95). The neuropsychic state, including cognitive capacities, is also improved by synbiotics, for example, by the *Bifidobacterium longum*-fructo-oligosaccharide combination (12). Neural inflammation, brain edema, and the resulting encephalopathy symptoms that are due to increased NH_3 concentrations in the brain tissue can be mitigated by minocycline, an inhibitor of microglia activation, and *N*-acetylcysteine, as well as by mild hypothermia; all these techniques produce neuro- and hepatoprotective effects (95).

Conclusion

The data presented in this review provide compelling evidence that a whole gamut of gaseous endogenous and microbial products perform important functions in neurophysiological, biochemical, microbiological, and medical terms. The (patho)physiological effects produced by most of these gases in the organs and tissues of the organism have been studied in detail. Unfortunately, determining the concentrations of these gases in target cells presents serious difficulties that are due to the limitations of currently available measurement techniques and the gases' high reactivity and short lifetime. Most endogenous and microbially produced SCFAs and gases readily pass through the mucosa layer and cell membranes and exert toxic effects on mammal cells if applied at high concentrations. In contrast, many gases behave as broad action spectrum regulators within the physiological concentration range. In particular, they influence the functions of the nervous system by serving as nutrients, metabolites, or regulators involved in the operation of various kinds of nervous cells. This is the reason for classifying them as *gasotransmitters* and/or *gasomodulators*.

In microorganisms, the aforementioned gaseous products function as intermediates in denitrification (the role of NO) and oxygen-free sulfate respiration (the function of H_2S) or as non-conventional carbon and energy sources (the role of CO). Gasotransmitters are of considerable importance not only for eukaryotes but also for bacteria. The gases can promote their survival and metabolic activity under various conditions, and specifically in the presence of antibiotics and in the host–microbiota system. Formation of gasotransmitters/gasomodulators by intestinal bacteria and host cells provides an illustrative example of (patho)physiological effects of both the microbiota and the diet (which influences gas production by the host and the microbiota). The synthesis and biological activities of each of the aforementioned gases are, to an extent, influenced by other gases. Therefore, most gas molecules are to be regarded as cooperatively functioning low molecular weight agents that control a specific function or a whole complex of functions (25, 28, 43, 95, 97). Gases differ in stability and, therefore, can produce their effect either directly on the site at which they are formed (H_2S) or on remote tissues/organs (e.g. CO and NH_3) (43). NO molecules can diffuse and influence metabolic processes within a distance of several cell diameters (50). Owing to their high reactivity, gases do not accumulate locally; instead, they rapidly reach their target cells where they interact with intracellular enzymes, transporters, and ion channel proteins (26, 27, 40).

A large family of genes coding for receptors in cation channels (TRP channels) has been revealed. They function as polymodal detectors (sensors) of a wide spectrum of extra- and intracellular signals. Receptor–signal interaction results in activating cation channels. In mammals, six similar protein families were detected; they form TRP channels (TRPC, TRPV, TRPM, TRPA, TRPP, and TRPML) (98). A large number of gas molecules, for example, O_2, NO, CO, H_2S, and CH_4, can penetrate cell membranes, which is a prerequisite for performing the aforementioned auto-, para-, or endocrine functions. The gases' capacity to influence cell-environment interaction, electrolyte homeostasis, and cell-cell electrochemical communication via changes in redox signaling, gap junction channels, and ion channel/transporter regulation enables them to regulate a plethora of physiological processes in cells and tissues (26, 27, 99). Many gases can form covalent bonds with prosthetic metal complexes in receptor proteins or non-covalent bonds with protein regulatory subunits. They occupy the space within and around a protein, which prevents other gases from accessing its functionally active sites (98). Ion channels, for example, TRPV1 and TRPA1, can behave as sensory protein molecules that transduce gas-conveyed signals into electric signals in neurons, including those of *nervus vagus*. The transmission of these signals allows the nervous system to initiate processes that enable, for example, pain perception.

At this point, the research on microbial gasotransmitters actually merges into mainstream neuroscience. It should be noted that behavioral analysis, monitoring neurotransmission in various pathways, exploring synaptic plasticity in models such as long-term potentiation (LTP) and LTD, are the bread and butter of present-day

neuroscience. Studies with animals revealed that some gasotransmitters or their precursors (at appropriate concentrations) are therapeutically efficient and safe, and it seems feasible to use them for treating a number of pathological processes in the human organism. In fact, some of the aforementioned gases have already been employed for therapeutic purposes (25, 28, 43, 56, 83).

We suggest using the recently coined term *psychobiotics* (16) to denote probiotic bacterial strains that are employed as biologically active additives or functional nutrients for optimizing the gasotransmitter pools of the human organism and for beneficially influencing brain processes and behaviors that are subject to regulation by SCFAs, nitric oxide, carbon monoxide, H_2S, and ammonia. Further comparative studies using germ-free and conventional animal models as well as tissue cultures (100) will enable us to elucidate in detail the molecular mechanisms and cell targets of the recently discovered world of gasotransmitters, regardless of whether they represent host-produced, microbial, or diet-derived substances.

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