

S.I. Kolesnikov, B.Ya. Vlasov, L.I. Kolesnikova

Scientific Center for Family Health and Human Reproduction Problems, Irkutsk, Russian Federation

Hydrogen Sulfide as a Third Essential Gas Molecule in Living Tissues

Представлены данные зарубежных исследователей за последние 15 лет, посвященные эндогенному биосинтезу и биологической роли сероводорода в микромолярных количествах, который дополнил уже два известных газотрансмиттера — OH и NO. Несмотря на незначительный период со дня открытия физиологических свойств сероводорода (около 20 лет), установлено, что этот газотрансмиттер играет ключевую роль в регуляции нервной (нейронная передача сигнала), сердечно-сосудистой (расслабление гладких мышц), иммунной (противовоспалительный и цитопротекторный агент) сенсорной, желудочно-кишечной (выход инсулина) системы, а также в метаболизме различных органов. В настоящее время ведется изучение роли H₂S в патогенезе различных заболеваний (нейродегенеративные болезни, сахарный диабет, сердечная недостаточность). Перспективными являются разработки по созданию препаратов, которые выступают либо в качестве экзогенных доноров H₂S, либо в роли блокаторов биосинтеза H₂S. С учетом того факта, что H₂S является представителем несинаптического способа межклеточной коммуникации, основанного на диффузии молекул неорганических соединений по межклеточному пространству во всех направлениях и действии на отдаленные от их места образования несинаптические рецепторы, предлагается строго дозированно использовать и экзогенный H₂S для лечения ряда заболеваний человека.

Ключевые слова: газотрансмиттеры, сероводород, биологические эффекты, перспективы лечебного применения.

(Для цитирования: Колесников С.И., Власов Б.Я., Колесникова Л.И. Сероводород как третья эссенциальная газовая молекула живых тканей. *Вестник РАМН.* 2015;70(2):237–241. Doi: 10.15690/vramn.v70i2.1318)

The data of foreign studies over the last 15 years devoted to endogenous synthesis and biological role of hydrogen sulfide in micromolar quantities which complemented the already two well-known gas transmitters — OH and NO are presented in this review. Despite the short period since the physiological properties of hydrogen sulfide were opened (about 20 years) it was found that this gas transmitter plays a key role in the regulation of nerve (neural signal transmission), cardiovascular (relaxation of smooth muscles), immune (anti-inflammatory and cytoprotective agent) sensory, gastrointestinal (output of insulin) systems and in the metabolism of various organs. Currently the role of H₂S in the pathogenesis of different diseases, neurodegenerative diseases, diabetes, heart failure) is being studying. The developments of drugs that act as either exogenous donors H₂S or blockers of the biosynthesis of H₂S are promising. With consideration the fact that H₂S is a representative of non-synaptic way of intercellular communication based on diffusion of molecules of inorganic compounds in the intercellular space in all directions and effect on distant from their place of formation non-synaptic receptors it is suggested to use exogenous H₂S in strict proportion for the treatment of a number of human diseases.

Key words: gasotransmitters, hydrogen sulfide, biological effects, possible medical application.

(For citation: Kolesnikov S.I., Vlasov B.Ya., Kolesnikova L.I. Hydrogen as a Third Essential Gas Molecule in Living Tissues. *Vestnik Rossiiskoi Akademii Meditsinskikh Nauk = Annals of*

Introduction

There are no doubt of existence, in addition to synaptic form of information transmission (from neuron to neuron or from neuron to effector cell), other means of information transmission, with the participation of gas molecules, such as nitric oxide (NO), carbon monoxide (CO), and most recently —hydrogen sulfide (H₂S) [1]. These gaseous inorganic compounds, unlike other biologically active molecules, easy to penetrate through the membrane of any cells, do not bind with any receptor on the surface of cells and interact directly with intracellular proteins [2]. Among the so kind known metabolic regulators, so far H₂S the least studied [3].

Direct studies of biological effects of hydrogen sulfide began at the turn of the 20th century, but they were devoted mainly to the study of the toxic properties of hydrogen sulfide [4], the toxicity of which 5 times higher than that of carbon monoxide (CO). H₂S consider as a signaling molecule that not only is the toxic agent, but also participates in the regulation of functional activity of the various cells of the animal body, began only at the end XX century. Real start of research in this field put Japanese scientists K. Abe and H. Kimura, who in 1996 for the first time described the possibility of synthesis of H₂S in the tissues of the brain and its ability to regulate cell functions [5].

Although hydrogen sulfide was detected in tissues of the brain in 1980s, initially it was considered to be an artifact forming due to the rapid increase in the concentration of sulfides in the tissues after death. This occurrence of H₂S was associated with his release of disulfide compounds of Sulphur (so-called sulfone sulfur) during the preparation of the tissues [6].

Currently, hydrogen sulfide, as NO and CO assigned to the group of so-called gastransmitters — gaseous intracellular signaling molecules, performing in the cells specific regulatory functions. H₂S is well soluble in lipophilic substances [1]. Its solubility in lipids is 5 times higher than the solubility in water, which makes a good penetration ability H₂S through the cell membrane structures and does not require the participation of special ion transporting systems. Constant permeability (P_m) of hydrogen sulfide through bilayer lipid membranes is quite high - 0.5 ± 0.4 cm/s (for comparison, P_m for oxygen is approximately 0.0050 ± 0.0006 cm/s) [7]. This allows to consider hydrogen sulfide to be a highly available molecule effectively carrying out its functions inside cells. In human and animal organisms H₂S high concentrations is presenting in the intestines due to bacteria, which utilizing

sulfates and sulfur-bearing amino acids (cysteine, methionine), as well as sulfur containing substances, polysaccharides and lipids [8]. H₂S physiological concentration varies in different organs from 1 to 100 nmol/g of tissue [9]. At high concentrations (greater than 1.0 mg/l) a single inhalation of H₂S can cause instant death [4].

Biosynthesis of hydrogen sulfide

Hydrogen sulfide intracellular synthesis is carrying out in different cells of the animal body. Currently known 3 enzyme, which activity participating in hydrogen sulfide synthesis: cystathionin- β -synthase (CBS) cistotionin- γ -liaza (CSE) and 3-merkaptopiruvat-sulfurtransferaza (3MST). The CBS carries out the H₂S synthesis primarily in the nerve cells. In vascular smooth muscle cells (which contraction and relaxation alters the vascular tonus), hydrogen sulfide synthesis carries by CSE enzyme [10, 11] and in vessels inside endothelial cells lining — 3MST [12, 13].

All 3 enzymes using sulfur-containing amino acid (L)-cysteine as a substrate for the synthesis of hydrogen sulfide, catalyzing the reaction of cysteine desulfurhydration: cleavage of the sulfur atom from cysteine molecule without its subsequent oxidation, leading to the formation of H₂S. CSE converts the cystine (cysteine disulfide) to thiocystein, pyruvic acid and ammonia followed by nonenzyme thiocystein conversion to cysteine and H₂S. At the same time CBS uses a slightly different H₂S pathway synthesis, which is the condensation of homocysteine with cysteine and subsequent formation of cystathionin [10]. As a substrate for the H₂S synthesis could be used other sulfur-containing amino acids, such as methionine and cystine.

3MST enzyme operates in conjunction with other enzyme-cysteine amino transferase (CAT). There are two forms of CAT – cytosol and mitochondrial. Using sulfur-containing amino acid (L) -cysteine and ketoglutarate as a substrate, CAT produces 3-merkaptopiruvat (3MP) from which H₂S synthesizing directly with participation of 3MST enzyme [14]. H₂S synthesis terminates in the α -ketoglutarate absence.

Biological effects of hydrogen sulfide

H₂S as the gastransmitter penetrates the cell membrane without the aid of specific transport molecules. Obtained from Na₂S or NaHS in vitro hydrogen sulfide in micromole concentrations [15, 16] has cytoprotective properties that can be associated with its ability to neutralize various active forms of molecules (e.g., peroxynitrites, hypochlorous acid and homocysteine). Effects of H₂S is associated with modulation of intracellular kinases or caspase's activity (p-38, c-JUN N-Terminal protein kinase 1/2, ERK1/2, PI3K), activation of

nuclear factor-kB and kB-dependent proteins (inducible NO-synthase, cyclo-oksigenaze-2, intercellular adhesive molecule-1) as well as with the decline of antiapoptotic factor Bcl-2 [2].

H₂S stimulates antioxidant system in the organism along with well-known antioxidants (including N-acetyl cysteine, glutathione and superoxide dismutase) and renders the cytoprotective effect [15]. Inhibition of H₂S endogenous synthesis increases cytotoxic impact on the body's cells of exogenous H₂S.

Endogenous H₂S needed for protection of the kidneys from injury and dysfunction during ischemia/reperfusion and NaHS injection reduces the occurrence of dysregulation and morphological changes in kidney disease [2].

At the same time, the high H₂S millimolar concentration have a cytotoxic effect on the cells, leading to activation of free radical processes, mobilization of calcium, use up of glutathione, intracellular release of iron, as well as the induction of cell death mitochondrial pathways.

H₂S promotes the synthesis of cyclic AMP (cAMP) in neurons by activating the adenylate cyclase and cAMP-dependent reactions [17] contributes to the induction of LPT (amplification of signal transmission between neurons) in the hippocampus, in the central nervous system through (N)-methyl-b-aspartate-receptor (NMDA) activation [6], which govern the redox processes and participating in neurotransmission [18].

H₂S increases intracellular concentration of Ca²⁺ [17] in astrocytes and glial cells. It can regulate synaptic activity by modulating the activity of neurons and glia [19]. An increase in extracellular potassium concentration in neurons activation leads to astrocytes depolarization and electrogenic cotransporters activation — sodium bicarbonate (Na⁺ / HCO₃⁻). Cotransporters activation leads to increasing astrocytes intracellular pH. As for other gasotransmitters, H₂S has no selective receptor responsible for its biological effects.

Hemoproteins are key molecular subjects involved in gas transport, accumulation, interaction and indications of gases. They have a wide range of ligands, including CO [20], NO [21] and H₂S [22]. Ligand linking with heme and dissociation of the complex form a competing ability between these gases.

Hemoprotein perform 4 main functions: transport, electron transport, oxidation-redox and sensory.

Hemoprotein transport function associating with the transport of oxygen by hemoglobin. Redox reactions occur in the catalytic sites of specific heme iron enzyme activating oxygen and forms the high valent status of enzymes that catalyze the reaction of oxygen-substrate, refer to oxygenase's (for example, hemeoxygenase, NO-synthase). Heme

groups transfer signal to protein functional site. Representatives of these enzymes are CBS and guanilatcyclase. Guanilatcyclase inhibitors do not affect the ability of the H₂S to relax the blood vessels, so the action of H₂S does not depend on this enzyme. A covalently bounded with heme H₂S modulates hemecontaining enzymes [23].

The NO signaling mechanism is linked to the nitrozolation process – such modification, in which nitrozole group join proteins in posttranslational period. In addition to the process of S-nitrozolation there is another process of cysteine thiol modification – S-sulphydration. By mass-spectrometric analysis determined that the accession of additional Sulphur to cysteine thiol groups (SH) leads to the hydropersulfide (SSH) formation . Thus, H₂S signal mechanism associated with sulphydration [22].

Unlike S-thiolation process (formation of mixed disulfide protein with glutathione), which is blocking the thiol groups of proteins, S-sulphydration process lead to the formation of SSH groups and increases the chemical reactiveness. β-tubulin and glyceraldehyde-3-phosphate dehydrogenase are the examples of the basal sulphydrated proteins. Their sulphydration process is taking out at physiological levels of *L*-cysteine (0.6-1 μmol/l) with maximal stimulation. GAPDH nitrozolation reduces its catalytic activity, whereas H₂S, synthesized from *L*-cysteine with CSE, increases its activity. In addition, sulphydration promotes actin polymerization, at the same time not affecting its depolymerization of [22].

H₂S hampers neutrophil leucocytes apoptosis by inhibiting protein p38 Caspase 3 [22]. The H₂S ions prolong the lifetime of granulocytes (with the exception of lymphocytes and acidophilic granulocytes) and also neutrophils, and preventing the development of new one, thus accelerating the inflammatory processes course. H₂S participates in metabolic reactions which results in appearance of per- and polysulfides. Sulfide linking to with Hem- or Myoglobin leads to the formation of sulfhemoglobin or sulfmyoglobin. H₂S regulates the cellular signal transduction pathways [24], leading to changes of the expression of different genes and their products, including thioredoxin reductase and Interleukin 1β.

Reduced H₂S synthesis was demonstrated in vessels during experimentally generated hypoxia (by NO-synthase blockade), as well as with pulmonary hypertension in spontaneously hypertensive rats. Induced exogenous donor H₂S caused pronounced significant therapeutic effects in these model objects.

Numerous studies have shown that one of the systems where hydrogen sulfide plays a key role as a signaling molecule is the cardiovascular system is the blood vessels in particular. Its regulatory action in the vessels of the arterial bed shows its active participation in the regulation of blood pressure [25–28].

It was shown that a group of people with normal blood pressure had blood plasma H₂S concentration rates approximately 34 μm, whereas in patients with arterial hypertension its concentration has been reduced to 20 μm. Inhalation of hydrogen sulfide by patients with arterial hypertension contributed to blood pressure reducing [29]. Research on rats found that intravenous injection of hydrogen sulfide solution caused dose-dependent blood pressure decreasing [10].

In *in vitro* conditions sodium hydrosulfide (NaHS) as a hydrogen sulfide donor actively used in the experiments, also caused of the various divisions of arterial and venous system relaxation: thoracic, mesenteric, renal artery, aorta, portal vein, etc. Despite the significant role of endothelium in the regulation of vascular tone, its removal does not have a significant impact on the effects of hydrogen sulfide in smooth muscle cells [10]. This shows the influence of hydrogen sulfide on live smooth muscle cells through inherent regulatory mechanisms. Relaxing effect of hydrogen sulfide on smooth muscle cells predominantly associated with opening the special structures in their membrane-potassium channels, sensitive to the concentration of intracellular energy source-adenosine triphosphate (ATP) [30, 31].

Communicating with these channels proteins sulfur groups hydrogen sulfide alters their spatial configuration and thus contributes to the opening of channels [13, 20, 32]. Opening of potassium channels leads to an increase in output of potassium ions out of the cell into the intercellular space. At the same time, activation of ATP-sensitive potassium channels accompanied by the inactivation potential sensitive L-type local calcium channels, ensuring the flow of calcium ions (Ca²⁺) in the cell. High intracellular concentration of Ca²⁺ is a necessary condition for the development of contractile response of the smooth muscle cell. Closing of calcium channels contributes to the reduction of the concentration of free intracellular Ca²⁺ [29]. These processes combine to trigger mechanisms of relaxation in smooth muscle cells, which ultimately leads to lower the tone of the blood vessels and blood pressure in general [33, 34].

Conclusion

These data demonstrate H₂S important role in the processes of intracellular metabolism and fundamental cellular processes controlling. Summing up, it should be noted that this signal molecule plays an important role in the regulation of nerve (neural processes of signal transduction), cardiovascular (relaxation of smooth muscle), immune (anti-inflammatory and cytoprotective agent), sensory, gastrointestinal (output of insulin), as well as the metabolism in various organs. Current studies revealed H₂S important role in the

pathogenesis of various diseases (neurodegenerative diseases, diabetes, congestive heart failure) [13]. It is highly promising designing and development of drugs, which act either as exogenous donors H₂S, or either blocking the biosynthesis of H₂S [31].

In view of the fact that the H₂S is a representative of the non-synaptic way of intercellular communication, diffusion-based molecules of inorganic compounds on the moist space in all directions and distant from non-synaptic receptors place of formation, it is important strictly dosed exogenous H₂S use for the treatment of diseases of animals and humans [2, 19, 35–38].

Source of funding

This work was supported by the grant provided by President's Council (No. HIII-5646.2014.7).

Conflict of interest

The authors have indicated they have no financial support / conflict of interest relevant to this article to disclose.

REFERENCES

1. Wang R. Signal transduction and the gasotransmitters: NO, CO and H₂S in biology and medicine. Totowa, Humana Press, 2004. 392 p.
2. Tripatara P., Patel N.S., Collino M., Gallicchio M., Kieswich J., Castiglia S. Generation of endogenous hydrogen sulfide by cystathionine gammalyase limits renal ischemia/reperfusion injury and dysfunction. *Lab. Invest.* 2008;88:1038-1048.
3. Carsten A.W. Hydrogen sulfide: a new gaseous signal molecule and blood pressure regulator. *J. Nephrol.* 2009;22:173-176.
4. Fiorucci S., Distrutti E., Cirino G., Wallace J.L. The emerging roles of hydrogen sulfide in the gastrointestinal tract and liver. *J. Gastroenterol.* 2006;131:259-271.
5. Abe K., Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci.* 1996;16:1066-1071.
6. Kimura Y., Kimura H. Hydrogen sulfide protects neurons from oxidative stress. *FASEB J.* 2004;18:1165-1167.
7. Mathaia J.C., Missnerb A., Ku P. et al. No facilitator required for membrane transport of hydrogen sulfide. *Proc. Natl. Acad. Sci. U.S.A.* 2009;106:16633-16638.

8. Li L., Moore P.K. Putative biological roles of hydrogen sulfide in health and disease: a breath of not so fresh air? *Trends Pharmacol. Sci.* 2008;29:84-90.
9. Reiffenstein R.J., Hulbert W.C. Toxicology of hydrogen sulfide. *Annu. Rev. Pharmacol. Toxicol.* 1992;32:109-134.
10. Lowicka E., Beltowski J. Hydrogen sulfide - the third gas of interest for pharmacologists. *Pharmacol. Reports.* 2007;59:4-24.
11. Wagner C.A. Hydrogen sulfide: a new gaseous signal molecule and blood pressure regulator *J. Nephrol.* 2009;22:173-176.
12. Kamoun P. Endogenous production of hydrogen sulfide in mammals. *Amino Acids.* 2004;26:243-254.
13. Stipanuk M.H., Beck P.W. Characterization of the enzymic capacity for cysteine desulphydration in liver and kidney of the rat. *Biochem. J.* 1982;206:267-277.
14. Shibuya N., Milkanai Y., Kimura Y., Nagahara N., Kimura H. Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide. *J. Biochem. Adv. Access.* 2009;146:623-626.
15. Whiteman M., Armstrong J.S., Chu S.H., Jia-Ling S., Wong B.S., Cheung N.S., Halliwell B. The novel neuromodulator hydrogen sulfide: an endogenous peroxynitrite «scavenger»? *J. Neurochem.* 2004;90:765-768.
16. Yan S.K., Chang T., Wang H., Wu L., Wang R., Meng Q.H. Effects of hydrogen sulfide on homocysteine-induced oxidative stress in vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* 2006;361:485-491.
17. Rinaldi L., Gobbi G., Pambianco M., Micheloni C., Mirandola P., Vitale M. Hydrogen sulfide prevents apoptosis of human PMN via inhibition of p38 and caspase 3. *Lab. Invest.* 2006;86:391-397.
18. Qu K., Lee S. W., Bian J.S., Low, C.M.; Wong, P.T. Hydrogen sulfide: neurochemistry and neurobiology. *Neurochem. Int.* 2008;52:155-165.
19. Kimura H., Nagai Y., Umemura K., Kimura Y. Physiological roles of hydrogen sulfide: Synaptic modulation, neuroprotection, and smooth muscle relaxation. *Antioxid. Redox. Signal.* 2005;7:795-803.
20. Antonini E., Brunori M. Hemoglobin and myoglobin in their reactions with ligands. Amsterdam, North-Holland Publishing, 1971. 393 p.
21. Yonetani T., Tsuneshige A., Zhou Y., Chen X. Electron paramagnetic resonance and oxygen binding studies of anitrosyl hemoglobin: a novel oxygen carrier having NO assisted allosteric functions. *J. Biol. Chem.* 1998;273:20323-20333.

22. Mustafa A.K., Gadalla M.M., Snyder S.H. Signaling by gasotransmitters. *Sci. Signal.* 2009;2:2.
23. Hill B.C., Woon T.C., Nicholls P., Peterson J., Greenwood C., Thomson A.J. Interactions of sulphide and other ligands with cytochrome c oxidase. An electron paramagnetic resonance study. *Biochem. J.* 1984;224:591-600.
24. Yang G., Cao K., Wu L., Wang R. Cystathionine γ -lyase overexpression inhibits cell proliferation via a H₂S-dependent modulation of ERK1/2 phosphorylation and p21Cip/ WAK-1. *J. Biol. Chem.* 2004;279:49199-49205.
25. Beltowski J. Hydrogen sulfide as a biologically active mediator in the cardiovascular system. *Postepy. Hig. Med. Dosw.* 2004;58:285-291.
26. Geng B., Yang J., Qi Y., Zhao J., Peng Y., Du J., Tang C. H₂S generated by heart in rat and its effects on cardiac function. *Biochem. Biophys. Res. Commun.* 2004;313:362-368.
27. Smith R.P. A short history of hydrogen sulfide. *American Scientist.* 2010;98:6.
28. Skovgaard N., Gouliaev A., Aalling M., Simonsen U. The Role of Endogenous H₂S in Cardiovascular Physiology. *Curr. Pharm. Biotechnol.* 2011;12:1385-1393.
29. Wang R. Hydrogen sulfide: the third gasotransmitter in biology and medicine. *Antioxid. Redox. Signal.* 2010;12:1061-1064.
30. John L.W. Physiological and pathophysiological roles of hydrogen sulfide in the gastrointestinal tract. *Antiox. Redox. Signal.* 2010;12:1125-1133.
31. Wang R. Two's company, three's a crowd: can H₂S be the third endogenous gaseous transmitter? *FASEB J.* 2002;16:1792-1798.
32. Jang G., Wu L., Liang W., Wang R. Direct stimulation of K (ATP) channels by exogenous and endogenous hydrogen sulfide in vascular smooth muscle cells. *Mol. Pharmacol.* 2005;68:1757-1817.
33. Zhao W., Wang R. H₂S induced vasorelaxation and underlying cellular and molecular mechanisms. *Am. J. Physiol. Heart. Circ. Physiol.* 2002;283:474-480.
34. Zhao W., Zhang J., Lu Y., Wang R. The vasorelaxant effect of H₂S as a novel endogenous gaseous K (ATP) channel opener. *EMBO J.* 2001;20:6008-6016.
35. Lavu M., Bhushan S., Lefler D.J. Hydrogen sulfide-mediated cardioprotection: mechanisms and therapeutic protection. *Clin. Sci.* 2011;120:219-229.
36. Nakao A., Sugimoto R., Billiar T. R., McCurry K. R. Therapeutic antioxidant medical gas. *J. Clin. Biochem. Nutr.* 2009;44:1-13.
37. Wei G., Ze-yu C., Yi-zhun Z. Hydrogen sulfide and translational medicine. *Acta Pharmacol. Sinica.* 2013;34:1284-1291.

38. Warenycia M.W., Steele J.A., Karpinski E., Reiffenstein R.J. Hydrogen sulfide in combination with taurine or cysteic acid reversibly abolishes sodium currents in neuroblastoma cells. *Neurotoxicology*. 1989;10:191-199.

CONTACT INFORMATION

Kolesnikov Sergei Ivanovich, PhD, professor, Member of the Russian Academy of Sciences (RAS), Chief Researcher in Scientific Center for Family Health and Human reproduction problems, distinguished scientist of the Russian Federation

Address: 16, Timiryazev Str., Irkutsk, 664003, **tel.:** +7 (3952) 20-76-36, **e-mail:** sikolesnikov2012@gmail.com

Vlasov Boris Yakovlevich (decedent) PhD, Professor, senior researcher in Scientific Center for Family Health and Human reproduction problems

Kolesnikova Lyubov' Il'yinichna, PhD, corresponding member of RAS, Professor, Director of the Sci.Center of Family Health and Human reproduction problems

Address: 16, Timiryazev Str., Irkutsk, 664003, **tel.:** +7 (3952) 20-76-36, **e-mail:** iphr@sbamsr.irk.ru