ОГЛЯДИ

UDC 546.221.1

HYDROGEN SULFIDE: METABOLISM, BIOLOGICAL AND MEDICAL ROLE

N. V. ZAICHKO, A. V. MELNIK, M. M. YOLTUKHIVSKYY, A. S. OLHOVSKIY, I. V. PALAMARCHUK

Pirogov Vinnytsya National Memorial Medical University, Ukraine
e-mail: nzaichko@mail.ru

Hydrogen sulfide (H_2S) is a signaling molecule that is actively synthesized in the tissues and is involved in the regulation of vascular tone, neuromodulation, cytoprotection, inflammation and apoptosis. In recent years, new data on animal and human H_2S metabolism and function under the effect of various endogenous and exogenous factors, including drugs were collected. This review is provided to introduce generalized information about the main and alternative H_2S metabolism and regulation, peculiarities of transport, signaling, biological role and participation in pathogenesis. Submitted data describe H_2S content and activity of H_2S-synthesizing enzymes in different organs, H_2S effect on blood coagulation and platelet aggregation based on our research results. The working classification of H_2S metabolism modulators, which are used in biology and medicine, is proposed: 1) agents that increase H_2S content in tissues (inorganic and organic H_2S donors; H_2S-synthesizing enzymes substrates and their derivatives, H_2S-releasing drugs; agents that contain H_2S-synthesizing enzymes cofactors and activators, agents that inhibit H_2S utilization); 2) agents that reduce H_2S content in tissues (specific and nonspecific inhibitors of H_2S-synthesizing enzymes), 3) agents with uncertain impact on H_2S metabolism (some medicines). It was demonstrated that vitamin-microelement and microelement complexes with H_2S-synthesizing enzymes cofactors and activators represent a promising approach for H_2S content correction in tissues.

K e y w o r d s: hydrogen sulfide, H_2S-signaling, enzymes, regulation, H_2S metabolism modulators, vitamin and microelement complexes.

Hydrogen sulfide (H_2S) is well-known as a toxic gas with obnoxious odour, which is mainly formed in the process of putrefaction of animal and plant proteins. Its role in living organisms was considered for a long time only from the viewpoint of exotoxicant and endotoxicant, meaning the inhibitor of cytochrome *c* oxidase, monoamine oxidase, neurotoxin, and lung irritant. The interest to H_2S significantly increased from the beginning of the 90's of the 20th century because of determination of its connection with regulation of animal and human physiological functions. The results of studies of Kazuho Abe та Hideo Kimura (1996) have triggered more deep research of H_2S biological role. The studies described peculiarities of this metabolite production in rats' brain and identified its first molecular target: N-methyl-D-aspartate receptors (NMDA-receptors) [1].

H_2S is up to date a significant member of gasotransmitter family, including nitrogen monoxide (NO) and carbon monoxide (CO), and is involved in vascular tone regulation, neuromodulation, cytoprotection, inflammation, apoptosis and other processes [2–7]. Despite of the great number of foreign articles describing the role of H_2S in biology and medicine, the Ukrainian works on this issue are scarce. New information has been accumulated recently on metabolism and H_2S functions in organisms under the effect of different endogenous and exogenous factors, including medicines, which we tried to generalize in this review.

H_2S physicochemical properties and membrane transport. H_2S is a short-living molecule with half-life of a few minutes [2]. In aqueous solutions 20-30% of H_2S exist in a non-dissociated form at pH 7.4 and 70-80% in a form of hydrosulfide anion (H_2S

$\leftrightarrow H^+ + HS^-$; p$K_a$ 6.89), partially transforming into sulfide anion (S^{2-}) [3, 8]. H_2S possesses a high lipophility and is solved in lipid membranes two times easier (partition coefficient – 2.0 ± 0.6), than in water [8]. H_2S is characterized by high permeability coefficient of lipid membrane (P_m = 3 cm·s^{-1}) and easily diffuses throughout cell membranes [8]. In conditions of organism at pH 7.4, the transmembrane diffusion of H_2S proceeds slower ($P_{m7.4}$ = 0.85 cm·s^{-1}), that leads to local accumulation of this molecule near a cell-producent. It has been determined throughout 3D-mathematic modelling that the sphere of H_2S biological action is determined by that distance, at which metabolite's concentration is no less than 10% of its concentration in the place of synthesis, and is spread over 200 neighbouring cells per 1 sec on the average [8]. Thus, H_2S acts as paracrine signaling molecule, but the distant effect is not excluded, because this metabolite is a part of blood plasma and may be transported by erythrocytes. It is determined that H_2S transport by human erythrocytes proceeds in 4 stages: 1) diffusion of H_2S throughout cell membranes or gas channels; 2) extracellular deprotonation of H_2S into HS^-; 3) arrival of HS^- into erythrocyte throughout anion transporter – protein AE1 at the exchange for Cl^-; 4) intracellular HS^- protonation to H_2S [9]. The membrane transport of H_2S can also occur throughout aquaporins – water channels [10].

Biosynthesis of H_2S. Major sources of endogenous H_2S in tissues are sulfur- containing amino acids – L-cysteine and L-homocysteine, which metabolized in reactions of transsulfuration and transamination with participation of pyridoxal 5′-phosphate dependent enzymes of cystathionine γ-lyase (CSE, EC 4.4.1.1), cystathionine β-synthase (CBS, EC 4.2.1.22); 2) and cysteine aminotransferase (CAT, EC 2.6.1.3) (Table 1). Key reactions providing production of H_2S in tissues of animals and humans are as follows: 1) desulfuration of L-cysteine to pyruvate (α, β-elimination) by CSE; 2) condensation of L-homocysteine with L-cysteine (β-replacement) and desulfuration of L-cysteine to L-serine (β-elimination) by CBS; 3) transamination of L-cysteine with α-ketoglutarate by CAT with production of 3-mercaptopyruvate, out of which H_2S is further emitted with participation of 3-mercaptopyruvate sulfur transferase (3-MST, EC 2.8.1.2). Cofactors of 3-MST in this reaction may be glutathione, thioredoxin and dihydrolipoic acid [11, 12].

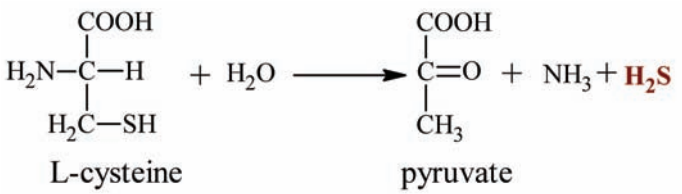
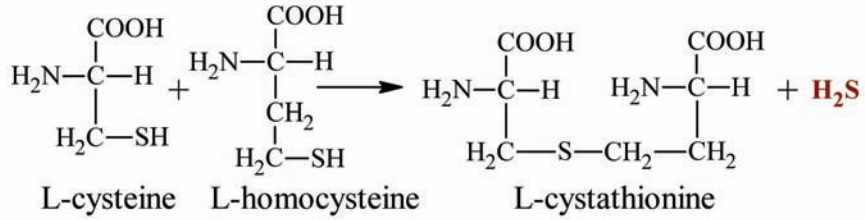
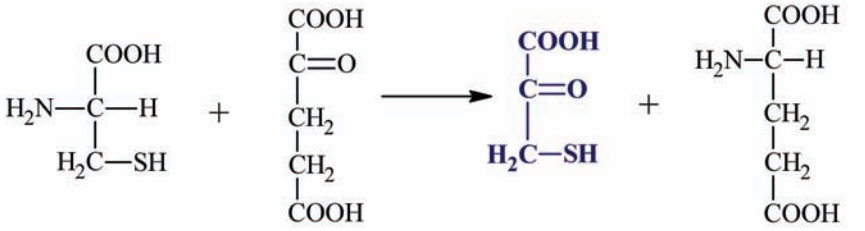
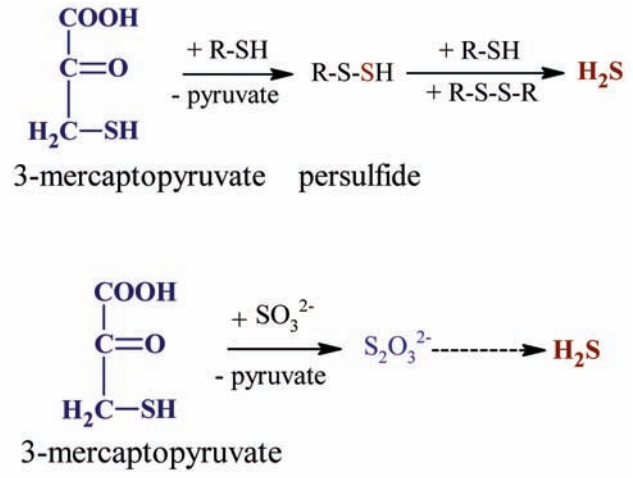
Other ways of enzymatic H_2S synthesis were detected recently, physiologic meaning of which is not finally clarified (Table 2) [12–15]. Alternative sources of H_2S are pyridoxal 5′-phosphate dependent reactions: 1) desulfuration of L-cystine to L-thiocysteine with next H_2S releasing (α, β-elimination) by CSE; 2) desulfuration of L-cysteine to L-serine (β-elimination) by CBS; 3) condensation of two molecules of L-homocysteine (γ-replacement) to L-homolanthionine by CSE; 4) desulfuration of L-homocysteine to L-homoserine by CSE; 5) condensation of two molecules of L-cysteine (β-replacement) to L-lanthionine with the participation of CSE or CBS; and also pyridoxal 5′-phosphate-independent reactions: 6) D-cysteine oxidation to 3-mercaptopyruvate by D-amino acid oxidase (DAAO, EC 1.4.3.3); 7) thiosulfate-anion reduction by thiosulfate-dithiol sulfurtransferase (TST, EC 2.8.1.5).

Kinetic parameters of key and alternative pyridoxal 5′-phosphate dependent reactions of H_2S synthesis, which have been studied *in vitro* on the example of human recombinant enzymes CSE and CBS, are significantly distinct. Investigations of Singh (2009) have shown that 96% of H_2S is produced in the condensation of L-cysteine with L-homocysteine with the participation of CBS with V_{max} 18.7 U/mg and K_m 3.2 mM. Alternative reactions of H_2S synthesis with the participation of CBS have such kinetic parameters: 1) desulfuration of L-cysteine into pyruvate with V_{max} 0.82 U/mg protein and K_m 27.3 mM; 2) lanthionine synthesis from L-cysteine with V_{max} 0.77 U/mg protein and K_m 45,6 mM [13].

Chiku et al. have shown (2009) that among reactions of H_2S synthesis with the participation of CSE only cysteine desulfuration (α, β-elimination) can play the main role because its kinetic parameters are V_{max} 0.6 U/mg protein and K_m 1.7 mM. It appeared that K_m of alternative CSE-dependent reactions of H_2S synthesis associated with lanthionine, homolanthionine and homoserine creation are significantly higher: 33; 5.9; 2.7 mM at V_{max} 1.2; 6.6; 1.2 U/mg, respectively [14]. Kinetic parameters of H_2S synthesis from cystine are not determined, because under conditions close to physiological ones, the reaction does not proceed.

3-MST is pyridoxal 5′-phosphate-independent enzyme, which is functionally connected with CAT and, possibly, with TST. As opposed to CSE and CBS, for which pH 8.5-9.0 is optimal, 3-MST effectively synthesizes H_2S from 3-mercaptopyruvate at pH 7.4. This enzyme provides creation of persulfides, from which H_2S can release under interaction with thioles (gluthathione, dihydrolipoic acid, thioredoxin) [16, 17]. Also, 3-MST may turn sulfite-

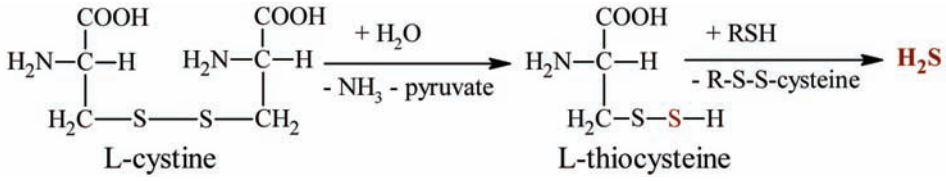
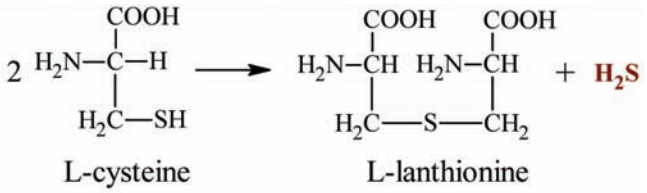
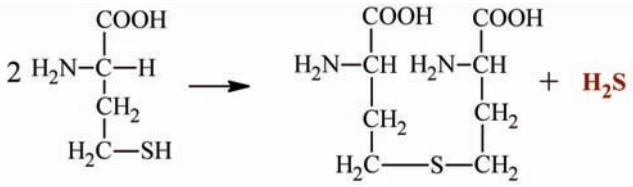
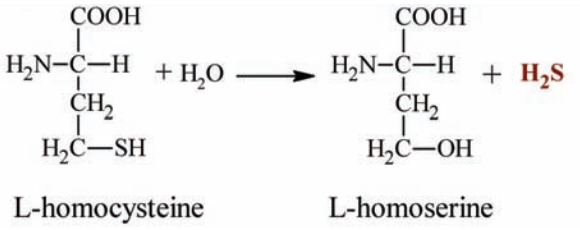
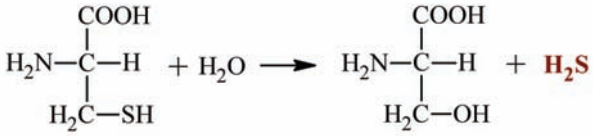
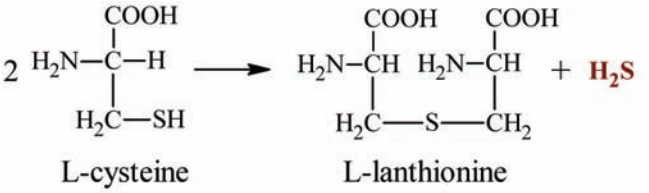
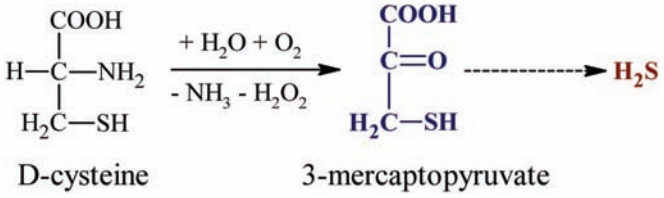
Table 1. Key enzymatic reactions of H_2S creation in tissues of animals and humans

Enzyme	Scheme of reaction	Ref.
Cystathionine γ-lyase (EC 4.4.1.1)		[13, 14]
Cystathionine β-synthase (EC 4.2.1.22)		[13, 14]
Cysteine aminotransferase (EC 2.6.1.3)		[13, 14]
3-mercaptopyruvate sulfur transferase (EC 2.8.1.2)		[11, 12]

anion into thiosulfite-anion, which is further reduced to H_2S with the participation of TST [11, 12, 18].

The question is how H_2S general production may provide reactions, which necessitate significantly high substrate concentrations (L-cysteine and L-homocysteine). As it is known, under organizm conditions, the pull of free sulfur-containing amino acids is significantly lower, than that of their bound and disulfide forms. The content of general homocysteine in human blood plasma does not exceed 15 μM, that of cysteine is 300 μM, but the content of their free (thiol) forms is about 0.13-0.17 and 24-27 μM, respectively [19]. Their quantity under pathology conditions may grow significantly: under homozygous deficit of CBS the level of general homocysteine in the blood plasm increases up to 100-500 μM and above [20], the content of free cysteine may 60 times exceed the norm under chronic kidney failure. Part of free cysteine in blood plasma of pre-hemodialysis patients was 40.9% compared

Table 2. Alternative enzymatic reactions of H_2S creation in tissues of animals and humans

Enzyme	Scheme of reaction	Ref.
Cystathionine γ-lyase (EC 4.4.1.1)		[13,14]
		[13,14]
		[13,14]
		[13,14]
Cystathionine β-synthase (EC 4.2.1.22)		[13,14]
		[13,14]
D-amino acid oxidase (EC 1.4.3.3)		[12]
Thiosulfate-dithiol sulfurtransferase (EC 2.8.1.5)	$S_2O_3^{2-} + 2R\text{-}SH \rightarrow SO_3^{2-} + R\text{-}S\text{-}S\text{-}R + H_2S$	[22,29]

to 1.6% in healthy persons [21]. Obviously, the role of alternative reactions of H_2S synthesis at ordinary concentrations of sulfur-containing amino acids in organism is insignificant, but it may increase under hyperhomocysteinemia or hypercysteinemia, which are often combined [19].

The role of pyridoxal phosphate-independent ways of H_2S creation (from D-cysteine and thiosulfate) in animal and human organism has not been studied completely. Studies of Shibuya and Kimura (2013) prove that optimal conditions for H_2S synthesis from D-cysteine exist in organism (pH 7.4). However, this amino acid is not formed in the cells and may arrive only from exogenous sources [12]. The role of thiosulfate-anion and TST in creation of H_2S in tissues is the least studied. We have no clear data as of today regarding the content of thiosulfate-anion in organs and tissues, thus it is difficult to estimate the importance of this enzyme. Allowing for the concentration of thiosulfate-anion in blood plasma and urine of humans (1.13 ± 0.11 mg/dl and 0.28 ± 0.02 mg/dl, respectively) [22], the above-mentioned way of H_2S formation in tissues is possible.

Catabolism and deposition of H_2S. H_2S catabolism may be performed by enzymatic and non-enzymatic ways (Table 3). In mytochodria HS^- is oxidated to thiosulfate-anion and sulfite-anion by sulfide quinone oxido-reductase system (SQR). Then sulfite is converted to sulfate by sulfite oxidase (EC 1.8.3.1). SQR consists of three enzymes: thiosulfate:cyanide sulfur-transferase (rhodanese, EC 2.8.1.1), sulfur dioxygenase (EC 1.13.11.18) and sulfide:quinone reductase (EC 1.8.5.4) [23]. H_2S may spontaneously react with mitochondrial hemoproteins – cytochrome oxidase and cytochrome *c* with creation of sulfane sulfur (S^0) and reactive thiol radical ($HS^{\bullet}$) [23, 24].

In cytosol H_2S methylates to methanethiol and dimethyl sulfide with participation of thiol S-methyltransferase (EC 2.1.1.9). Utilization of H_2S in erythrocytes proceeds in non-enzymatic way through formation of sulfhemoglobine [3, 2, 23].

H_2S catabolism by SQR and its direct reaction with thiols results in formation of unstable persulfides (R-S-S*-H, thiotaurine, thiocysteine) which contain active sulfane sulfur (S^0) [23]. H_2S is deposited in sulfane sulfur form in different tissues (brain, heart, liver, kidney) and can be released on demand. 3-MST and CAT provide H_2S deposition in polysulfides [25, 26]. The quantity of polysulfides in cells with 3-MST and CAT expression is twice as much in comparison with cells without these enzymes [26].

H_2S participates in formation of nitrosothiols (RSNO) known as depot of NO in cells [27, 28]. $HS^{\bullet}$ та HS^- may interact with active forms of nitrogen ($NO^{\bullet}$, $ONOO^-$) with creation of the smallest nitrosothiol – thionitrous acid (HSNO) [27]. HSNO, however, reacts with thiols with formation of nitrosothiols and H_2S [27].

Features of H_2S tissue metabolism and its regulation. Organs and tissues are distinguished by the ability to produce H_2S-synthesizing enzymes

Table 3. Utilization and deponation of H_2S in tissues

Enzyme	Scheme of reaction	Ref.
Sulfide:quinone reductase (EC 1.8.5.4)	H_2S +R-SH + quinone → R-S-**S***-H + hydroquinone	[24]
Sulfur dioxygenase (EC 1.13.11.18)	R-S-**S***-H + O_2 + H2O → R-SH + SO_3^{2-} + $2H^+$	[23]
Thiosulfate:cyanide sulfur-transferase (EC 2.8.1.1)	**$2HS^-$** + $2O_2$ → **$S_2O_3^{2-}$** + H_2O $S_2O_3^{2-}$ + CN- → SCN^- + **SO_3^{2-}**	[23]
Sulfite oxidase (EC 1.8.3.1)	**SO_3^{2-}** + Fe^{3+} (cytochrome *c*) → **SO_4^{2-}** + Fe^{2+} (cytochrome *c*)	[3, 23]
Thiol S-methyltransferase (EC 2.1.1.9)	**H_2S** → CH_3-SH → CH_3-S-CH_3	[23]
Non-enzymatic way	**H_2S** + methemoglobine → sulfhemoglobine **HS^-** + Fe^{3+} (cytochrome *c*) → Fe^{2+} (cytochrome *c*) + **$HS^{\bullet}$**	[23]
Non-enzymatic way	**$HS^{\bullet}$** + $NO^{\bullet}$ → HSNO HSNO + RSH ↔ RSNO + **H_2S**	[27, 28]

and ability to produce H_2S (Table 4). In animal and human organism H_2S is formed in the liver where all H_2S-synthesizing enzymes (CBC, CSE, CAT, 3MST) are expressed [5, 29–31]. Also H_2S is intensively formed in kidneys. CSE and CBS are expressed in renal cortex and medulla, proximal tubules, interstitial tissue [32], but 3-MST is expressed in glomerules [33].

CBS is considered the major producer of H_2S in the central nerve system; it is expressed in the hippocampus, cerebellum, cortex and brain stem [5, 15, 34, 35]. Creation and deposition of H_2S as polysilfude in brain are provided by CAT and 3-MST, which are expressed in the cortex, cerebellum and retina [25]. CBS is localized in astrocytes, and 3-MST – in neurons [17]. Contribution of CSE to production of H_2S in brain is the least, however, this way is considered important in support of redox homeostasis of the brain [36]. CSE activity in the mice brain is about 1% of its activity in the liver. CSE is present in neurons of human brains and its activity is several times higher than that in mice [36].

In blood vessels H_2S is synthesized with participation of CSE which is mainly expressed in smooth myocytes, and with CAT/3-MST, which are expressed in endothelium. The expression of these enzymes has been found in the aorta, lung artery, mesenteric and kidney arteries [3, 5, 11, 17]. CAT/3MST and CSE are expressed in the myocardium [37–39], but CAT/3-MST, CSE and CBS are expressed in skeletal muscles [40]. Considerably high ability of H_2S creation was detected in the myometrium of rats and humans, in which CBS and CSE are expressed [41, 42]. The expression of CSE and CBS is detected in the intestine, stomach, β-cells of pancreas [43–45], adipocytes [46], lungs [47, 48].

H_2S synthesis regulation. CBS and CSE cells are localized in the cytozole, while 3-MST and CAT – in the mitochondria and cytozole (division of their fractions depends on tissue) [17, 26, 49].

Human and rat CBS is homotetramer and consists of 4 equal subunits with molecular weight of 63 kDa. Each subunit is bound to the cofactors pyridoxal 5′-phosphate, heme and S-adenosylmethionine [5]. S-adenosylmethionine is an allosteric activator of CBS. That is why CBS-dependent H_2S production may increase, if S-adenosylmethionine concentration is elevating in cells [1]. CBS activity is inhibited by CO, which binds with high affinity with heme (K_i 5.6 μM), and NO – only in high supraphysiological concentrations (K_i 360 μM) [50]. One more mechanism of impact on CBS activity, which proves possible participation of enzyme in epigenetic regulation and gene expression, was detected recently. C-terminal fragment of CBS contains a tandem of two domains, which undergoes SUMO-modification (SUMO – small ubiquitin-like modifier) of lysine in position 211 that causes a decrease of enzyme's activity. CBS sumoylation is inhibited by cystathionine [51]. Biological importance of SUMO-modification of CBS remains unidentified so far. Hypothetically, such form of CBS translocation into nucleus with

Table 4. Main H_2S-synthesizing enzymes expression in laboratory animals and human tissues

Organ, tissue	H_2S-synthesizing enzymes	Ref.
Liver	CSE[1,2,3], CBS[1,2,3], CAT[1], 3-MST[1]	[5, 29–31]
Kidneys	CSE[1,2,3], CBS[1,2,3], CAT[1], 3-MST[1]	[5, 29, 30, 32, 33, 38]
Brain	CBS[1,2,3], 3-MST[1], CSE[1,2,3]	[5, 17, 25, 35, 36]
Myocardium	CSE[1,2], 3-MST[1]	[37–39]
Aorta, endothelium	CAT[1], 3-MST[1], CSE[2]	[5, 11]
Aorta, smooth myocytes	CSE[1]	[52, 101]
Myometrium, placenta	CBS[1], CSE[1]	[41, 42]
Skeletal muscles	CSE[1], CBS[1], 3-MST[1]	[40]
Adipocytes	CSE[1]	[46]
Stomach, intestine	CBS[1,2], CSE[12]	[43, 78]
Pancreas, β-cells	CBS[2], CSE[2]	[45, 44]
Lungs	CSE[1,3]	[47, 48]

Notation. Enzyme expression in [1]rat (*Rattus norvegicus*), [2]mouse (*Mus musculus*) and [3]human tissues

further desumoylation and renovation of ability to H_2S synthesis can take place [51].

H_2S formation by CSE is activated under the action of calmodulin in the presence of 1-2 mM Ca^{2+} [52]. However, basic concentration of Ca^{2+} in cells is around 100 nM, that is why the role of Ca^{2+}/calmodulin in regulation of CSE-dependent H_2S production necessitates further study [6]. Under *in vitro* conditions the ability of recombinant human CSE to sumoylation is established, and this process is not blocked by cystathionine [51]. Whether SUMO-modification of CSE under conditions of organism exists and what kind of role this process plays has not been determined yet. Under hypoxia when concentration of cytosolic Ca^{2+} increases, CSE translocation from cytosole to mitochondrium may happen, the process is accompanied by the increase of mitochondrial H_2S production and ATP synthesis [26].

In contrast to CSE, H_2S formation with participation of CAT/3-MST is dose-dependently inhibited by Ca^{2+} (with full blocking in the presence of 2.9 μM Ca^{2+}) and does not depend on calmodulin [17]. CAT activity decreases with L-aspartate increase in the medium [49]. 3-MST-dependent H_2S production increases with the increase of thioredoxin and dihydrolipoic acid content in cells [17].

Concentration of endogenous H_2S. H_2S content in blood plasma of animals (rats) and humans is around 50-80 μM [2, 3, 53]. H_2S presence is more considerable in tissues, in particular, the content of this metabolite in the animal brain is around 50-160 μM [17, 2]. According to other data, H_2S content in the brain, myocardium and kidneys of rats is 2.6; 11.4 and 6.7 μg/g of tissue, respectively [54]. We have to mention that measurement of H_2S content in tissues in the majority of investigations is performed by colorimetric method (in compliance with the reaction with N,N-dimethyl-p-phenylenediamine in the presence of $FeCl_3$), which needs highly acidic medium and does not exclude H_2S release from the tissue depot. Presumably, intracellular free H_2S concentration is much more less, because pH is around 7-8 in the mitochondria. Free H_2S concentration determined by the method of gas chromatography, was 0.12 μmol/kg of protein (14 nM) in the rat brain, and concentration of acid-labile sulfur was 916 μmol/kg of protein [55].

According to the results of our own studies (Table 5), general H_2S content and specific activity of H_2S-synsethizing enzymes in rats' tissues are on the highest level in the liver and kidneys, slightly lower in myocardium, brain and aorta. Determination of H_2S content and production in tissues was performed as it has been described [22, 56–58].

H_2S biological role and molecular targets. H_2S in organism plays a role of signaling molecule, gasotransmitter; no specific receptors have been found for it. Different ion channels, receptors, enzymes and proteins, regulating numerous biochemical and physiological processes, play a role of H_2S molecular targets (Table 6).

A key mechanism of H_2S-signaling is S-sulfhydration of proteins, post-translation modification with convertation of -SH groups into -SSH, which significantly increases reactivity of cysteine residues and increases functional activity of molecular targets as well [6, 17, 59]. H_2S also reduces disulfide bonds of cystine residues with -SH groups releasing [23]. Redox-modification of proteins with participation of H_2S may be of independent regulatory importance as well as serve as a preparatory stage for S-sulfhydration and other kinds of post-translational S-modification (S-nitrosylation, S-homocysteinylation, S-glutathionylation). For example, activation of NMDA-receptors with participation of H_2S proceeds in two stages: 1) NMDA-receptors become active under reduction of their disulfide form into thiol form, 2) transition of thiol form into persulfide form

Table 5. H_2S content and activity of H_2S-synthesizing enzymes in rats' organs (M ± m, n = 10)

Organ, tissue	H_2S, nmol/mg protein	Enzyme activity, nmol H_2S/min·mg protein			
		CSE	CBS	CAT/3-MST	TST
Liver	3.85 ± 0.21	3.09 ± 0.15	2.75 ± 0.18	2.47 ± 0.10	13.0 ± 0.55
Kidneys	3.27 ± 0.16	1.65 ± 0.11	2.17 ± 0.14	2.49 ± 0.18	3.29 ± 0.14
Brain	1.52 ± 0.08	0.27 ± 0.03	0.57 ± 0.03	0.13 ± 0.03	1.25 ± 0.08
Myocardium	2.37 ± 0.10	0.23 ± 0.03	0	0.52 ± 0.07	1.20 ± 0.06
Aorta	1.28 ± 0,06	0.67 ± 0.04	0	0.59 ± 0.05	1.98 ± 0.08

Table 6. H_2S-signaling: mechanism, targets, biological processes

Mechanisms	Molecular targets	Target cells	Biological processes
1. *Modification of proteins:* - S-sulfhydration R-SH ↔ R-S-SH - Redox-modification R-S-S-R ↔ 2R-SH 2. *Modification of prosthetic groups* (heme, Cu) 3. *Interaction with NO, participation in S-nitrosylation of protein* $HS^{\bullet} + NO^{\bullet} \rightarrow$ HSNO →RSNO 4. *Interaction with ROS, generation of* $HS^{\bullet}$, $S_2O_3^{2-}$, SO_3^{2-}, SO_4^{2-} 5. *Interaction with electrophile lipid derivatives* (4-hydroxynonenal and others)	*Ion channels:* - K^+-ATP-channels - K^+ (Ca^{2+})-channels - T- or L-type of Ca^{2+}-channels - TRP- channels ($TRPV_1$, $TRPA_1$) - Cl^--channels *Receptors:* NMDA receptors *Signaling enzymes, transcription factors:* adenylate cyclase protein kinase A protein kinase C protein kinase B (Akt) phosphoinositide 3-kinase (PI3K) phosphodiesterase MAPK, ERK NF-κβ, Nrf2, HIF-1α *Others:* glyceraldehyde-3-phosphate dehydrogenase glutamate-cysteine ligase thioredoxin reductase cytochrome *c* oxidase cytochrome *c* monoamine oxidase actin, β-tubulin cystine/glutamate antiporters excitatory amino acid transporters	neurons astrocytes smooth myocytes cardiomyocytes skeletal myocytes pancreatic β-cells endothelium hepatocytes platelets leukocytes renal tubular epithelium gastrointestinal epithelium	neurotransmission nociception vascular tone regulation myocardial contractility cytoprotection inflammation apoptosis angiogenesis platelet aggregation insulin secretion cysteine transport into cells glutathione synthesis tissue respiration aging circadian rhythm gastroduodenal motility

(S-sulfhydration) with a further, more considerable increase of NMDA-receptors activity [17].

Mustafa et al. (2009) obtained direct proofs that S-sulfhydration raises the activity of ATP-sensitive potassium channels (-SSH modification of Kir6,1 subunit), glyceraldehyde-3-phosphate dehydrogenase (-SSH modification Cys150) and increases actin ability to polymerization [59]. S-sulfhydration increases neuroprotective activity of ubiquitin E3 ligase [15]. H_2S impact over other molecular targets, calcium channels of different types, TRP (transient receptor potential) channels ($TRPV_1$, $TRPA_1$), protein kinases, factors of transcription, is going on presumably through S-sulfhydration/ desulfhydration. This is proved by the results of numerous studies *in vivo* and *in vitro* with usage of H_2S donors (NaHS), inhibitors of H_2S-synthesizing enzymes, knock-out of CSE and CBS genes in animals.

H_2S together with NO participates in S-nitrosylation of proteins and low-molecular thiols [28, 27]. S-nitrosylation, in contrast to S-sulfhydration, decreases cystein's ability to reaction and leads

to further lost of activity of molecular targets. [6, 17, 59]. For example, glyceraldehyde-3-phosphate dehydrogenase is inactivated during S-nitrozylation Cys150 [59]. H_2S, in its turn, may express NO, activating guanilate cyclase and its own signaling ways, from nitrosothiols [27, 28].

H_2S also acts through interaction with prosthetic groups of metalloproteins, reactive oxygen species (ROS), low-molecular electrophilic derivatives. H_2S in high concentrations blocks the activity of cytochrome *c* oxidase, reducing by electrons heme *aa3*, *CuB* and cytochrome *c* [23]. H_2S may reduce Fe^{3+} into Fe^{2+} of methemoglobin with its conversion into a form able to bind and transport oxygen [23]. Under H_2S interaction with ROS, generation of thiyl radical and thiosulfate anion which is transformed into oxygen-containing sulfur derivatives (sulfites and sulfates) is going on. It is not excluded that this way also mediates H_2S signaling, because SO_2 participates in regulation of vascular tonus and contraction of the myocardium [60]. It is proved that Na_2SO_3/$NaHSO_3$ causes dose-dependent relaxation of isolated rings of rats' aorta *in vitro* [61]. As a nucleophile, H_2S easily interacts with electrophile lipid derivatives, including 4-hydroxynonenal, which is a strong modulator of oxidative stress, cell proliferation, apoptosis [62].

Biological effect of H_2S may be significantly distinct depending on tissue localization of molecular target. H_2S impact on ion channels, for example, is going on in such directions [7, 15, 26, 63, 64]: 1) activation of ATP-K^+-channels of smooth myocytes of blood vessels, that is associated with vasodilation, lowering of arterial pressure, cardioprotection under ischemia/reperfusion, inhibition of insulin secretion by the β-cells, anti-inflammatory, antinociceptive and anti-apoptosis effects; 2) inhibition of Ca^{2+}-channels of L-type in cardiomyocytes (with Ca^{2+} level decrease) and their activation in neurons (with an increase of Ca^{2+} level); inhibition of large $K^+(Ca^{2+})$-channels in carotide sinuses and their activation in pituitary cells; 3) activation of Ca^{2+}-channels of T-type and $TRPV_1$ channels, that is associated with H_2S-induced hyperalgesia and pronociceptive effect; activation of $TRPA_1$-channels with transportation of Ca^{2+} into astrocytes; 4) H_2S-induced contraction of out-vascular smooth muscles and increase Cl^- secretion in gastrointestinal tract are connected with activation of $TRPV_1$ and $TRPA_1$ channels; 5) Cl^- channels activation and prevention from neurons oxytosis.

Year after year more and more mechanisms of H_2S-signaling are identified, where kinases, transcription factors, growth factors and other regulator molecules are involved. H_2S action may be mediated through:

1) modulation of adenylate cyclase, phosphodiesterase activity and cAMP content in cells;

H_2S causes inhibition of adenylate cyclase and decrease of cAMP content in neurons of morphin-dependent mice [65]; phosphodiesterase activity increase in juxtaglomerulal cells of rats' kidneys with renovascular hypertension [66]; cAMP level and activity of protein kinase A increase but cAMP-dependent phosphodiesterase activity decrease in isolated mitochondria of rats' hepatocytes [67]; cAMP synthesis increase in culture of neurons and glial cells [68].

2) modulation of protein kinases C, PI3K, B (Akt), MAPK, ERK activity;

H_2S induces an increase of activity and expression of protein kinase C [69]; phosphoinositide 3-kinase (PI3K), protein kinaseB (p-Akt), kinase-3β glycogen synthetase (GSK-3β) and protein Bcl-2 [70]; inhibits expression of mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinases (ERK) [4, 71], that decreases inflammatory response and disorder of cells under action of different factors.

3) modulation of activity of transcription factors Nrf2, NF-*κ*B, HIF-1α;

H_2S stimulates translocation in the nucleus of Nrf2 – nuclear factor (erythroid-derived nuclear factor of transcription), that activates antioxidant response pathway and up-regulates thioredoxin reductase, glutathione S-transferase, thioredoxin-1 expression [23] and glutathione synthesis [72]; inhibits NF-κB expression (nuclear factor kappaB) and down-regulates proinflammatory cytokines expression [73, 74]; up-regulates expression of HIF-1α (hypoxia-inducible factor-1) and vascular endothelial growth factor (VEGF), which stimulates angiogenesis [23].

4) modulation of NO and CO synthesis and their signaling pathways;

H_2S up-regulates eNOS expression and increases endothelial NO production [23]; decreases iNOS production and nitrosative stress [75]; up-regulates heme oxygenase-1 expression and CO production in cardiomyocytes and other cells [75, 76].

5) impact on aging and circadian rythm genes (*SIRT1, Klotho*) expression, through which formation of age-associated changes in tissues is mediated [77].

Workers of Blood Circulation Physiology Department of the O. O. Bohomolets Institute of Physiology of NAS of Ukraine (Kyiv) under leadership of V. F. Sahach, Corresponding Member of NAS of Ukraine, doctor of medical science, assessed that H_2S is involved into opening of mitochondrial permeability transition pore, which is a key player in development of apoptosis and necrosis, and this effect is realized through K^+-ATP-channels modulation [102–104].

Direction of H_2S action depends on its content in tissues. At low (physiological) concentrations H_2S shows its vasodilating, cytoprotector, antioxidant, anti-inflammatory and anti-apoptotic effects. It also increases sensibility of NMDA-receptors of neurons to glutamate, stimulates Ca^{2+} transportation to astrocytes and increases synaptic activity. H_2S activates cystine-glutamate antiporters, stimulates transportation of cysteine into mitochondria, and increases activity of γ-glutamylcysteine synthetase (glutamate-cysteine ligase, EC 6.3.2.2) and glutathione synthesis in neurons and other cells [4, 17, 23, 26, 68]. H_2S in supraphysiological and toxic concentration induces mithohondrial dysfunction, blocks tissue respiration and oxidative phosphorylation, increases vascular tonus, causes hyperalgesia, promotes inflammation and apoptosis, inhibits synaptic transmission. H_2S enables biogen amins effects (γ-aminobutiric acid, glutamate, serotonin, dophamine, epinephrine and norepinephrine) and acetylcholine by up-regulation of their receptors exression and inhibition of monoamine oxidase and acetylcholine esterase activity [17, 68]. Low H_2S concentrations increase basal tension, smooth muscle contractions and motility of the gastric antrum, but high H_2S concentrations, on the contrary, decrease all the above-mentioned functions [78]. H_2S in concentration of 0.1-1 μM increased electron transport and ATP content, and being in a concentration of 3-30 μM it inhibited cytochrome *c* oxidase and oxidative phosphorylation in mitochondria of isolated hepatocytes [79].

Different pathological conditions are associated with disorder of H_2S content in tissues. A decrease of basic H_2S content in blood plasma is noticed in patients with arterial hypertension, ischaemic heart disease, deep venous thrombosis, Alzheimer's disease, hyperhomocysteinemia [3, 19, 53]. An increase of H_2S content is observed in patients with Down's syndrome, with decompensated liver cirrhosis, sepsis, ischaemic stroke, chronic obstructive pulmonary diseases [19, 3].

H_2S metabolism modulators in biology and medicine. The following basic approaches are used to study H_2S role *in vivo* and *in vitro*: 1) introduction of inorganic and organic H_2S donors; 2) introduction of specific inhibitors of H_2S-synthesizing enzymes; 3) modification of efficiency and toxicity of pharmacological remedies with the help of H_2S; 4) modification of H_2S metabolism with the help of pharmacological drugs. Examples of some H_2S metabolism modulators are presented in Table 7.

H_2S under conditions of organism generally works in the form of HS-, that is why NaHS or Na_2S are used as its donors. Information on doses, ways and duration of administration to animals of inorganic H_2S donors varies [80]. NaHS/Na_2S, according to most studies, are injected parenterally (intraperitoneally, intravenously) in doses from 100 μg/kg to 3 mg/kg of the animal body weight, and duration of experiment may vary from several hours to 2-3 weeks [80]. In long-term studies (8-10 weeks) NaHS is added to drinking water in concentrations of about 30 μmol/l; animals drink it *ad libitum* [81]. We should note that LD_{50} for NaHS under parenteral injection is 14.6 ± 1.0 mg/kg [82], in compliance with other data it is 60.2 mg/kg, and conventionally therapeutic doses of NaHS are about 1/20 LD_{50} [83]. NaHS/Na_2S in such concentration range decrease the ischaemic-reperfusion damages under conditions of myocardial infarction, kidney ischemia, intestinal ischemia, show antioxidative, cerebroprotective, anti-inflammatory features [7, 17, 34, 68, 80, 81].

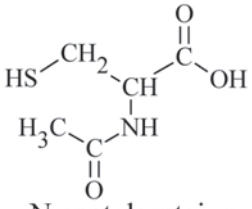
Vasodilatory, cytoprotective, antioxidative, anti-inflammatory effects of NaHS/Na_2S in studies *in vitro* are realized in broader range concentrations from 1 to 1000 μM [80].

We have shown that H_2S donors demonstrate antiaggregation and anticoagulant effect: NaHS in concentration of 1 mM inhibited aggregation of human platelets, induced by ADP and collagen, and decreased amidolytic activity of thrombin (IC_{50} = 65.3 ± 3.76 μM) *in vitro*; the injection of $Na_2S{\cdot}9H_2O$ (Sigma, USA) to rats in the amount of 3.36 mg/kg once a day during 7 days caused the increase of prothrombin time and activated partial thromboplastic time and decrease of Xa factor activity [19]. According to data of Nishikawa et al. (2013), NaHS inhibits thrombocyte aggregation, induced by ADP and collagen in plasma enriched by thrombocytes in concentrations of 0.1-0.3 mM, and in a suspension of washed up thrombocytes of rabbits in concentrations of 1-3 mM, respectively [84].

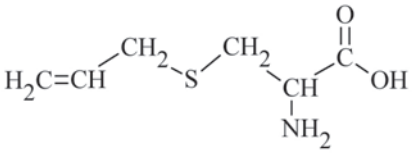
Table 7. Examples of H_2S metabolism modulators

H_2S donors and cysteine derivatives

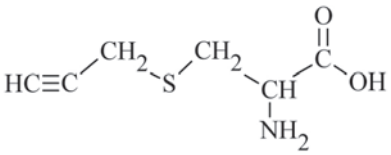
Na_2S NaHS



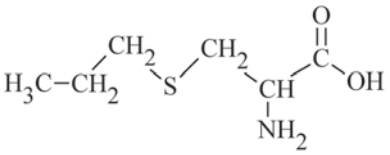
N-acetylcysteine



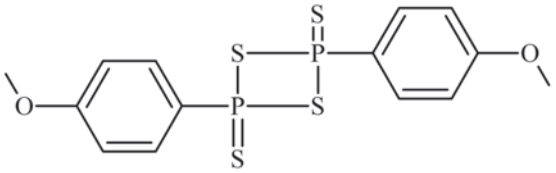
S-allyl-L-cysteine



S-propargyl-L-cysteine

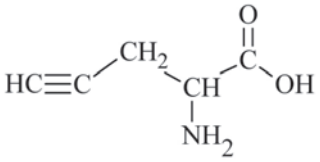


S-propyl-L-cysteine

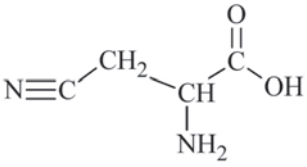


Lawesson's reagent

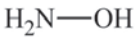
Inhibitors of H_2S-synthesizing enzymes



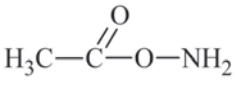
DL-propargylglycine



β-cyanoalanine

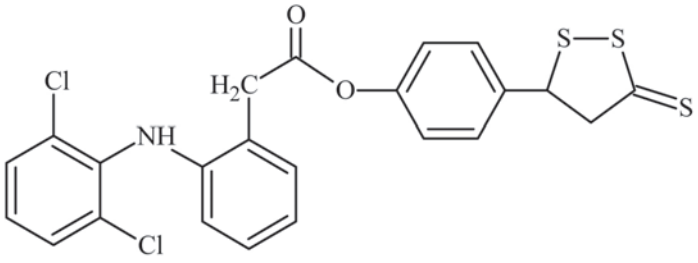


hydroxylamine

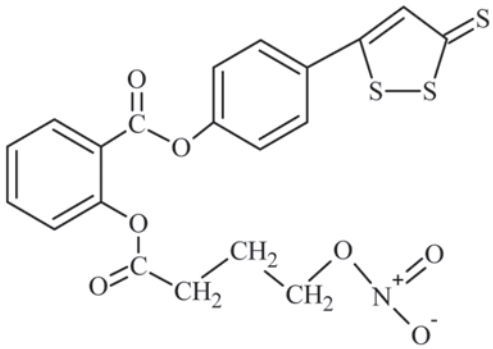


aminooxyacetate

H_2S – derivatives NSAIDs



ACS 15 (S-diclofenac)



NOSH-1 (aspirin-NO+H_2S)

It has been showed in works of N. A. Strutynska, O. M. Semenykhina et al (2011, 2013) that NaHS та L-cystein in physiological concentrations improve functional conditions of mitochondria in the heart, prevent Ca^{2+}-induced opening of mitochondrial pore and swelling of mitochondria at the time when CSE inhibitor propargylglycine increases sensitivity of mitochondria pore up to the action of Ca^{2+} inductor

and causes mitochondria swelling [102–104]. Gradual swelling of rats' heart's mitochondria is observed in potassium-free medium in the presence of NaHS in concentrations of 10^{-12}– 10^{-8} M [102]. Age distinctions regarding H_2S impact over pore creation in the heart mitochondria have been observed: NaHS protector effect in adult rats is realized in a more broad range of concentrations (10^{-6}, 10^{-5} and $5{\cdot}10^{-5}$ M) than in old rats (10^{-5} M) [102]. NaHS in the range of concentrations 10^{-9}–10^{-6} M causes dose-depending decrease of oxygen absorption by isolated mitochondria of adult rats' heart in the presence of succinate and ADP at concentration of 10^{9} and 10^{-8} M and increases connection between oxidation and phosphorylation [103]. Preincubation of isolated mitochondria with inhibitor of K^+-ATP-channels 5-hydroxydecanoate significantly decreased the ability of NaHS (10^{-4} M) to prevent mitochondrial swelling under ischemia/ reperfusion of myocardium [104].

Inhalation of H_2S aimed at cytoprotection is used in some studies, and concentrations of 50-400 ppm which cause mitochondrial dysfunction, are sublethal. H_2S in concentration up to 10 ppm does not create disorder in activity of mitochondrial enzymes [26]. The perfusion of lungs by gas mixture with H_2S in concentration of 50 and 100 μmol/l decreased oxidative disorders at pulmonal ischemia/ reperfusion in mice [80].

Inorganic H_2S donors quickly increase its content in blood plasma and tissues, but they are also quickly eliminated from the organism in a form of sulfides, thiosulfates, sulfites, sulfates. More slow increase of H_2S content in tissues provide H_2S-synthesing enzymes substrates such as L-cysteine, D-cysteine; L-cysteine derivatives such as N-acetylcysteine, S-propalgyl-L-cysteine, S-propyl-L-cysteine, S-allyl-L-cysteine; polysulfides (diallyl disulfide, diallyl trisulfide) and artificial organic donor of H_2S - Lawesson's reagent [68, 85, 105]. Sodium thiosulfate may also play the role of inorganic substrate for endogenous H_2S production. For example, the injection of L-cysteine (15 and 100 mg/kg) stimulated epithelialization of gastric ulcer in rats [86]. D-cysteine protects neurons of cerebellum from oxidative stress and decreases damage of kidneys under condition of ischeamia-reperfusion more effectively than L-cysteine [12]. S-propalgyl-L-cysteine, S-propyl-L-cysteine, S-allyl-L-cysteine showed cardioprotective action under acute myocardial infarction in rats, increased H_2S content and increased activity of superoxide dismutase (Mn-SOD) in cardiomyocytes [105]. Administration of sodium thiosulfate (3 mg/ml with drinking water for 6 weeks) normalized cardial H_2S production in rats with chronic heart failure [87].

To reach a decrease of H_2S endogenous production the H_2S-synthesizing enzymes have been used: inhibitors of CSE – DL-propargylglycine, β-cyanoalanine or inhibitors of CBS – hydroxylamine, aminooxyacetate. As a rule, inhibitors of H_2S-synthesizing enzymes demonstrate vasoconstriction, cytotoxic, pro-oxidant and pro-inflammatory action [7, 15, 17, 34, 68, 80, 81]. For example, the injection of DL-propargylglycine caused a decrease in activity of mitochondrial electron transport chain Complex I, decrease of glutathione content and development of oxidative stress in the brain of mice [36]; increased ischaemia-reperfusion damages and increased size of myocardial infarction, worsened functional condition of kidneys and increased content of creatinine in blood serum of rats [80]. Seven-day injection of propargylglycine (50 mg/kg) caused activation of blood coagulation and increased ADP-induced aggregation of thrombocytes in rats [88]. With excessive H_2S in tissues, the inhibitors of H_2S-synthesizing enzymes may show cytoprotector action. Propargylglycine injection (8-10 mg/kg) decreased features of acute turbular necrosis, prevented the increase of serum creatinine and decreased H_2S creation in rats' kidneys under gentamicyne administration [89].

Taking into consideration H_2S physiological effects, the attempts have been made to modify effectiveness and toxicity of medicines with the help of its donors. Thus there appeared H_2S-releasing drugs, and H_2S-derivatives of nonsteroidal anti-inflammatory drugs (NSAIDs) became their first example. It has been proved that one of the mechanisms of gastroduodenal toxicity of NSAIDs is a decrease of H_2S endogenous production [90]. H_2S-derivatives, S-diclofenac, S-naproxen, S-aspirin possess a higher anti-inflammatory action and lower gastrotoxicity than their prototypes [86, 91]. (H_2S+NO)-derivatives of aspirin and H_2S-derivatives of other pharmacological means – L-DOPA (ACS_{83-86}), sildenafil (ACS6) and mesalamin (ATB-429) were made later [68].

Pharmaceutical means may influence H_2S content in animals' organs in different ways. For example, paracetamol decreased H_2S concentration in brain, but increased its concentration in the heart, liver and kidneys of mice [92]. Amplodipin caused a decrease of H_2S level in the brain and liver of mice

in a dose of 3 and 10 mg/kg, but caused its increase in the heart and kidneys in a dose of 3 mg/kg and its decrease in a dose of 10 mg/kg [56]. It has been demonstrated as well that aspirin decreased H_2S level in the brain and increased its level in the liver of mice [93]. Carvedilol (in a dose of 10 mg/kg) caused an increase of H_2S content in the heart, kidneys and brain of mice [94]. The same effect was caused by digoxin [54], atorvastatin [92], ramipril [95], and metformin [96].

Medicines' influence on H_2S content in tissues, and their interaction with H_2S donors and inhibitors of H_2S-synthesising enzymes are not known well. It is proved that atorvastatin increases H_2S content in perivascular adipose tissue at the expence of inhibition of its mitochondria utilization, but does not influence the CSE activity. Atovastatin disrupts endogenous production of cofactor of sulfide quinone oxidoreductase – ubiquinone that significantly decreases its content in the blood plasma and tissues of animals [97]. Other drugs may be non-specific activators or inhibitors of H_2S-synthesizing enzymes. We have shown that a single injection of cysplatine (7 mg/kg) causes a significant decrease of CSE, CBS and CAT activity in the rat kidneys, that is associated with a decrease of H_2S level and increase of homocysteine level in the blood plasma. The injection of DL-propargylglycine (50 mg/kg) potentiated nephrotoxic effect of cysplatine, while NaHS injection (3 mg/kg), on the contrary, decreased cysplatine-induced nephropathia, increased glutathione content and glutamate-cysteine ligase in the rat kidneys [98].

It is not improbable that inorganic and organic donors of H_2S may significantly impact biotransformation of medicines. NaHS peroral administration (5 mg/kg) caused a decrease of CYP2C9 activity, increase of CYP3A4 activity and had no influence on CYP2B6, CYP2D6 and CYP2C19 activity in rats [99].

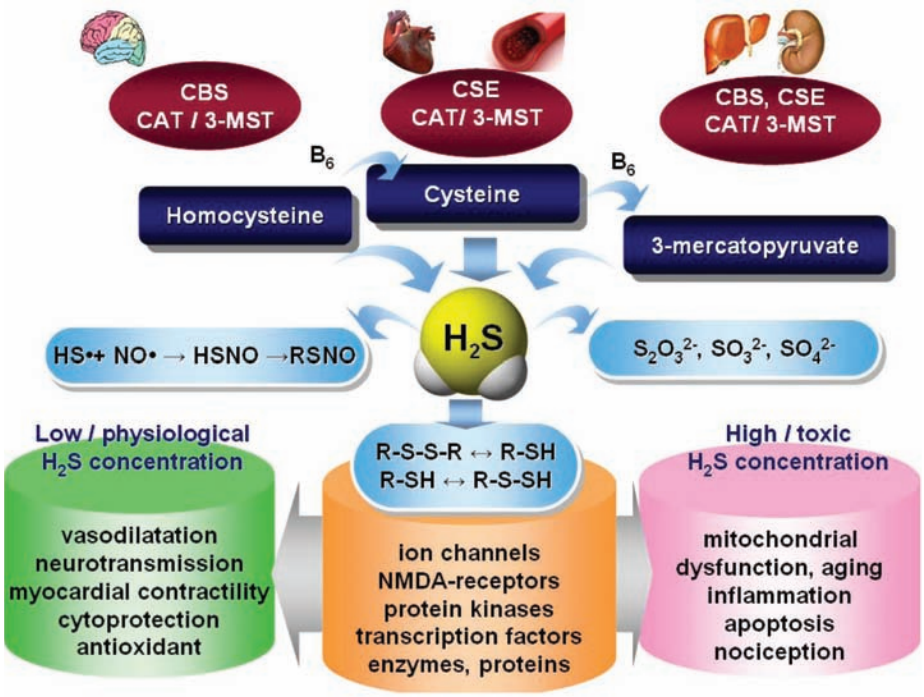
Search for safe and effective H_2S modulators is continued. In our opinion, the drugs containing activators and cofactors of H_2S-synthesizing enzymes, vitamin-microelements complexes, are perspective. We have showed in some studies that under long-term hyperhomocystenemia in rats H_2S content in the blood plasma and H_2S-synthesizing enzymes activity (CSE, CBS, CAT, TST) in the liver, kidneys, aorta is decreased. Administration of vitamin-microelement complex (VMC), which contains vitamins B_6, B_9, B_{12} and coordinating compositions of zinc (Zn^{2+}) and chrome (Cr^{3+}) with N-2,3-dimethylphenylanthranilic acid and ammonium vanadate (V^{5+}), effectively increased endogenous H_2S production from cysteine, homocysteine, thiosulfate in rats' organs. The H_2S production was also increased under the condition of combination of cysteine, homocysteine, thiosulfate in rats' organs with hyperhomocysteinemia and also under its combination with nitric oxide synthase inhibitor L-NAME [57]. A complex of essential microelements (iron, copper, zinc, cobalt, manganese, chrome) and oxygen-containing salts of ultramicroelements (vanadium, molybdenum and selenium) – esmin (Esmin, PC "Kyiv Vitamin Factory") in a dose of 35 mg/kg decreased age-associated reduction of H_2S production in the myocardium, aorta and kidneys of rats [100]. The above-mentioned microelements are necessary for the broad range of biochemical processes; in particular, they are cofactors or activators of antioxidant enzymes and enzymes of sulfur-containing amino acids metabolism, involved in cardiovascular homeostasis and tissue respiration.

Thus a great body of scientific information about H_2S role in biology and medicine (Scheme) was accumulated within the last ten years. H_2S metabolism modulation has assumed great significance in determining mechanisms of formation of different pathological conditions, in development of new approaches to their preventive maintenance and correction, in increasing effectiveness and safety of pharmacotherapy and in development of many other directions, which amount increases. In our opinion, there is a necessity for systematization of H_2S metabolism modulators and creation of their working classification. We propose to divide modulators of H_2S metabolism, which are used in biology and medicine, into groups and subgroups in respect of their impact on endogenous H_2S in tissues and mechanism of their action (Table 8):

1) means that increase H_2S content in tissues (with regard to action mechanism, they are divided into H_2S donors; H_2S-synthesising substrates of enzymes and their derivatives; H_2S-releasing drugs; remedies containing cofactors and activators of H_2S-synthesizing enzymes; drugs inhibiting H_2S utilization);

2) means that decrease H_2S content in tissues (with regard to action mechanism, they are divided into specific and non-specific inhibitors of H_2S-synthesizing enzymes);

3) means with indeterminate mechanism of impact on H_2S metabolism. This group contains phar-



Scheme. Main directions of metabolism and biological effects of H_2S

Table 8. Classification of H_2S metabolism modulators, which are used in biology and medicine

Groups		Representatives
1. Agents that increase H_2S content in tissues		
1.1	H_2S donors	Inorganic – NaHS, Na_2S; organic – Lawesson's reagent
1.2	Substrates of H_2S-synthesizing enzymes and theirs derivatives	L-cysteine, D-cysteine, N-acetylcysteine, S-allyl-L-cysteine, S-propargyl-L-cysteine, S-propyl-L-cysteine, sodium thiosulfate
1.3	Agents with H_2S releasing effect (H_2S-releasing drugs)	H_2S-derivatives of non-steroidal anti-inflammatory drugs (S-aspirin, S-diclofenac, S-naproxen), L-DOPA (ACS83-86), sildenafil (ACS6)
1.4	Agents that contain cofactors and activators of H_2S-synthesizing enzymes	Vitamin-microelement and polymicroelement complexes (VMC and esmin)
1.5	Agents that inhibit H_2S utilization	atorvastatin
2. Agents that decrease H_2S in tissues		
2.1	Specific inhibitors of H_2S-synthesizing enzymes	DL-propargylglycine, β-cyanoalanine, β-aminooxyacetate, hydroxylamine
2.2	Nonspecific inhibitors of H_2S-synthesizing enzymes	non steroidal anti-inflammatory drugs (diclofenac, ketoprofen, indomethacin, aspirin), cisplatin
3. Agents with indefinite mechanism of action on H_2S metabolism		
3.1	Increase H_2S in tissues	carvedilol, digoxin, ramipril, paracetamol (in liver, kidneys), metformin, amlodipin
3.2	Decrease H_2S in tissues	paracetamol (in brain), amlodipin

macology means, influence of which on H_2S metabolism necessitates further study.

It is obvious that the proposed classification of H_2S will change with the extension of the range of its representatives, with determination and clarification of their action mechanisms. There are many unsolved problems concerning molecular targets, ways of H_2S-signaling realizations, regulation mechanisms of H_2S synthesis and degradation under the effect of different endogenous and exogenous factors. Solution of these problems opens new prospects in development of medical biochemistry and pharmacology.

ГІДРОГЕНСУЛЬФІД: МЕТАБОЛІЗМ, БІОЛОГІЧНЕ ТА МЕДИЧНЕ ЗНАЧЕННЯ

Н. В. Заїчко, А. В. Мельник, М. М. Йолтухівський, О. С. Ольховський, І. В. Паламарчук

Вінницький національний медичний університет ім. М. І. Пирогова, Україна;
e-mail: nzaichko@mail.ru

Гідрогенсульфід (H_2S) є сигнальною молекулою, яка активно синтезується в тканинах і бере участь у регуляції судинного тонусу, нейромодуляції, цитопротекції, в запаленні, апоптозі. В останні роки накопичились нові дані про метаболізм та функції H_2S в організмі тварин та людини в умовах дії різних ендогенних та екзогенних чинників, у тому числі і лікарських засобів. У представленому огляді узагальнено інформацію про основні та альтернативні шляхи метаболізму H_2S та їх регуляцію, особливості його транспортування, сигналінгу, біологічну роль, участь в розвитку патологічних станів. Наведено дані щодо вмісту H_2S та активності H_2S-синтезуючих ензимів у різних органах, щодо впливу H_2S на процеси зсідання крові та агрегації тромбоцитів з урахуванням результатів власних досліджень. Запропоновано робочу класифікацію модуляторів обміну H_2S, які використовуються в біології та медицині: 1) засоби, що підвищують вміст H_2S у тканинах (неорганічні та органічні донори H_2S; субстрати H_2S-синтезуючих ензимів та їх деривати; засоби з ефектом вивільнення H_2S; засоби, що містять кофактори та активатори H_2S-синтезуючих ензимів; засоби, які інгібують утилізацію H_2S); 2) засоби, що знижують вміст H_2S у тканинах (специфічні та неспецифічні інгібітори H_2S-синтезуючих ензимів); 3) засоби з невизначеним механізмом впливу на обмін H_2S (окремі фармакологічні засоби). Показано, що перспективними засобами для корекції вмісту H_2S у тканинах є вітамінно-мікроелементні та мікроелементні комплекси, які містять кофактори та активатори H_2S-синтезуючих ензимів.

Ключові слова: гідрогенсульфід, H_2S-сигналінг, ензими, регуляція, модулятори обміну H_2S, комплекси вітамінів та мікроелементів.

ГИДРОГЕНСУЛЬФИД: МЕТАБОЛИЗМ, БИОЛОГИЧЕСКОЕ И МЕДИЦИНСКОЕ ЗНАЧЕНИЕ

Н. В. Заичко, А. В. Мельник, Н. М. Йолтуховский, А. С. Ольховский, И. В. Паламарчук

Винницкий национальный медицинский университет им. Н. И. Пирогова, Украина;
e-mail:nzaichko@mail.ru

Гидрогенсульфид (H_2S) является сигнальной молекулой, которая активно синтезируется в тканях и участвует в регуляции сосудистого тонуса, нейромодуляции, цитопротекции, воспалении, апоптозе. В последние годы накопились новые данные о метаболизме и функции H_2S в организме животных и человека под влиянием различных эндогенных и экзогенных факторов, в том числе и лекарственных средств. В представленном обзоре обобщена информация об основных и альтернативных путях метаболизма H_2S и их регуляции, особенности его транспорта, сигналинга, биологической роли, участие в развитии патологических состояний. Приведены данные о содержании H_2S и активности H_2S-синтезирующих энзимов в различных органах, о влиянии H_2S на процессы свертывания крови и агрегации тромбоцитов с учетом результатов собственных исследований. Предложена рабочая классификация модуляторов обмена H_2S, которые используются в биологии и медицине: 1) средства, повышающие содержание H_2S в тканях (неорганические и органические доноры H_2S; субстраты H_2S-синтезирующих энзимов и их дериваты, средства с эффектом высвобождения H_2S; средства, содержащие кофакторы и активаторы H_2S-синтезирующих энзимов; сред-

ства, ингибирующие утилизацию H_2S), 2) средства, снижающие содержание H_2S в тканях (специфические и неспецифические ингибиторы H_2S-синтезирующих энзимов), 3) средства с неопределенным механизмом влияния на обмен H_2S (отдельные фармакологические средства). Показано, что перспективными средствами для коррекции содержания H_2S в тканях является витаминно-микроэлементные и микроэлементные комплексы, содержащие кофакторы и активаторы H_2S-синтезирующих энзимов.

Ключевые слова: гидрогенсульфид, H_2S-сигналинг, энзимы, регуляция, модуляторы обмена H_2S, комплексы витаминов и микроэлементов.

References

1. Abe K., Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci.* 1996;16(3):1066-1071.
2. Wang R. Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter? *FASEB J.* 2002;16:1792-1798.
3. Lowicka E., Beltowski J. Hydrogen sulfide (H_2S) – the third gas of interest for pharmacologists. *Pharmacol. Rep.* 2007;59:4-24.
4. Miasoedova O. A., Korzhov V. I. Role of hydrogen sulfide in the realization of organism's physiological functions. *J. NAMS Ukraine.* 2011;17(3):191-200. (In Russian).
5. Gadalla M. M., Snyder S. H. Hydrogen sulfide as a gasotransmitter. *J. Neurochem.* 2010;113:14-26.
6. Kimura H. Hydrogen sulfide: its production and functions. *Exp. Physiol.* 2011;96(9):833-835.
7. Wang J. F., Li Y., Song J. N., Pang H. G. Role of hydrogen sulfide in secondary neuronal injury. *Neurochem. Int.* 2014;64:37-47.
8. Cuevasanta E., Denicola A., Alvarez B., Moller M. N. Solubility and Permeation of Hydrogen Sulfide in Lipid Membranes. *PLoS ONE*. 2012;7(4): e34562. doi:10.1371/journal.pone.0034562.
9. Jennings M. L. Transport of hydrogen sulfide and hydrosulfide anion across the human red blood cell membrane. Rapid H_2S diffusion and AE1-mediated Cl-/HS- exchange. *Am. J. Physiol. Cell Physiol.* 2013;305(9):941-950.
10. Mathai J. C., Missner A., Kugler P., Saparov S. M., Zeidel M. L., Lee J. K., and Pohl P. No facilitator required for membrane transport of hydrogen sulfide. *Proc. Natl. Acad. Sci. USA.* 2009;106(39):16633-16638.
11. Shibuya N., Mikami Y., Kimura Y., Nagahara N., Kimura H. Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide. *J. Biochem.* 2009;146(5):623-626.
12. Shibuya N., Kimura H. Production of hydrogen sulfide from d-cysteine and its therapeutic potential. *Front Endocrinol. (Lausanne).* 2013;4:87. doi: 10.3389/fendo.2013.00087.
13. Singh S., Padovani D., Leslie T. Chiku R. A., Banerjee R. Relative contributions of cystathionine beta-synthase and gamma-cystathionase to H_2S biogenesis via alternative trans-sulfuration reactions. *J. Biol. Chem.* 2009;284(33):22457-22466.
14. Chiku T., Padovani D., Zhu W., Singh S., Vitvitsky V., Banerjee R. H_2S biogenesis by human cystathionine gamma-lyase leads to the novel sulfur metabolites lanthionine and homolanthionine and is responsive to the grade of hyperhomocysteinemia. *J. Biol. Chem.* 2009;284(17):11601-11612.
15. Kimura H. Physiological role of hydrogen sulfide and polysulfide in the central nervous system. Neurochem. Int. 2013;63(5):492-497.
16. Yadav P. K., Yamada K., Chiku T., Koutmos M., Banerjee R. Structure and kinetic analysis of H_2S production by human mercaptopyruvate sulfurtransferase. *J. Biol. Chem.* 2013;288(27):20002-20013.
17. Kimura H., Shibuya N., Kimura Y. Hydrogen sulfide is a signaling molecule and a cytoprotectant. *Antioxid. Redox Signal.* 2012;17(1):45-57.
18. Tanizawa K. Production of H_2S by 3-mercaptopyruvate sulphurtransferase. *J. Biochem.* 2011;149(4):357-359.
19. Zaichko N. V. Homocysteine, cysteine, and hydrogen sulfide blood level in patients with thrombophylia: relationship with methylenetetrahydrofolate reductase C677T polymorphism. *Exp. Clin. Physiol. Biochem. J.* 2010;(4):35-41. (In Ukrainian).
20. Yang F., Tan H.-V., Wang H. Hyperhomocysteinemia and atherosclerosis.*Acta Physiol. Sin.* 2005;57(2):103-114.
21. Nakanishi T., Hasuike Y., Otaki Y., Hama Y., Nanami M., Miyagawa K, Moriguchi R, Nishikage H., Izumi M., Takamitsu Y. Free

cysteine is increased in plasma from hemodialysis patients. *Kidney Int.* 2003;63:1137-1140.
22. Zaichko N. V., Pentiuk N. A., Melnik A. V., Shtatko E. I., Andrushko I. I. Production of hydrogen sulfide in organs of rats. *Med. Chem.* 2009;11(4):7-13. (In Ukrainian).
23. Stein A., Bailey Sh. M. Redox biology of hydrogen sulfide: Implications for physiology, pathophysiology, and pharmacology. *Redox Biology.* 2013;(1):32-39.
24. Jackson M. R., Melideo S. L., Jorns M. S. Human sulfide:quinone oxidoreductase catalyzes the first step in hydrogen sulfide metabolism and produces a sulfane sulfur metabolite. *Biochemistry.* 2012;51(34):6804-6815.
25. Zhao H., Chan S-J., Ng Y-K., Wong PT-H. Brain 3-mercaptopyruvate sulfurtransferase (3MST): cellular localization and downregulation after acute stroke. *PLoS ONE.* 2013;8(6):e67322. doi:10.1371/journal.pone.0067322.
26. Guo W., Kan J. T., Cheng Z. Y., Chen J. F., Shen Y. Q., Xu J., Wu D., Zhu Y. Z. Hydrogen sulfide as an endogenous modulator in mitochondria and mitochondria dysfunction. *Oxid. Med. Cell Longev.* 2012;2012:878052 doi: 10.1155/2012/878052.
27. Filipovic M. R., Miljkovic J. Lj., Nauser T., Royzen M., Klos K., Shubina T., Koppenol W. H., Lippard S. J., Ivanović-Burmazović I. Chemical characterization of the smallest S-nitrosothiol, HSNO; cellular cross-talk of H_2S and S-nitrosothiols. *J. Am. Chem. Soc.* 2012;134(29):12016-12027.
28. Kolluru G. K., Shen X., Kevil C. G. A tale of two gases: NO and H_2S, foes or friends for life? *Redox Biol.* 2013;23(1):313-318.
29. Stipanuk M. H., Beck P. W. Characterization of the enzymic capacity for cysteine desulphhydration in liver and kidney of the rat. *Biochem. J.* 1982;206(2):267-277.
30. Bao L., Vlcek C., Paces V., Kraus J. P. Identification and tissue distribution of human cystathionine beta-synthase mRNA isoforms. *Arch. Biochem. Biophys.* 1998;350(1):95-103.
31. Levonen A. L., Lapatto R., Saksela M., Raivio K. O. Human cystathionine gamma-lyase: developmental and in vitro expression of two isoforms. *Biochem. J.* 2000;347(1.):291-295.
32. Yamamoto J., Sato W., Kosugi T., Yamamoto T., Kimura T., Taniguchi S., Kojima H., Maruyama S., Imai E., Matsuo S., Yuzawa Y., Niki I. Distribution of hydrogen sulfide (H_2S)-producing enzymes and the roles of the H_2S donor sodium hydrosulfide in diabetic nephropathy. *Clin. Exp. Nephrol.* 2013;17(1):32-40.
33. Fan Q. L., Yang G., Liu X. D., Ma J. F., Feng J. M., Jiang Y., Wang L. N. Effect of losartan on the glomerular protein expression profile of type 2 diabetic KKAy mice. *J. Nephrol.* 2013;26(3):517-526.
34. Guo W., Cheng Z. Y., Zhu Y. Z. Hydrogen sulfide and translational medicine. *Acta Pharmacol. Sin.* 2013;34(10):1284-1291.
35. Zhang M., Shan H., Wang T., Liu W., Wang Y., Wang L., Zhang L., Chang P., Dong W., Chen X., Tao L. Dynamic change of hydrogen sulfide after traumatic brain injury and its effect in mice. *Neurochem. Res.* 2013;38(4):714-725.
36. Diwakar L., Ravindranath V. Inhibition of cystathionine-gamma-lyase leads to loss of glutathione and aggravation of mitochondrial dysfunction mediated by excitatory amino acid in the CNS. *Neurochem. Int.* 2007;50(2):418-426.
37. Wang Q., Liu H.R., Mu Q., Rose P., Zhu Y.Z. S-propargyl-cysteine protects both adult rat hearts and neonatal cardiomyocytes from ischemia/hypoxia injury: the contribution of the hydrogen sulfide-mediated pathway. *J. Cardiovasc. Pharmacol.* 2009;54(2):139-146.
38. Nagahara N., Ito T., Kitamura H., Nishino T. Tissue and subcellular distribution of mercaptopyruvate sulfurtransferase in the rat: confocal laser fluorescence and immunoelectron microscopic studies combined with biochemical analysis. *Histochem. Cell Biol.* 1998;110(3):243-250.
39. Chen Y., Zhao J., Du J., Xu G., Tang C., Geng B. Hydrogen sulfide regulates cardiac sarcoplasmic reticulum $Ca^{(2+)}$ uptake via K(ATP) channel and PI3K/Akt pathway. *Life Sci.* 2012;91(7-8):271-278.
40. Du J. T., Li W., Yang J. Y., Tang C. S., Li Q., Jin H. F. Hydrogen sulfide is endogenously generated in rat skeletal muscle and exerts a protective effect against oxidative stress. *Chin. Med. J. (Engl).* 2013;126(5):930-936.
41. You X. J., Xu C., Lu J. Q., Zhu X. Y., Gao L., Cui X. R., Li Y., Gu H., Ni X. Expression of cystathionine β-synthase and cystathionine γ-lyase in human pregnant myometrium and their roles in the control of uterine contractility. *PLoS One.* 2011;6(8):e23788. doi:10.1371/journal.pone.0023788.

42. Patel P., Vatish M., Heptinstall J., Wang R., Carson R. J. The endogenous production of hydrogen sulphide in intrauterine tissues. *Reprod. Biol. Endocrinol.* 2009;7:10 doi:10.1186 /1477-7827-7-10.
43. Martin G. R., McKnight G. W., Dicay M. S., Coffin C. S., Ferraz J. G., Wallace J. L. Hydrogen sulphide synthesis in the rat and mouse gastrointestinal tract. *Dig. Liver Dis.* 2010;42(2):103-109.
44. Taniguchi S., Niki I. Significance of hydrogen sulfide production in the pancreatic β-cell. *J. Pharmacol. Sci.* 2011;116(1):1-5.
45. Okamoto M., Yamaoka M., Kimura T. Hydrogen sulfide and its effect on pancreatic beta-cells. *Nihon Rinsho.* 2013;71(1):175-80.
46. Feng X., Chen Y., Zhao J., Tang C., Jiang Z., Geng B. Hydrogen sulfide from adipose tissue is a novel insulin resistance regulator. *Biochem. Biophys. Res. Commun.* 2009;380(1):153-159.
47. Chen Y. H., Wu R., Geng B., Qi Y. F., Wang P. P., Yao W. Z., Tang C. S. Endogenous hydrogen sulfide reduces airway inflammation and remodeling in a rat model of asthma. *Cytokine.* 2009;45(2):117-123.
48. Fang L. P., Lin Q., Tang C. S., Liu X. M. Hydrogen sulfide attenuates epithelial-mesenchymal transition of human alveolar epithelial cells. *Pharmacol. Res.* 2010;61(4):298-305.
49. Akagi R. Purification and characterization of cysteine aminotransferase from rat liver cytosol. *Acta Med. Okayama.* 1982;36(3):187-197.
50. Taoka S., Banerjee R. Characterization of NO binding to human cystathionine beta-synthase: possible implications of the effects of CO and NO binding to the human enzyme. *J. Inorg. Biochem.* 2001;87(4):245-251.
51. Agrawal N., Banerjee R. Human polycomb 2 protein is a SUMO E3 ligase and alleviates substrate-induced inhibition of cystathionine beta-synthase sumoylation. *PLoS One.* 2008;3(12):e4032. doi: 10.1371/journal. pone.0004032.
52. Yang G., Wu L., Jiang B., Yang W., Qi J., Cao K., Meng Q., Mustafa A. K., Mu W., Zhang S., Snyder S. H., Wang R. H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science.* – 2008;5901(322):587-590.
53. Zaichko N. V., Pentiuk N. O., Pentiuk L. O., Melnik A. V., Shtatko E. I., Andrushko I. I.. Determination of hydrogen sulfide in blood serum. *J. Res.* 2009;(1):29-32. (In Ukrainian).
54. Wiliński B., Wiliński J., Somogyi E., Piotrowska J., Góralska M., Macura B. Carvedilol induces endogenous hydrogen sulfide tissue concentration changes in various mouse organs. *Folia Biol. (Krakow).* 2011;59(3-4):151-155.
55. Furne J., Saeed A., Levitt M. D. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2008;295(5):1479-1485.
56. Wiliński B., Wiliński J., Somogyi E., Piotrowska J., Góralska M. Amlodipine affects endogenous hydrogen sulfide tissue concentrations in different mouse organs. *Folia Med. Cracov.* 2011;51(1-4):29-35.
57. Melnik A. V. The activity of transsulfuration and methylation enzymes in rats kidney under prolonged administration of homocysteine thiolactone, its combination with L-NAME and correction of violations by the vitamin and microelement complex. *Biomed. Biosocial. Anthropol.* 2010;(14):58-62. (In Ukrainian).
58. Olhovskiy A. S., Zaichko N. V., Melnik A. V. Age-related differences in production of hydrogen sulfide in heart and aorta of rats. *Urgent Probl. Mod. Med.* 2011;11(4):133-137. (In Ukrainian).
59. Mustafa A. K., Gadalla M. M., Sen N., Kim S., Mu W., Gazi S. K., Barrow R. K., Yang G., Wang R., Snyder S. H. H_2S signals through protein S-sulfhydration. *Sci. Signal.* 2009;2(96):72. doi: 10.1126/scisignal.2000464.
60. Wang X. B., Jin H. F., Tang C. S., Du J. B. The biological effect of endogenous sulfur dioxide in the cardiovascular system. *Eur. J. Pharmacol.* 2011;670(1):1-6.
61. Du S. X., Jin H. F., Bu D. F., Zhao X., Geng B., Tang C. S., Du J. B. Endogenously generated sulfur dioxide and its vasorelaxant effect in rats. *Acta Pharmacol. Sin.* 2008;29(8):923-930.
62. Schreier S.M., Muellner M.K., Steinkellner H., Hermann M., Esterbauer H., Exner M., Gmeiner B.M., Kapiotis S., Laggner H. Hydrogen sulfide scavenges the cytotoxic lipid oxidation product 4-HNE. *Neurotox. Res.* 2010;17(3):249-256.
63. Tang G., Wu L., Wang R. Interaction of hydrogen sulfide with ion channels. *Clin. Exp. Pharmacol. Physiol.* 2010;37(7):753-763.
64. Kawabata A., Ishiki T., Nagasawa K., Yoshida S., Maeda Y., Takahashi T., Sekiguchi F., Wada T.,

Ichida S., Nishikawa H. Hydrogen sulfide as a novel nociceptive messenger. *Pain.* 2007;132(1-2):74-81.
65. Yang H. Y., Wu Z. Y., Wood M., Whiteman M., Bian J. S. Hydrogen Sulfide Attenuates Opioid Dependence by Suppression of Adenylate Cyclase/cAMP Pathway. *Antioxid. Redox Signal.* 2014;20(1):31-41.
66. Lu M., Liu Y. H., Ho C. Y., Tiong C. X., Bian J. S. Hydrogen sulfide regulates cAMP homeostasis and renin degranulation in As4.1 and rat renin-rich kidney cells. *Am. J. Physiol. Cell Physiol.* 2012;302(1):59-66.
67. Módis K., Panopoulos P., Coletta C., Papapetropoulos A., Szabo C. Hydrogen sulfide-mediated stimulation of mitochondrial electron transport involves inhibition of the mitochondrial phosphodiesterase 2A, elevation of cAMP and activation of protein kinase A. *Biochem. Pharmacol.* 2013. 86(9):1311-1319.
68. Caliendo G., Cirino G., Santagada V., Wallace J. L. Synthesis and biological effects of hydrogen sulfide (H_2S): development of H_2S-releasing drugs as pharmaceuticals. *J. Med. Chem.* 2010;53(17):6275-6286.
69. Pan T. T., Neo K. L., Hu L. F., Yong Q. C., Bian J. S. H_2S preconditioning-induced PKC activation regulates intracellular calcium handling in rat cardiomyocytes. *Am. J. Physiol. Cell Physiol.* 2008;294(1):169-177.
70. Zhang Q., Fu H., Zhang H., Xu F., Zou Z., Liu M., Wang Q., Miao M., Shi X. Hydrogen sulfide preconditioning protects rat liver against ischemia/reperfusion injury by activating Akt-GSK-3β signaling and inhibiting mitochondrial permeability transition. *PLoS One.* 2013;8(9):e74422. doi:10.1371/journal.pone.0074422.
71. Xu W., Wu W., Chen J., Guo R., Lin J., Liao X., Feng J. Exogenous hydrogen sulfide protects H9c2 cardiac cells against high glucose-induced injury by inhibiting the activities of the p38 MAPK and ERK1/2 pathways. *Int. J. Mol. Med.* 2013;32(4):917-925. doi: 10.3892/ijmm.2013.1462.
72. Koike S., Ogasawara Y., Shibuya N., Kimura H., Ishii K. Polysulfide exerts a protective effect against cytotoxicity caused by t-buthylhydroperoxide through Nrf2 signaling in neuroblastoma cells. *FEBS Lett.* 2013;587(21):3548-3555.
73. Gao C., Xu D. Q., Gao C.J., Ding Q., Yao L.N., Li Z. C., Chai W. An exogenous hydrogen sulphide donor, NaHS, inhibits the nuclear factor κB inhibitor kinase/nuclear factor κb inhibitor/nuclear factor-κB signaling pathway and exerts cardioprotective effects in a rat hemorrhagic shock model. *Biol. Pharm. Bull.* 2012;35(7):1029-1034.
74. Guo R., Wu K., Chen J., Mo L., Hua X., Zheng D., Chen P., Chen G., Xu W., Feng J. Exogenous Hydrogen Sulfide Protects against Doxorubicin-Induced Inflammation and Cytotoxicity by Inhibiting p38MAPK/NFκB Pathway in H9c2 Cardiac Cells. *Cell Physiol. Biochem.* 2013;32(6):1668-1680.
75. Hua W., Chen Q., Gong F., Xie C., Zhou S., Gao L. Cardioprotection of H_2S by downregulating iNOS and upregulating HO-1 expression in mice with CVB3-induced myocarditis. *Life Sci.* 2013;93(24):949-954.
76. Majid A. S., Majid A. M., Yin Z. Q., Ji D. Slow regulated release of H_2S inhibits oxidative stress induced cell death by influencing certain key signaling molecules. *Neurochem. Res.* 2013;38(7):1375-1393.
77. Zhang Y., Tang Z. H., Ren Z., Qu S. L., Liu M. H., Liu L. S., Jiang Z. S. Hydrogen sulfide, the next potent preventive and therapeutic agent in aging and age-associated diseases. *Mol. Cell Biol.* 2013;33(6):1104-1113.
78. Huang X., Meng X. M., Liu D. H., Wu Y. S., Guo X., Lu H. L., Zhuang X.Y., Kim Y. C., Xu W. X. Different regulatory effects of hydrogen sulfide and nitric oxide on gastric motility in mice. *Eur. J. Pharmacol.* 2013;720(1-3):276-285.
79. Módis K., Coletta C., Erdélyi K., Papapetropoulos A., Szabo C. Intramitochondrial hydrogen sulfide production by 3-mercaptopyruvate sulfurtransferase maintains mitochondrial electron flow and supports cellular bioenergetics. *FASEB J.* 2013;27(2):601-611.
80. Nicholson C. K., Calvert J. W. Hydrogen sulfide and ischemia-reperfusion injury. *Pharmacol. Res.* 2010;62(4):289-297.
81. Mishra P. K., Tyagi N., Sen U., Givvimani S., Tyagi S. C. H_2S ameliorates oxidative and proteolytic stresses and protects the heart against adverse remodeling in chronic heart failure. *Am. J. Physiol. Heart Circ. Physiol.* 2010;298(2):451-456.
82. Warenycia M. W., Goodwin L. R., Benishin C. G., Reiffenstein R. J., Francom D. M., Taylor J. D.,

Dieken F. P. Acute hydrogen sulfide poisoning. Demonstration of selective uptake of sulfide by the brainstem by measurement of brain sulfide levels. *Biochem. Pharmacol.* 1989;38(6):973-981.

83. Voloshchuk N. I., Taran I. V. Acute toxicity of hydrogen sulfide and its influence on the antiinflammatory effect of diclophenac in experiment. *Med. Chem.* 2011;13(4):88-90. (In Ukrainian).
84. Nishikawa H., Hayashi H., Kubo S., Tsubota-Matsunami M., Sekiguchi F., Kawabata A. Inhibition by hydrogen sulfide of rabbit platelet aggregation and calcium mobilization. *Biol. Pharm. Bull.* 2013;36(8):1278-1282.
85. Gu X., Zhu Y. Z. Therapeutic applications of organosulfur compounds as novel hydrogen sulfide donors and/or mediators. *Expert Rev. Clin. Pharmacol.* 2011;4(1):123-133.
86. Wallace J. L., Dicay M., McKnight W., Martin G. R. Hydrogen sulfide enhances ulcer healing in rats. *FASEB J.* 2007;21:4070-4076.
87. Sen U., Vacek T. P., Hughes W. M., Kumar M., Moshal K. S., Tyagi N., Metreveli N., Hayden M. R., Tyagi S. C. Cardioprotective role of sodium thiosulfate on chronic heart failure by modulating endogenous H_2S generation. *Pharmacology.* 2008;82(3):201-213.
88. Zaichko N. V., Platonova T. N., Chernishenko T. M., Grinenko T. V., Yusova E. I. Influence of hydrogen sulfide on hemostasis in rats. *Med. Perspect.* 2010;15(1):15-20. (In Ukrainian).
89. Francescato H. D., Chierice J. R., Marin E. C., Cunha F. Q., Costa R. S., Silva C. G., Coimbra T. M. Effect of endogenous hydrogen sulfide inhibition on structural and functional renal disturbances induced by gentamicin. *Braz. J. Med. Biol. Res.* 2012;45(3): 244-249.
90. Fiorucci S., Distrutti E., Cirino G., Wallace J. L. The emerging roles of hydrogen sulfide in the gastrointestinal tract and liver. *Gastroenterology.* 2006;131(1):259-271.
91. Wallace J. L., Caliendo G., Santagada V., Cirino G. Markedly reduced toxicity of a hydrogen sulphide-releasing derivative of naproxen (ATB-346). *Br. J. Pharmacol.* 2010;159(6):1236-1246.
92. Wiliński B., Wiliński J., Somogyi E., Piotrowska J., Góralska M. Atorvastatin affects the tissue concentration of hydrogen sulfide in mouse kidneys and other organs. *Pharmacol. Rep.* 2011;63(1):184-188.
93. Bilska A., Iciek M., Kwiecień I., Kaniecki K., Paliborek M., Somogyi E., Piotrowska J., Wiliński B., Góralska M., Srebro Z., Włodek L. Effects of aspirin on the levels of hydrogen sulfide and sulfane sulfur in mouse tissues. *Pharmacol. Rep.* 2010;62(2):304-310.
94. Wiliński B., Wiliński J., Somogyi E., Piotrowska J., Góralska M. Digoxin increases hydrogen sulfide concentrations in brain, heart and kidney tissues in mice. *Pharmacol. Rep.* 2011;63(5):1243-1247.
95. Wiliński J., Somogyi E., Góralska M., Wiliński B., Czarnecka D. Ramipril enhances the endogenous hydrogen sulfide tissue concentration in mouse heart and brain. *Folia Med. Cracov.* 2008;49(3-4):123-130.
96. Wiliński B., Wiliński J., Somogyi E., Piotrowska J., Opoka W. Metformin raises hydrogen sulfide tissue concentrations in various mouse organs. *Pharmacol. Rep.* 2013;65(3):737-742.
97. Bełtowski J., Jamroz-Wiśniewska A. Modulation of H(2)S metabolism by statins: a new aspect of cardiovascular pharmacology. *Antioxid. Redox Signal.* 2012;17(1):81-94.
98. Yoltukhivskyy M. M. Effect of DL-Propargylglycine and sodium hydrosulfide on cisplatin-induced changes of renal functions in rats. *J. VNMU.* 2012;16(2):257-262. (In Ukrainian).
99. Wang X., Han A., Wen C., Chen M., Chen X., Yang X., Ma J., Lin G. The Effects of H_2S on the activities of CYP2B6, CYP2D6, CYP3A4, CYP2C19 and CYP2C9 *in vivo* in rat. *Int. J. Mol. Sci.* 2013;14(12):24055–24063.
100. Zaichko N. V., Olhovskiy A. S., Yurchenko P. A. Microelements complex esmin protects the age-related changes of hydrogen sulfide tissues formation in rats. *Materials of the 7th Lviv-Lublin Conference of Experimental and Clinic Biochemistry.* Lviv, Ukraine, 2013. P. 191.
101. Yang G., Pei Y., Teng H., Cao Q., Wang R. Specificity protein-1 as a critical regulator of human cystathionine gamma-lyase in smooth muscle cells. *J. Biol. Chem.* 2011;286(30):26450-26460.
102. Strutyns'ka N. A., Semenykhina O. M., Chorna S. V., Vavilova H. L., Sahach V. F. Hydrogen sulfide inhibits $Ca^{(2+)}$-induced mitochondrial permeability transition pore opening in adult and old rat heart. *Fiziol. Zhurn.* 2011;57(6):3-14. (In Ukrainian).

103. Semenykhina O. M., Strutyns'ka N. A., Bud'ko A. Iu., Vavilova H. L., Sahach V. F. Effect of hydrogen sulfide donor NaHs on the functional state of the respiratory chain of the rat heart mitochondria. *Fiziol. Zhurn.* 2013;59(2):9-17. (In Ukrainian).
104. Shimanskaia T. V., Strutinskaia N. A., Vavilova G. L., Goshovskaia Iu. V., Semenikhina E. N., Sagach V. F. Cyclosporin A-sensitive mitochondrial pore as a target of cardioprotective action of hydrogen sulfide donor. *Ross. Fiziol. Zhurn. im. I. M. Sechenova.* 2013;99(2):261-272. (In Russian).
105. Wang Q., Wang X. L., Liu H. R., Rose P., Zhu Y. Z. Protective effects of cysteine analogues on acute myocardial ischemia: novel modulators of endogenous H(2)S production. *Antioxid. Redox Signal.* 2010;12(10):1155-1165.

Received 15.03.2014