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NEUROTOXIC AND NEUROPROTECTIVE EFFECTS OF HOMOCYSTEINE AND HYDROGEN SULFIDE

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Abstract

Homocysteine is a sulfhydryl-containing amino acid derived from methionine. The cellular concentration of homocysteine is regulated by two key pathways: remethylation back to methionine or transsulfuration to cysteine with simultaneous production of hydrogen sulfide (H_2S). Homocysteine levels increase in different conditions, including genetic factors, diet, life style or miscellaneous medication. Elevated levels of the homocysteine, called hyperhomocysteinemia (hHcy), are associated with a higher risk of neurovascular diseases, dementia, developmental impairments or epilepsy. Oxidative stress is one of the common mechanisms of homocysteine-induced disorders. H_2S as an established gasotransmitter implicated in the regulation of numerous physiological functions is also well-known for its neuroprotective potential. The recent data indicate that the level of H_2S decreases in hHcy conditions, which may mediate homocysteine-induced neurotoxicity. This review summarizes the available data on homocysteine and H_2S metabolism and mechanisms of H_2S mediating neuroprotection and can be helpful in searching for ways to prevent homocysteine-induced neurotoxicity.

Keywords: homocysteine, hyperhomocysteinemia, cystathionine beta synthase, hydrogen sulfide, oxidative stress, glutamate receptors, Ca^{2+}-activated K^+-channels, neurodegeneration, cognitive dysfunctions

Introduction

Homocysteine is a sulfur-containing amino acid derived from the metabolism of methionine. Enhanced homocysteine concentrations underlie cellular disturbances in different systems [1]. An increase of the total plasma homocysteine level of more than 15 μM in humans has been defined as hyperhomocysteinemia (hHcy). hHcy is a risk factor of cardiovascular diseases, cognitive impairments, increased risk of Alzheimer disease, vascular dementia, and cerebrovascular stroke [2, 3]. hHcy during pregnancy is frequently associated with health complications, such as pregnancy-induced hypertension, placental abruption, thromboembolic events, neural-tube defects, and intrauterine growth restrictions. Infants born from mothers with hHcy suffer from mental and physical retardation [1]. Elevated levels of homocysteine alter hippocampal plasticity and synaptic transmission resulting in learning and memory deficits [4]. Hydrogen sulfide (H_2S) is one of the homocysteine metabolites produced by cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE), enzymes of the transulfuration pathway of methionine metabolism [5]. In addition to its role as an important neuromodulator

[6–8], H_2S acts as a neuroprotector against oxidative stress and apoptosis in several pathophysiological conditions [5].

A protective role of H_2S in homocysteine-induced oxidative stress was shown in brain endothelial cells, memory deficit, neurodegeneration, neuro-inflammation, and cerebrovascular remodeling [9, 10]. A recent study demonstrated that H_2S ameliorates homocysteine-induced cerebrovascular diseases, blood-brain barrier, and synaptic disruption [11]. Perturbation of H_2S generation was suggested to contribute in homocysteine-induced neurotoxicity [12, 13]. The results indicate that H_2S is able to provide protection against neurodegeneration and cognitive dysfunction in homocysteine-exposed rats. In this review, we discuss the connection of homocysteine with H_2S and possible neuroprotective effects of H_2S in homocysteine-induced neurotoxicity.

Metabolism of Homocysteine

Homocysteine is a non-essential, nonproteinogenic amino acid derived from S-demethylation of L-methionine [14]. The concentration of homocysteine is regulated by two key processes: remethylation back to methionine or irreversible transsulfuration with cysteine and H_2S production [2]. Remethylation is catalyzed by methionine synthase using 5-methyl tetrahydrofolate (5-MTHF) or betaine (in liver and kidney) as methyl donors and requires folic acid as a cofactor. 5-MTHF is synthesized by 5,10-methylenetetrahydrofolate reductase (5,10-MTHFR). Transsulfuration depends on vitamin B6 and converts homocysteine to cystathionine and, subsequently, to cysteine used in glutathione synthesis. CBS, the first enzyme of the transsulfuration pathway, catalyzes the condensation of Hcy with serine to form cystathionine and H_2S. CSE then converts cystathionine to cysteine, which is used in the synthesis of glutathione [1, 15]. An increased level of total plasma homocysteine (up to more than 15 μM) in humans is associated with hHcy. According to the total plasma homocysteine level, it is classified as mild (15–25 μM), moderate (25–50 μM), and severe (50–500 μM) hHcy [16]. A high tissue level of homocysteine depends on several factors: age, genetics, lifestyle, smoking, alcohol abuse, renal dysfunction, and intake of miscellaneous medications. The plasma levels of homocysteine are higher in men than in women and increase from 10.8 μM at the age of 40–42 years up to 12.4 μM between the age of 65 and 67 years [17]. hHcy may be induced by an increase of methionine in the diet, vitamin deficiency (folate, B12, or B6), mutations in the genes encoding MTHFR, thereby limiting the cell's methylating capacity, or by CBS activity [18].

Increased homocysteine levels are associated with certain disorders: cardio- and cerebrovascular diseases, as well as neurodegenerative pathologies, such as epilepsy, stroke, Alzheimer disease, Parkinson disease, and dementia [2, 18]. Migraine with aura is a chronic disabling neurovascular condition, which is suggested to be triggered by high homocysteine levels [20]. Elevated homocysteine levels contribute to pathophysiology of psychiatric disorders, including schizophrenia and affective disorders [21]. hHcy is associated with common pregnancy complications, such as preeclampsia, placental abruption, intrauterine growth retardation, and neural-tube defects [22–24]. In our laboratory, we investigated the physical development, reflex ontogeny, locomotion and exploratory activity, muscle strength and motor coordination of rat pups with maternal hHcy during four weeks of postnatal development. Our results indicate a significant decrease in the litter size and body weight of pups born from

dams fed with methionine-rich food. The main features of the physical maturation were not different from the control group; however, the formation of sensory-motor reflexes in the pups of the hHcy group was delayed. Also locomotor and exploratory activity, muscle strength and coordination were decreased in rats from the hHcy group [24, 25].

Mechanisms of Homocysteine-Induced Neurotoxicity

Homocysteine exerts toxic effects on the nervous system by several mechanisms underlying neurodegeneration, including oxidative stress, DNA damage, protein thiolation and homocysteinylation triggering apoptosis and excitotoxicity [2, 15, 21].

Homocysteine and oxidative stress. Oxidative stress is one of the main mechanisms of homocysteine-induced neurotoxicity. Oxidative stress is a serious imbalance between the production and utilization of reactive species and can result from an increased production of reactive oxygen species (ROS) or/and low levels of antioxidant defense [26]. Homocysteine itself can undergo autooxidation of its free thiol group; it binds to plasma proteins, low-molecular plasma thiols, a second Hcy molecule or interferes with the generation of ROS, such as superoxide, hydrogen peroxide, or hydroxide radicals [27, 28]. Indirect oxidative effects of hHcy may include generation of superoxide from xanthine oxidase or uncoupled endothelial nitric oxide synthase, downregulation of antioxidant enzymes or depletion of intracellular glutathione [29]. Generation of superoxide during hHcy may result also from the activation of NADPH oxidases [2, 30]. An increased level of ROS can cause oxidation of various functionally important proteins, lipids, and nucleic acids [31]. An augmentation of the H_2O_2 level and lipid peroxidation was shown in brain tissues during severe hHcy [32, 33]. Intracerebroventricular injections of homocysteine in rats induced lipid peroxidation and increased the level of ROS followed by neurovascular dysfunctions, blood-brain barrier disruption, and decreased expression of synaptic proteins in the hippocampus [10, 11].

Endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), are in the first line of cellular defense against oxidative injury, decomposing of O^{2-} and H_2O_2 to prevent reactive hydroxyl radical (HO^-) formation. The elevated levels of homocysteine induced impairment of endogenous antioxidant enzymes depending on the tissue type or the treatment with homocysteine. In the models of rat hHcy, SOD deficiency and elevated lipid peroxidation were found in the brain samples [2, 32, 34]. Catalase activity was reduced [35] or increased in the hHcy group [2]. The decreased expression and activity of GPx-1 induced by homocysteine was shown by the in vitro and in vivo studies [28, 36]. Rats fed on a methionine-rich diet exhibit a lower activity of glutathione peroxidase [22]. In our laboratory experiments with biochemical assays, we found an increased level of malondialdehyde, the end product of lipid peroxidation, and decreased activity of the antioxidant enzymes – superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the brains of rats from the hHcy group [25].

Role of homocysteine and glutamate receptors in synaptic plasticity. Homocysteine is an endogenous agonist of glutamate N-methyl-D-aspartate (NMDA) receptors whose activation induces Ca^{2+} flux in the cells [37–39]. Excessive activation of the NMDA receptor by the high concentrations of homocysteine led to rapid and sustained phosphorylation of the MAP kinase and transient phosphorylation of the cAMP

response element-binding protein (CREB), which resulted in oxidative stress and neuronal death [40]. Homocysteine effects on NMDA receptors depend on the subunit composition and concentration of glycine, because homocysteine acts as a partial antagonist at the glycine-binding site [37]. Moreover, depending on the GluN2 subunit compositions, homocysteine can differently modulate the peak amplitudes of NMDA mediated currents and reduce NMDA receptor desensitization [38]. In fact, homocysteine induced an increase of the peak amplitude of GluN2A-containing NMDA receptors and a decrease of GluN2B-mediated currents [38]. Therefore, homocysteine is a high-affinity agonist of NMDA receptors at the GluN1/2A subunits. At the same time, homocysteine caused desensitization of GluN2B-containing the NMDA receptors which limited homocysteine neurotoxicity [39]. The regular injections of homocysteine during four weeks to adult rats or acute application of homocysteine to the brain slices induced bidirectional changes of the basic synaptic transmission and long-term potential (LTP) of hippocampal CA1 pyramidal cells [4, 41]. Homocysteine at low concentrations caused LTP impairment at < 100 μM, which is similar to the NMDA effects, but there was LTP enhancement at ≥ 500 μM [4].

Homocysteine can also activate the group I metabotropic glutamate receptors [42], which indirectly increases intracellular Ca^{2+} levels and activates several kinases in the hippocampus [43]. A high level of homocysteine caused neuronal death through activation of the NMDA receptors and mGluR5 [44]. Therefore, an increased level of homocysteine enhances excitatory glutamatergic neurotransmission in different brain areas, whereby neuronal damage derives from excessive Ca^{2+} influx and generation of reactive oxygen.

Homocysteine and neuronal excitability. Chronic hHcy increases the excitability of nervous circuits and may provoke seizures [45]. The clinical studies reported that antiepileptic drugs induced elevation of plasma homocysteine levels [46]. In addition, the systemic administration of homocysteine metabolites induced age-dependent convulsing action in rats; however, its effects were different from the effects of kainic acid (KA) and NMDA, indicating additional complicating effects of homocysteine on the nervous tissue [47]. Anticonvulsant effects against homocysteine-induced seizures could be treated by a combination of low subthreshold doses of NMDA and non-NMDA receptor antagonists [48]. Homocysteine elicits an increase of the neuronal excitability, which may contribute to neurological impairment and seizures associated with hHcy [49, 50]. The analysis of intrinsic electrophysiological properties of pyramidal neurons in the hippocampal slices of rats with prenatal hHcy revealed a decrease of the threshold for generation of action potentials and reduced frequency adaptation during repetitive firing [50]. We observed an increase of neuronal firing and network activity in the hippocampal slices of neonatal rats with prenatal hHcy [50]. At the same time, a decrease of the frequency of giant depolarizing potentials – a typical network activity in the hippocampus of neonatal rats – was found in hHcy animals, thereby suggesting impairments of the neuronal synchronization in the CA3 hippocampal network. One of the possible explanations is reduction of the GluN2B-current amplitude and fast desensitization of GluN2B-containing receptors during the chronic exposure of homocysteine to the immature hippocampus [51].

In the peripheral synapse of the motor neuron, it was shown that homocysteine largely increased the inhibitory effect of oxidative stress on the transmitter release,

via the activation of NMDA receptors. This combined effect of homocysteine and local oxidative stress can specifically contribute to the alteration of presynaptic terminals in neurodegenerative motor neuron diseases, including amyotrophic lateral sclerosis [52].

Homocysteine and Ca^{2+}-activated K^+-channels. Ca^{2+}-activated K^+ (BK)-channels were shown to be a target of homocysteine action. It was shown that homocysteine induces inhibitory effects on the BK currents in smooth muscle cells by either direct interaction [53] or indirectly via generation of intracellular superoxide anions [30]. Recently, we demonstrated with the use of GH3 cells of the rat hypophysis that high homocysteine concentrations observed during the hHcy conditions are able to activate oxidized BK-channels by acting from the internal side of the cell membrane and decrease exocytosis of the secretory granules containing growth hormone [54]. These effects may underlie the adverse impacts of homocysteine on prenatal development [55]. Furthermore, multiple functional disorders associated with the elevated levels of homocysteine, including neurodegenerative diseases, such as Alzheimer disease or amyotrophic lateral sclerosis, may be related to the growth hormone level [1, 56]. Notably, the growth hormone level was significantly decreased in the experiments on mice with hHcy, whereas growth hormone had a protective role on hHcy-induced glomerular injury with the increased levels of ROS [57].

Endogenous Production of H_2S in the Brain and Its Cellular Targets

Hydrogen sulfide (H_2S), a gaseous molecule characterized by a strong rotten egg smell, has long been known as a toxic gas. Recently, H_2S has been recognized as the third gasotransmitter along with nitric oxide (NO) and carbon monoxide (CO). Gasotransmitters have been found to regulate numerous physiological and pathological processes, including inflammation, blood pressure, neuroprotection and tumorigenesis [5]. H_2S, amongst its many other functions, exerts neuro-protectant effects by preventing oxidative stress [58] and by increasing glutathione levels [59]. Furthermore, H_2S participates in the pathophysiology of central nervous system diseases, such as epilepsy, stroke, neurodegenerative diseases, and hHcy [5]. The neurotoxic or neuroprotective action of H_2S is critically dependent on its concentration and cellular location [60].

Enzymatic production of H_2S. As an endogenous signaling molecule, the production of H_2S is tightly and precisely controlled in cells. CBS and CSE, enzymes involved in the transsulfuration pathway, were initially thought to be the primary pathways for H_2S biosynthesis. Both enzymes are pyridoxal 5'-phosphate (PLP) dependent and generate H_2S via β-elimination reactions from cysteine [61]. In the neonatal brain, CBS is mainly localized to astrocytes and the basal H_2S level in unstimulated human astrocytes is 3.0 mol/g protein, which is 7.9-fold higher than in cultured microglia [5]. Additionally, in the adult brain, CBS is highly expressed in Purkinje neurons and hippocampal neurons [62]. CBS and CSE have equal capacity for H_2S production in the liver, while CBS is a major contributor to H_2S production in the brain and kidney [5]. H_2S is metabolized by sulfur dioxygenase, which is present in higher levels in the liver and kidney than in the brain. Even small deviations in the rates of H_2S production and clearance may lead to rapid and dramatic changes in the levels of H_2S, providing an essential feature for a signaling molecule [63]. Cysteine aminotransferase (CAT), another PLP dependent enzyme, is able to convert L-cysteine

into 3-mercaptopyruvate (3MP), which serves as a substrate of 3-mercaptopyruvate sulfurtransferase (3MST) for the production of H_2S mostly in mitochondria [5].

Mechanisms of H_2S Action

H_2S effects in the central nervous system. Role of NMDA receptors. In the central nervous system, H_2S induces LTP in the hippocampus [6]; it modulates neuronal excitability of the subfornical organ, the nucleus of the solitary tract [64], and trigeminal neurons [65], as well as mediates central inhibition of the respiratory rhythm [66]. Interestingly, H_2S effects in the central nervous system are dependent on the brain region and the age of experimental animals. The expression of CBS in the nervous system during the embryonic period is generally low and increases in the late embryonic to the early postnatal period [67]. The significance of this developmental action is unknown. A possible role of CBS may comprise a reduction of the homocysteine level, which in high concentrations contributes to pathologies of the nervous system during prenatal development [68]. An increase of the H_2S signal in the limbic system in vivo can improve the fear memory in aged rats by H_2S-dependent regulation of the activity of GluN2B-containing NMDA receptors in the amygdala [69]. A protective role of H_2S was shown in rat models of recurrent febrile seizure, which is the most common seizure type in children, often causing hippocampal damage [70]. Our experiments support this notion by the finding of a complete depression of bicuculline-induced interictal-like activities in the neonatal rat hippocampus by NaHS, which can be explained by network desynchronization due to neuronal depolarization [7]. In the hippocampal slices of neonatal rats, we found that H_2S induces a biphasic effect on spontaneous network and neuronal spiking activity. The initial increase of activity was due to a transient depolarization mediated partially by a reduction of outward potassium currents. The second inhibitory phase was mediated by a right shift in the activation curve of Na^+ current, thus decreasing neuronal excitability and preventing network activity. Furthermore, NaHS decreases NMDA-mediated currents in neonatal rats without affecting AMPA and GABA responses [7].

Recently, it has also been shown that H_2S upregulates γ-aminobutyric acid B receptor (GABA(B)), a G protein-coupled receptor located at pre- and post-synaptic sites [70]. At pre-synaptic sites, GABA(B) receptors regulate the release of neurotransmitters, such as GABA and glutamate, by inhibiting voltage sensitive Ca^{2+}-channels. Upregulation of GABA(B) receptor expression by H_2S implies that H_2S may participate in maintaining the excitation/inhibition balance in the brain [71].

NMDA receptors are the targets of H_2S in the brain. H_2S alone does not induce any apparent currents, but significantly increases the NMDA-induced inward current [5]. It was suggested that H_2S enhanced the induction of LTP by activating NMDA receptors [6]. In our laboratory, we observed that NMDA receptor-mediated currents were modulated by H_2S in the opposite direction, dependent of the animal age and subunit specificity. Specifically, we demonstrated that NaHS decreased NMDA responses in the neonatal slices, whereas a significant increase of NMDA-evoked currents was found in the older animals (2–3 weeks old) [7]. The expression of NMDA receptors composed of GluN1/2A or GluN1/2B subunits in HEK293T cells revealed that NaHS induced the potentiation of GluN1/2A receptor-mediated currents and the inhibition of GluN1/2B receptor-mediated currents [51]. The activating effect of H_2S was previously

shown on GluN1/2A NMDA receptors expressed in *Xenopus* oocytes, where NaHS decreased the onset time of NMDA responses [72]. In both cortex and hippocampus, the GluN2B subunit dominated in early development and slowly declined during maturation. In contrast, the GluN2A subunit was increasingly expressed during development and becomes dominant in adult neurons [73]. This developmental shift could explain the age-dependent effects of H_2S on NMDA evoked currents.

H_2S and neuromuscular transmission. H_2S effects were studied in peripheral neuromuscular junctions. We demonstrated using the frog and mice neuromuscular junction that H_2S enhanced both spontaneous and evoked neurotransmitter release without changing focally recorded presynaptic responses. We also found that the substrate of H_2S synthesis, L-cysteine, cause an increase of evoked transmitter release, whereas the inhibitors of CSE and CBS induced the opposite action [8, 74]. The analysis of the intracellular mechanisms of H_2S action suggested a role of cAMP and ryanodine receptors (RyR) in the effects of H_2S [8, 74–77]. Indeed, NaHS increases the transmitter release after inhibition of adenylate cyclase, indicating that H_2S does not affect the enzyme activity. However, the effects of NaHS decreased after the application of cAMP analogues [75]. It seems that the initial application of cAMP induced phosphorylation of intracellular proteins, which are involved in H_2S action, such as Ca^{2+}-channels in the presynaptic plasma membrane or the endoplasmic reticulum membrane [78]. This hypothesis is supported by data showing that H_2S increases the intracellular Ca^{2+} concentration in glial cells [79] and in cultured neurons [80]. H_2S also exerts stimulating effects on the release of tachykinines in capsaicin-sensitive sensory nerve endings by activation of TRPV-1 receptors which are non-selective cation channels permeable to Ca^{2+} [81]. It is known that H_2S modifies protein molecules, including ion channels and receptors, by its reducing action on disulfide bonds or by S-sulfhydration of cysteine residues [82]. These modifications can change the proteins of the SNARE complex responsible for the process of exo- and endocytosis of synaptic vesicles [83]. In fact, it was shown by using the endocytosis marker FM 1–43 that H_2S accelerated synaptic vesicle cycling in motor nerve endings by increasing exocytosis and fast endocytosis of synaptic vesicles during high-frequency stimulation [84, 85].

The modulatory effect of H_2S increases the safety factor of neuromuscular transmission and may have a protective effect on the nerve terminals known to be inhibited by reactive oxygen species [86] induced during various pathological conditions, including amyotrophic lateral sclerosis (ALS), and aging [31, 58].

H_2S and Ca^{2+}-activated K^+ channels. H_2S can change neuronal excitability through modulation of Na^+ channels [87] and different types of K^+-channels [88–92], Cl^--channels [12], and Ca^{2+}-channels [93]. In rat pituitary tumor cells (GH3), H_2S increased the activity of big conductance BK channels by its reducing action on the channel protein [90]. H_2S effect on BK channels in different cell lines – GH3, GH4 and GH4 STREX was determined by their phosphorylation state and phosphorylation by protein kinase G primes the channels for activation by NaHS [89]. H_2S also induced a dose-dependent hyperpolarization and truncation of spontaneous action potentials in rat pituitary GH3 cells as a result of the activation of K_{ATP} channels. This results in a decrease of the growth hormone release as indicated by the fluorescence experiments with the use of FM 1–43 that showed the inhibition of basal and evoked exocytosis of secretory granules after H_2S incubation [91].

H_2S and nociception. Increasing evidence suggests that H_2S plays a role in the emergence and conductance of somatic and visceral pain [94]. Intracolonical administration of NaHS, a H_2S donor, induced nociceptive behavior with abdominal hyperalgesia/allodynia [95]. NaHS produced mechanical hyperalgesia in the rat hind paw in response to the intraplantar administration [96]. On the other hand, NaHS activates K_{ATP} channels in different tissues [97], which may underlie the antinociceptive effects of NaHS [98]. Although several reports have shown that H_2S activates sensory neurons, the molecular targets of H_2S action in trigeminal nociception, implicated in migraine, remain controversial. It was revealed that NaHS, similarly to capsaicin, induced the release of calcitonin gene-related peptide and substance P from the sensory nerves in the airways of guinea pigs, which causes in vivo bronchoconstriction [81]. A number of in vitro and in vivo experiments indicate that H_2S can activate TRPV1 or TRPA1 receptors [99].

Indirect evidence shows that H_2S induced mechanical hyperalgesia and allodynia requires activation of TRPA1 channels in mice [94], whereas visceral pro-nociceptive effects of H_2S are independent of TRPA1 [100]. In our laboratory investigations, we observed that H_2S induces an increase of the firing activity in rat trigeminal nerve and this effect is mediated by the activation of both TRPV1 and TRPA1 receptors. In the soma of trigeminal neurons, NaHS causes a dual effect on capsaicin-evoked currents. In one fraction, NaHS induces an initial increase of the current amplitude with a subsequent decrease. In another fraction, NaHS induces a constant decrease of TRPV1 currents. Moreover, H_2S locally applied to trigeminal neurons elicits inward currents which were inhibited by TRPV1 antagonist capsazepine, but not sensitive to the inhibitor of TRPA1 – HC 030031. Furthermore, NaHS induces Ca^{2+} transients in trigeminal ganglion neurons which were prevented by inhibitors of TRPV1 and TRPA1 receptors [101].

Chemical protein modification by H_2S. There are three main routes by which H_2S exerts its biological effects: metal center interactions reactive oxygen species (ROS)/reactive nitrogen species scavenging or/and S-persulfidation [102]. Although the first two routes have significance, S-persulfidation is accepted as the key process by which H_2S acts as signaling route. S-persulfidation (more commonly but less accurately called S-sulfhydration) is the process in which a thiol (R–SH) is converted into a perthiol (R–SSH, also called persulfide). S-persulfidation modulates the biological activity of proteins due to a decrease in the acid dissociation constant and an increase in nucleophylity of perthiols with respect to thiols. For example, S-persulfidation of K_{ATP} channels contributes to H_2S-induced vasodilation [103].

Some of the H_2S signaling mechanisms are mediated by the reduction of disulfide bonds at channel proteins [90,104]. In our experiments, we observed that H_2S alters the response of NMDA channels in the hippocampal slices of rats to glutamate agonists by disrupting disulfide bonds in the GluN1 subunits and dithiothreitol (DTT), as well as other sulfhydryl-reducing agents which diminish the effects of H_2S donors [51].

H_2S may easily react with other compounds, especially with reactive oxygen and nitrogen species (ROS and RNS). It has been demonstrated that H_2S reacts with at least four different ROS, superoxide radical anion, hydrogen peroxide, peroxynitrite, and hypochlorite [105]. Additional mechanisms through which H_2S may exert antioxidant effects involve stimulation of cysteine transport into cells and enhancement

of glutathione synthesis [106]. H_2S has been shown to stimulate heme oxygenase expression and CO production and to exert bidirectional effects on extracellular signal-regulated kinases (ERK) and inducible NO synthase [105].

Another mechanism involved in the beneficial action of H_2S could be linked to sulfhydration of parkin at different cysteine residuals which are critical to its physiological function [107]. It is thought that alterations of synaptic monoamine levels produce secondary neuroplastic changes. H_2S has been found to stimulate glutathione biosynthesis that alleviates oxidative stress [108].

Homocysteine versus Hydrogen Sulfide

Homocysteine and H_2S are the metabolites of methionine [61]. However, they exert opposite effects on the viability of neuronal cells. A protective role of H_2S was shown in homocysteine induced oxidative stress in brain endothelial cells, in neurodegeneration, and neuroinflammation [10]. Homocysteine initiates ROS production and stimulates excitotoxicity [109], while H_2S scavenges ROS and prevents oxidative stress-induced neuronal death [58, 110]. It has been reported that H_2S serves as a protective gaseous signaling molecule by preventing endoplasmic reticulum stress [5].

Homocysteine, as a risk factor for Alzheimer's disease, induces cognitive dysfunction. A recent study demonstrated that H_2S ameliorated homocysteine-induced Alzheimer disease-like cerebrovascular pathology. Intracerebral homocysteine injections in mice induced a disruption of the blood brain barrier and caused synaptic dysfunction, revealed by altered expression of important proteins regulating blood brain barrier permeability, adhesion molecules, pre-synapse and post-synapse markers and brain derived nerve factors. The impaired memory functions and increased expression of the NMDA receptor (NR1 subunit) and synaptosomal Ca^{2+} were shown in the homocysteine-injected brain of mice along with the decreased expression of CBS and CSE [10, 11].

Reactive aldehydes, the products of lipid peroxidation, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), accumulate in the brain tissues during neurodegenerative diseases, including Alzheimer disease and Parkinson disease [111, 112]. Homocysteine exposure induced an elevation of MDA and 4-HNE and decreased the activity and expression of aldehyde-dehydrogenase (ALDH2), a key enzyme involved in detoxifying aldehydes that metabolizes acetaldehyde to reactive aldehydes [113]. Treatment with H_2S ameliorated the cognitive dysfunction, diminished the reactive aldehyde level, upregulated the glutathione level, as well as ALDH2 activity and expression in the homocysteine-exposed rats [114]. Skeletal muscle malfunction was observed in hHcy conditions and a protective role of H_2S was shown during metabolic syndromes [115]. As we found shown earlier, the H_2S donor – NaHS increased the synaptic transmission in the mammalian neuromuscular junction, whereas the inhibition of CBS induced a decrease of the evoked transmitter release, which may also impact the muscle function [8]. Thus, the deficit of H_2S production may be one plausible reason of muscle weakness observed during the hHcy conditions.

Altered H_2S signaling was suggested to contribute to homocysteine-induced neurotoxicity [12, 13]. Indeed, intra-cerebroventricular administration of homocysteine decreased CBS expression and endogenous H_2S generation in the hippocampus of rats along with learning and memory dysfunctions [116]. In hHcy conditions, the H_2S

content appeared to be diminished as was shown in the recent studies [13]. In the liver, homocysteine at higher levels has been reported to inhibit the CSE activity and to suppress the H_2S level in the plasma [117]. The results suggest that H_2S is effective in providing protection against neurodegeneration and cognitive dysfunction in homocysteine-exposed rats. Current therapies for hHcy are limited to vitamin supplements, which serve as cofactors in the pathways of homocysteine metabolism. These therapies lower the level of homocysteine, but generally do not alter disease consequences [118]. Therefore, elucidation of the mechanisms of neuroprotective effects of H_2S may develop a new approach aimed to prevent homocysteine deleterious effects.

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Нейропротекторные и нейротоксические эффекты гомоцистеина и сероводорода

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Аннотация

Гомоцистеин представляет собой тиолсодержащую аминокислоту, синтезируемую из метионина. Концентрация гомоцистеина регулируется двумя основными метаболическим реакциями: реметилирование обратно в метионин или транссульфирование до цистеина с одновременным выделением сероводорода (H_2S). Различные факторы – диета, образ жизни, прием лекарств, гене-

тическая предрасположенность – могут вызывать повышение уровня гомоцистеина. Высокая концентрация гомоцистеина в крови называется гипергомоцистеинемией, которая связана с риском развития сердечно-сосудистых заболеваний, деменции, нарушений развития и эпилепсии. Окислительный стресс является одним из распространенных механизмов нарушений, вызванных действием гомоцистеина. Недавно было обнаружено, что один из трех газотрансмитеров – H_2S является сигнальной молекулой, регулирует различные физиологические функции и оказывает нейропротекторное действие во многих системах организма. Было показано, что в условиях гипергомоцистенемии наблюдается низкий уровень H_2S. В обзоре рассматриваются современные представленияы о метаболизме гомоцистеина и H_2S, а также механизмы опосредованной H_2S нейропротекции как возможного терапевтического агента для предотвращения нейротоксичности, вызванной гомоцистеином.

Ключевые слова: гомоцистеин, гипергомоцистеинемия, цистеин бета-синтаза, сероводород, оксидативный стресс, глутаматные рецепторы, Ca^{2+}-активируемые K^+-каналы, нейродегенерация, когнитивные нарушения

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