Bachelor thesis

Therapeutic potential of H_2S and neurodegenerative diseases

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1. Abstract

Neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD) are growing in occurrence due to their age-related onset and a global increase in life expectancy. To prevent growing worldwide costs in healthcare, it is important to find therapeutic targets. Despite the differences in pathogenic onset, neurodegenerative diseases share common mechanisms including neuro-inflammation, oxidative stress and neuro-apoptosis. Hydrogen sulfide (H_2S), known as a toxic gas with the smell of rotten eggs, is increasingly proposed as potential therapeutic target that influences these mechanisms in different ways. Abnormal H_2S generation and metabolism were also found in most neurodegenerative disorders. Interestingly, in the central nervous system (CNS) H_2S exerts anti-inflammatory, antioxidant and anti-apoptotic effects which are related to neurodegenerative diseases. The aim of this paper is to present the current understanding of H_2S-induced functions in the brain, and its potential therapeutic value in neurodegenerative diseases. The emphasis will be on AD, PD, amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD). It is concluded that due to its neuroprotective functions, H_2S is a promising target for future research and treatment of neurodegenerative diseases.

Keywords: Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, neurodegeneration, hydrogen sulfide, therapeutic potential

2. Introduction

2.1 Neurodegenerative diseases

Neurodegeneration is a process which shows progressive loss of structure or functions of neurons. It leads to, or characterizes various diseases such as Alzheimer's, Parkinson's, Amyotrophic lateral sclerosis (ALS) and Huntington's, which are therefore classified as neurodegenerative diseases. Despite their different pathogenic onsets and clinical features, common mechanisms have been found between these diseases[4]. Alzheimer's disease (AD) represents the fourth highest source of overall disease burden in the high-income countries, according to WHO statistics (2004)[1]. In the Alzheimer's Disease International (ADI) 2010 report[2], 35.6 million people suffer from AD worldwide. In 2050, this number is estimated to grow to 115.4 million people. WHO also states that especially in low and middle income countries, the healthcare costs of dependent older people will increase dramatically. For Parkinson's disease (PD), the prevalence is about 1% for people over 60, with estimates of up to 4% for people in the highest age groups[3] in industrialized countries. The risk of developing Parkinson's disease rises at a very high rate with age over 60 years.

The world population's life expectancy is increasing. Thus neurodegenerative diseases which are often age dependent will also be more prevalent and associated economic costs will rise at a high rate. To accommodate these increases in healthcare, worldwide policies and plans have to be made for future provisioning and financing. However, it is prioritized that novel treatments become available.

The symptoms of neurodegenerative diseases are well known, however its mechanisms are still not understood. It is clear that there are shared mechanisms between different neurodegenerative diseases, and these mechanisms are expressed on many different levels[4] (Fig.1.). AD is associated with plaques and tangles in the cortex and hippocampus[15], suggesting a protein misfolding or breakdown, followed by protein accumulation. Increasing evidence is showing a possible role of oxidative stress in the onset of AD. For PD pathogenesis, mitochondrial dysfunction, oxidative stress, and protein mishandling are thought to play a crucial role[16]. The common neurodegenerative nature is a burden for patients, but simplifies the understanding of possible mechanisms and

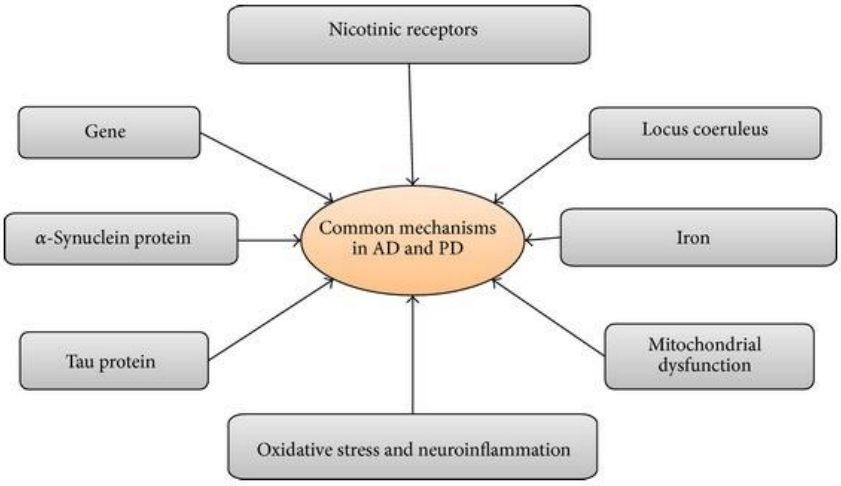

Fig. 1. Possible shared mechanisms in the onset of AD and PD

finding potential therapeutic targets for all neurodegenerative diseases.

2.2 Current treatment

There are some treatments that improve a patient's diagnosis. Patients with AD can be treated with cholinesterase inhibitors in mild to moderate AD, and NMDA receptor antagonists like in moderate to severe AD[5]. There have also been several reports of beneficial effects of vitamin E, possibly resulting in a slower functional decline of AD patients[6]. Despite the beneficial effects of these treatments, they only facilitate a limited benefit on the progression of AD. In other words, there is no cure yet. For Parkinson's and other neurodegenerative diseases, treatment is only available to help relieve the symptoms and maintain quality of life. There are dopaminergic and non-dopaminergic therapeutic strategies, however a better understanding of the mechanisms are essential for ongoing advances[7]. Especially since neurodegeneration probably has multiple causes, which cannot be readily targeted.

2.3 H_2S

H_2S, or hydrogen sulfide, is a colorless gas with the distinctive smell of rotten eggs. It is a poisonous, flammable and explosive compound. Still, H_2S is a physiologically important gaseous mediator. In fact, it is hypothesized to be the third gasotransmitter, following nitric oxide (NO) and carbon monoxide[8], indicating physiological and pathophysiological functions in various body systems. Increasing evidence show neuroprotective effects of H_2S, making it an interesting target of research for neurodegenerative diseases.

2.3.1 History

The discovery of H_2S acting as a NMDA receptor enhancer in the central nervous system (CNS) in 1996 by Abe and Kimura[9] has resulted in a surge of research concerning H_2S and its neurological properties. Today, H_2S has been reported and discussed by neurochemistry and signaling properties[10, 11]. Research is still ongoing, since H_2S has shown promising effects on neurodegenerative diseases, but may also have many other applications. The properties and functions of H_2S have been reviewed earlier, but the assumptive relationship of neurodegenerative diseases and H_2S is scarce or outdated.

2.3.2 Production pathways

H_2S can be produced through different pathways. Often these pathways are related to specific tissues. Kimura, H. has done a lot of research on H_2S and its functions. He has reported H_2S to be produced by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE)

and 3-mercaptopyruvate sulfurtransferase (3MST) along with cysteine aminotransferase (CAT) [12]. Recently, a novel pathway was identified which involves D-amino acid oxidase (DAO) together with 3MST[13]. CBS is expressed in kidney, liver, brain, ileum, uterus, placenta and pancreatic islets. CSE is expressed in kidney, liver, thoracic aorta, ileum, portal vein, uterus, pancreatic islets and placenta. 3MST/CAT on the other hand, is present in all cells' mitochondria and cytosol. To investigate the effects of H_2S on neuronal tissue, CBS and 3MST are therefore often inhibited or overexpressed. However, the regulation of H_2S-producing enzymes is not well understood, and their activation involve many different molecules which are still being identified. Research directing these enzymes will not be easy and H_2S is a gaseous substance, therefore these production pathways are mandatory to facilitate local H_2S release[14].

3. H_2S functions

3.1 Neuroprotection

There is *in vivo* and *in vitro* evidence that H_2S is beneficial in neurodegenerative diseases on three different neuronal levels: anti-inflammation; anti-apoptosis and anti-oxidation. This section is outlining and discussing the possible underlying mechanisms of H_2S and its neuroprotective properties.

3.1.1 Anti-inflammation

The progressive nature of neurodegenerative diseases is commonly ascribed to inflammation. Inflammatory processes have been identified in AD[37], PD[37], HD[38] and ALS[39]. It is a biological response by the immune system to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammatory processes attempt to remove these stimuli, so that a healing process can follow. Since neuro-inflammation, is a key factor in neurodegeneration, it is an interesting therapeutic target in delaying or stopping the progression of neurodegenerative diseases.

Lipopolysaccharide (LPS) induces neuroinflammation, neuronal ultrastructure impairment and cognitive defects. It binds to immune cells such as monocytes, dendritic cells, macrophages and B cells, promoting the secretion of pro-inflammatory cytokines, nitric oxide (NO) and eicosanoids[40]. NaHS treatment has shown to reduce LPS-induced inflammation in both primary cultured microglia and immortalized murine microglial cells[41]. It is hypothesized that H_2S inhibits NO-synthase and p38 MAPK signaling pathways in a concentration-dependent matter. The inhibition of H_2S production by silencing CSE in LPS-activated macrophages has shown an increased production level of NO, also suggesting the crucial role of H_2S in anti-inflammation[45]. However, the levels of pro-inflammatory cytokines were significantly lower after CSE silencing[45]. This result contradicts our current understanding of the mechanism, and should therefore be further examined.

Microglia and astrocytes are immune cells that reside in the brain and spinal cord and form the main active immune defense of the central nervous system. Their Inflammatory activation cause induction of nuclear factor-κB(NF- κB) and a release of inflammatory mediators tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and nitrite ions, as well as a down-regulation of CBS and H_2S[51]. These inflammatory factors induce tissue repair, but also further aggravates tissue injury and cause cell death. This effect was partially reversed in pretreated cells with NaSH, indicating anti-inflammatory effects of H_2S[46]. Again, the exact mechanism has not yet been documented. It may be a direct effect of H_2S on astrocytes and microglia, or an indirect effect by inhibiting the released pro-inflammatory factors.

AMP-activated protein kinase (AMPK) is increasingly recognized as a central factor in inflammation[44]. A recent study in 2014 suggest

the inhibition of neuroinflammation by the activation of AMPK by H_2S[42]. The study supports other earlier findings on the inflammation-inhibiting effects of the activation of AMPK. Also AMPK has earlier been documented as therapeutic intervention for several diseases[43], but the discovery of H_2S induced AMPK activation through the calmodulin-dependent protein kinase kinase β (CaMKKβ) makes H_2S an interesting anti-inflammatory target.

Concluding, H_2S shows promising anti-inflammatory functions, due to its interaction with inflammation related LPS, microglia, astrocytes and AMPK.

3.1.2 Anti-oxidation

When there is an excess of reactive oxygen species (ROS) and/or decrease in antioxidant levels, oxidative stress occurs which is harmful in any tissue. It causes the production of toxic peroxides and free radicals, which in turn damages all components of the cells. Free-radical-mediated oxidative injury in acute stroke or trauma, as well as in chronic diseases like neurodegenerative disorders, has been documented in the CNS.

H_2S can function as a ROS scavenger. Earlier was mentioned that 3MST and CAT are primarily localized in mitochondria, the place where most ROS are produced. Administration of H_2S by sodium hydrosulfide (NaHS), an H_2S donor, was found to significantly inhibit hypochlorous acid (HOCl) and peroxynitrite ($ONOO^-$) induced cytotoxicity, intracellular protein oxidation and lipid peroxidation in human neuroblastoma cells[20, 21]. However, the contribution of the direct antioxidative effects of H_2S to ROS is questionable. A research in 2010 examined the reductive potential of H_2S and found that not only H_2S is a poor reductant, it is also present in relatively low concentrations[22].

Glutathionine (GSH) is an important antioxidant, preventing damage from ROS and free radicals[17]. NaHS administration increases GSH levels and indirectly functions antioxidative[18]. Also after inhibition of H_2S producing enzymes in bacteria, an increase in vulnerability for antibiotics was shown, indicating cytoprotective effects of H_2S[19]. This research claims H_2S to be a universal defense mechanism that functions from bacteria to mammals. However, research on cytoprotective effects of H_2S is limited, and it is different from neuroprotection, making this statement bold but possible.

Homocysteine (Hcy) is a thiol-containing amino acid, and was shown to be toxic to neuronal cells[23]. Moreover, Hcy made neuronal cells significantly more vulnerable to excitotoxic and oxidative injury[24]. Elevated levels of Hcy have been reported in neurodegenerative and neurovascular diseases such as AD[25], making it a risk factor and potential therapeutic target. The antioxidant properties of H_2S could provide protection from Hcy. Recently, a mouse model was used to explore the neuroprotective role of H_2S on Hcy-induced neurodegeneration and neurovascular function[26]. Their findings suggest that treatment with NaHS could reduce redox homeostasis of the brain. Malondialdehyde (MDA), which is a lipid peroxidation product, was measured as an index of oxidative stress. NaHS+Hcy significantly reduced oxidative stress opposed to the Hcy and control group. Also GSH, an important antioxidant as mentioned before, was significantly decreased in the Hcy-group. This decrease was almost normalized in the NaHS+Hcy group (Fig. 2).

To summarize, H_2S has strong antioxidative effects on different levels, either directly through acting as a ROS scavenger, or indirectly through increasing GSH levels or preventing Hcy neurotoxicity. The contributions of different antioxidative actions of H_2S are not clear, however in most literature the direct antioxidative effects are presumably much less important.

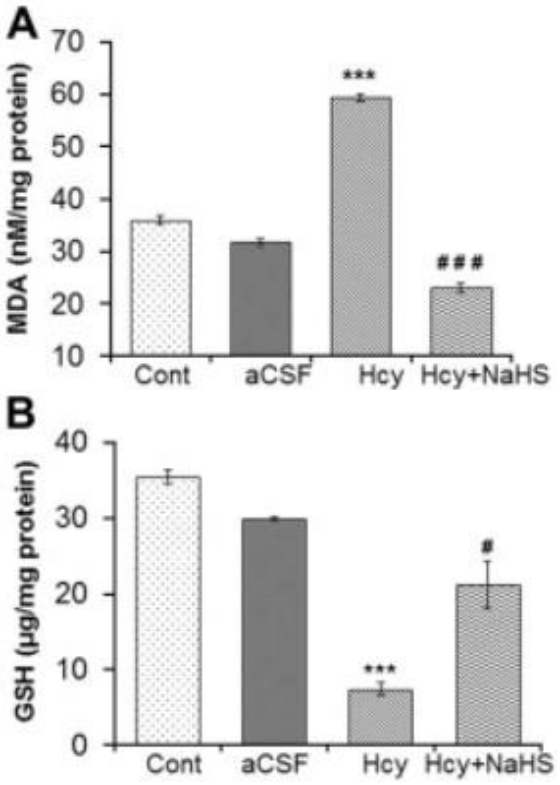

Fig. 2. Effect of NaHS on Hcy-induced alterations in malondialdehyde and intracellular-reduced glutathione (GSH). (A) MDA: [F(3, 16) = 1.23; P < 0.001] and (B) GSH: [F(3, 16) = 2.2; P < 0.005 denotes Hcy significantly increased oxidative stress. A significant protection was observed with NaHS treatment. Data represent mean ± SE from n = 5 per group; ∗∗∗P < 0.0001 vs. control group, #P < 0.05, ###P < 0.0001 vs. to Hcy-treated group[26]

3.1.3 Anti-apoptosis

Proliferative tissues have to maintain a constant size to function properly. Therefore, older cells must die to make way for new cells. A 'programmed' cell death, also called apoptosis, is the phenomenon that allows the cell to die without adversely affecting neighbouring cells. Neurons commonly survive for the entire lifetime of the organism, which is necessary to maintain the function within neuronal circuits. In contrast, neuronal apoptotic activity is a characteristic of neurodegenerative diseases. Fig. 3. shows brain regions in which neurodegenerative conditions are typified by selective apoptosis of neurons[27]. In AD, death of hippocampal and cortical neurons (Fig. 3. a) is observed. In PD it is the death of midbrain neurons that use the neurotransmitter dopamine located in the substantia nigra. Huntington's disease (HD) involves the death of neurons in the striatum (Fig. 3. b), which control body movements. Amyotrophic lateral sclerosis (ALS) shows death of spinal cord motor neurons.

Increasing evidence is being found on an anti-apoptotic effect on neuronal cells by H_2S. NaHS at low concentrations (<300µM) inhibits the apoptosis of PC12 and SH-SY5Y cells and primarily cultured hippocampal neurons which injuries were induced by various common neurodegenerative disease-like agents that resemble protein aggregation, oxidative stress, apoptotic factors and neurotoxicity[28].

Also Homocysteine (Hcy) seems to play a role in neuronal cell death, as well as being a neurotoxic substance. A study in 2014 used a rat model to intracerebroventricularly inject Hcy (0.6µmol/day) for 7 days. They found

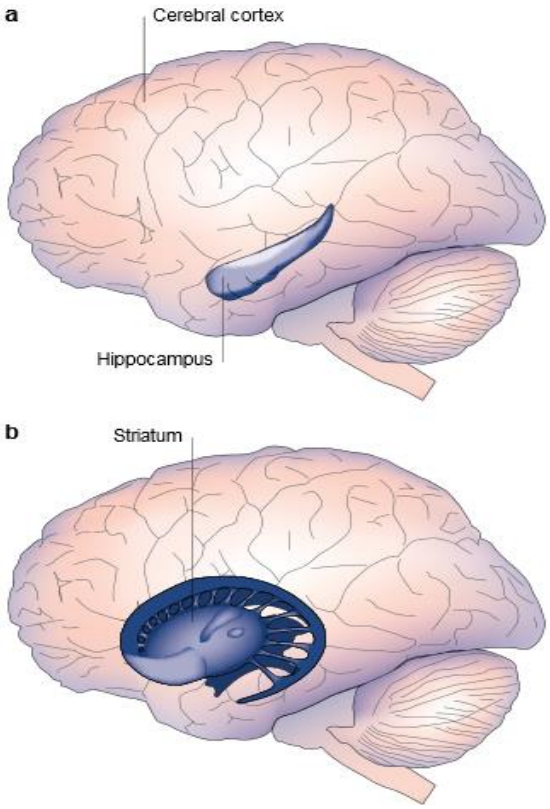

Fig. 3. Brain regions in which neurodegenerative conditions are typified by selective apoptosis of neurons[27]

increased apoptotic neurons in hippocampal coronal slices and an endogenous H_2S inhibition, causing stress on the endoplasmic reticulum (ER). Treatment with NaHS however attenuated the apoptotic effects[32]. The same model showed that intracerebroventricular Hcy injection leads to learning and memory dysfunctions[33]. The researchers suggest that the disturbance of hippocampal endogenous H_2S generation and the increase in ER stress in the hippocampus are related to Hcy-induced defect in learning and memory. The same was found by another research group in 2013, which also focused on neurovascular dysfunction. Here NaHS treatment also attenuated Hcy-induced cerebrovascular remodeling[34]. Other anti-apoptotic effects of H_2S on vascular dementia induced injury in hippocampal neurons were found in 2009[30]. This gives strong evidence for the neuroprotective function of H_2S by inhibiting the neurodegenerative functions of Hcy.

Yet, most literature indicate that the anti-apoptotic effects of H_2S are mainly because of its ability to preserve mitochondrial integrity, thereby suppressing the mitochondrial apoptotic pathway. For example, a study in 2014 showed that a novel mitochondria-targeted H_2S donor 'AP39' exerts antioxidant and cytoprotective effects under oxidatively stressed endothelial cells in vitro[29]. The proposed mechanism is the inhibition of cytochrome C, which once released from mitochondria into the cytosol acts as an apoptogenic factor inducing cell death[35].

The exact mechanism between H_2S and the apoptotic pathway is unclear. Moreover, NaHS has shown to induce apoptosis in cortical neurons instead of inhibiting it, when the dosage was high (200 to 1000µM, much higher than the physiological range of H_2S in the brain) [31]. This indicates either a two-way mechanism of H_2S being anti-apoptotic at low concentrations, and apoptosis-inducing at high concentrations, or it could indicate H_2S being toxic itself in high concentrations (much higher than physiological range) which makes this fact irrelevant as far as therapeutic potential.

4. H_2S and Alzheimer's Disease

Key elements in the onset of AD are the formation of extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles, mainly in the cortex and hippocampus. Aβ plaques are generated by γ- and β-secretases that process the amyloid precursor protein (APP) to Aβ peptides which accumulate. Neurofibrillary tangles are composed of hyperphosphorylated tau protein[48]. To date, multiple genetic mutations have been investigated that lead to familial AD (with an early age onset) [47]. However the vast majority of AD patients are sporadic (with a late age onset), in which the specific causes are still unknown. Neuroinflammation is a decisive event in AD and other neurodegenerative diseases that eventually leads to neurodegeneration. The generation of inflammatory cytokines, complement components, and toxic free radicals are among the many species that are generated. Microglia attack the pathological entities and may inadvertently injure host neurons, all as a result of Aβ accumulation[49]. Neuroinflammation is therefore proposed as the main drive for the progressive nature of AD.

Interestingly, reduced levels of H_2S were found in human brain tissue, as well as one of its activators CBS[50]. Also, the plasma levels of endogenous H_2S and homocysteine in patients with AD were observed as negatively correlated with the severity of the disease[53]. Attenuation of LPS-induced cognitive defects and neuronal ultrastructure impairment by NaHS treatment has been observed in a rat model, by inhibiting LPS-induced secretion of pro-inflammatory cytokines and NF- κB activation[55]. An Aβ accumulation-induced astrocytic and microglial response as well as inflammatory cytokines expressions was decreased by NaHS treatment in another

study[56]. These findings suggest the possible therapeutic potential and biomarker function of H_2S in the neuroinflammatory manifestation of AD.

In addition to the inhibition of inflammation by attenuating pro-inflammatory factors by H_2S, it has also been proposed that elevated levels of these pro-inflammatory cytokines may inhibit phagocytosis of Aβ in AD brains[52]. Thus the removal of Aβ plaques by microglia could be hindered by their own products. Besides beneficial effects on Aβ-accumulation, there is also evidence of reduced tau hyperphosphorylation induced by H_2S. Mitogen-activated protein kinases (MAPK) play an important role in tau hyperphosphorylation, as well as the transcription of several pro-inflammatory cytokines and apoptosis-related genes. An AD mouse model was used to examine the activity of different MAPK members. In comparison to the wild-type control mice, AD mice showed an increased activity of MAPK members JNK, p38 and ERK. Treatment with an H_2S-rich spa-water induced a significant reduction of such increase[54]. In fact, the same study also showed a reduced Aβ deposit and tau protein at critical sites, and diminished morphological damage including a decrease in neuronal death.

Oxidative stress is another prominent factor of AD. CBS and CSE mediate the total serum level of Hcy and GSH through H_2S, previously mentioned as 2 important molecules in oxidative stress. Hcy has even been established as a strong, independent risk factor of AD[61]. However both H_2S-producing enzymes are reported as dysfunctional and at low concentrations in AD patients, leading to a decrease in H_2S production[57]. This results in elevated Hcy levels and reduced antioxidant GSH levels, which causes neurotoxicity and renders neuronal cells vulnerable to excitotoxic and oxidative injury. Subsequently, it leads to inhibition of endogenous H_2S production and down-regulation of expression and activity of CBS, as observed in PC12 cells[58], indicating a progressing vicious cycle effect. Other oxidative stress-inducing molecules such as maldondialdehyde (MDA), carbonyl proteins, 4-hydroxynonenal (HNE), superoxide anions, hydrogen peroxide and hypochlorous acid, which are markedly increased in the brains of patients with severe AD, have been attenuated by NaHS treatment in vitro[59, 60, 63, 64].

Because Hcy also has pro-apoptotic effects, H_2S treatment will most likely protect neuronal cell death in various neuronal types including hippocampal and cortical neurons that occurs in AD[62]. In vitro, NaHS treatment has a protective anti-apoptotic effect on PC12 cells with increased Aβ and Hcy levels[65]. Formaldehyde is another cytotoxic and apoptosis-inducing substance of which accumulated levels were found in AD patients. NaHS also significantly protects cells in vitro against formaldehyde-induced apoptosis[66]. Again, the proposed mechanism in which H_2S inhibits apoptosis is mainly due to the preservation of mitochondrial function.

AMPK could possibly be a potential therapeutic target for AD, since it is activated by H_2S and thereby inhibits neuroinflammation. However, concerning evidence shows that AMPK activation has non-neuroprotective properties including Aβ generation and tau phosphorylation[83]. Therefore, it is still unclear whether AMPK could serve as a potential therapeutic target for AD, and directly compromises the therapeutic potential of H_2S. Further studies will be needed to clarify the role of AMPK in AD.

5. H_2S and Parkinson's Disease

PD is characterized by a progressive loss of dopaminergic neurons in the substantia nigra (SN), a brain structure located in the mesencephalon, which results in a depletion of the neurotransmitter dopamine in the striatum. Increasing evidence indicates that deficits in mitochondrial function, oxidative stress, the accumulation of aberrant or misfolded proteins, and ubiquitin-proteasome

system dysfunction may represent the principal molecular pathways or events that commonly underlie the pathogenesis of PD[67]. Just as in AD, the exact mechanisms of the onset of PD are unclear. Oxidative stress, inflammation and neuronal apoptosis are likely to be important contributors. Especially oxidative stress, which has been demonstrated to be an early sign that often precedes and triggers neuronal death in PD[74].

H_2S treatment has been examined in different PD models, however, not as extensively as in AD. Reduced levels of H_2S were found in a PD mouse model[72], focusing on the SN and striatum. Additionally, an H_2S-induced attenuation of neurodegeneration and movement disorders was observed, which are characteristic symptoms of PD[72]. Similar neuroprotective results of H_2S were later established in a PD rat model, where NaHS administration dramatically attenuated the progression of movement dysfunction and loss of tyrosine hydroxylase positive-neurons in both SN and striatum induced by PD[73]. Additionally, a new ratiometric two-photon fluorescent probe technique revealed a live reduction in H_2S production in PD astrocytes and living brain slices[70].

In PD, elevated levels of Hcy are found as well[68], assumedly affecting both oxidative stress and neuro-apoptosis in the pathogenesis of PD. In various studies, the protective impact of H_2S induced Nrf2-dependant signaling has been reported as an important component in preventing oxidative stress. This was characterized by the upregulation of antioxidant and detoxification proteins by the Nrf2-pathway in H_2S treated PD mouse models[71, 72]. GSH levels did not show any significant changes in tissue homogenates of the SN and striatum in these models. GSH levels were however altered in the AD model, this difference could be due to the administration of H_2S (NaHS in AD studies and inhalated H_2S in PD studies) or the detection methods.

An upregulation of inflammatory cytokines was found in the brains and cerebrospinal fluid of patients with PD, and activated glial cells have been observed in humane PD post-mortem material[69]. NaHS treatment in an induced PD rat model inhibits the microglial activation in the SN and the accumulation of proinflammatory factors such as TNF-α and NO in the striatum[73]. Supporting evidence of NaHS treatment in PD rats show similar results[75].

6. H_2S and other Neurodegenerative Diseases

Even though AD and PD are the neurodegenerative diseases that cover the most patients by far, the pathogenesis of all neurodegenerative diseases are related. Despite the lower occurrence of other neurodegenerative diseases like Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS), they are often not less lethal. Much less research is done in neurodegenerative diseases other than AD and PD, but they all share neurodegenerative characteristics on which H_2S could provide potential therapeutic value. The lack of sufficient literature has limited this paper to include only HD and ALS.

HD is a progressive, fatal, neurodegenerative disorder caused by an expanded polyglutamine (CAG) repeat in the huntingtin gene, which encodes an abnormally long polyglutamine repeat in HD. Though the gene and its corresponding huntingtin protein have been identified as the main cause for the onset of HD, its function is unclear and under heavy investigation. Neurodegeneration already occurs years before clinical symptoms of HD, and is accompanied by subtle cognitive, motor and psychiatric changes. As earlier mentioned, CBS and 3MST are considered as most important H_2S-producing enzymes in the brain. However, in a recent study high amounts of CSE were found in whole-brain lysates of mice[81]. More interestingly, CSE expressions was significantly reduced in various parts of the

brain in their HD mouse model (Fig. 4.), while in previous literature CBS and 3MST have been established as main H_2S-producing enzymes in the brain. This not only indicated lower H_2S concentrations in HD, possibly leading to a decrease in neuroprotection, but also questions the current understanding of the restricted localization of H_2S-producing enzymes, and their correspondent role in neurodegeneration. Additionally, plasma Hcy levels are elevated in HD patients and even implicates a correlation between Hcy aberration and the pathogenesis of HD[82].

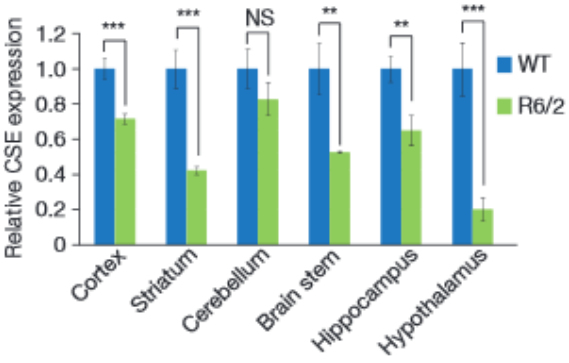

Fig. 4. Relative CSE expression of whole-brain lysates. Wt, wild type. R6/2, 13-week-old R6/2 (HD-induced) mice [81]

ALS is the most common yet rare motor neuron disease of which the exact mechanisms are poorly known. It is a fatal, rapid progressive and invariable neurodegenerative disease, characterized by motor neuron degeneration and paralysis[76]. Like AD and PD, a major known cause of the pathogenesis of ALS is toxic protein aggregation. Mutations in copper/zinc superoxide dismutase (SOD1), resulting in a toxic pro-apoptotic gain of function, have been detected in aggregates in motor neurons of mice models and patients[77, 78]. Despite this mutation in an antioxidative enzyme, the SOD1 mutations appear not to affect their antioxidative function. Yet oxidative stress does occur through the interaction of SOD1 mutants with hydrogen peroxide or superoxide anions, also disturbing mitochondrial function, and is proposed as a possible mechanism for the neurodegeneration in ALS[80]. Glial cells have been implicated in the pathogenic cascades for ALS. Very recently the important role of NF-κB localization and signaling caused microglial-mediated motor-neuron death in vitro, and subsequent deletion of NF- κB signaling extended survival in ALS mice by impairing pro-inflammatory microglial activation[79].

7. Discussion

This paper has evaluated the neuroprotective properties of H_2S and its relevance with various neurodegenerative diseases. H_2S attenuates neurodegeneration on three frontiers. First, evidence shows that H_2S functions anti-inflammatory by interacting with inflammation-inducing LPS and AMPK, and inhibiting activation of microglia and astrocytes. H_2S also functions anti-oxidative by being a ROS scavenger itself, but more importantly by increasing antioxidant GSH levels and decreasing toxic Hcy levels. Finally, anti-apoptotic effect on neuronal cells are shown by H_2S treatment by the interaction with Hcy, but also by maintaining mitochondrial integrity which suppresses the mitochondrial apoptotic pathway. Based on our current understanding of the mechanisms of various neurodegenerative diseases described in this paper, links can be established between possible H_2S functions and the pathogenesis of specific diseases (Table 1.).

There are however contradicting findings to this model, indicating the complexity of the mechanism involving H_2S. Contradictions discussed in this paper are based on single studies with often questionable methods, though these cannot be neglected. Some studies have shown opposite effects of H_2S on neuroprotection, such as the apoptotic effects found in high H_2S concentrations. However, since H_2S concentrations are still hard to measure, especially in the brain, the amount of H_2S could be so high that it becomes cytotoxic and would not per se indicate the suggested neurodegenerative function of H_2S. An easy

	AD	PD	HD	ALS
Neuro-inflammation	Yes	Yes	Yes	Yes
-AMPK	Yes	*Unknown*	*Unknown*	*Unknown*
-Microglia/astrocytes	Yes	Yes	*Unknown*	Yes
Oxidative stress	Yes	Yes	Yes	Yes
-GSH inhibition	Yes	No	*Unknown*	*Unknown*
-Hcy	Yes	Yes	Yes	*Unknown*
Neuro-apoptosis	Yes	Yes	Yes	Yes
-Hcy	Yes	Yes	Yes	*Unknown*
-Mitochondrial stress	Yes	Yes	Yes	Yes
Mutant protein accumulation	Contradictory	No	*Unknown*	*Unknown*

Table 1. Established targets of H_2S in different neurodegenerative diseases, according to studies evaluated in this paper

experiment could give us a better understanding of concentration-dependent (anti-)apoptosis, by measuring apoptotic and cytotoxic markers in increasing H_2S concentrations. Questions were also raised on the function and localization of H_2S producing enzymes. Detection methods of these enzymes are done on tissue after euthanizing animals or on deceased human material, which can influence these enzymes. H_2S localization and functional findings however are more solid. Novel methods might give us a better view of the localization and abundance of H_2S producing enzymes, leading to better research and therapeutic possibilities. The two-photon probe technique, or the use of isotopes might provide more accurate results.

Neuro-inflammation, oxidative stress and neuro-apoptosis are intimately connected processes. Often one process precedes, influence and/or worsen the progression of others. This leads to much confusion, especially when trying to place every event in the complete picture of neurodegenerative mechanisms. It becomes even harder when attempting to incorporate the neurodegenerative mechanisms in the different pathologies, which all have distinct pathogenic onsets. Although evidence for the neuroprotective functions of H_2S is convincing, it cannot always be directly translated to neurodegenerative diseases. Suggestively, H_2S can be of therapeutic potential in neurodegenerative diseases, but it does not solve the root of the problem. It can inhibit the progression of neurodegenerative diseases by keeping neurons alive, but it might be limited as a supplementary treatment in combination with treatments that engage protein accumulation or genetic deficits. Further research and possible clinical trials have to shed more light on this hypothesis before conclusions can be made. Moreover, alterations in H_2S could also be a symptom of a crashing protein homeostasis. It is not yet possible to assign cause and effect, which compromises our understanding of the mechanisms induced by, or inducing alterations in H_2S.

Another considerable problem is the administration of H_2S. H_2S is a gas, which means it has to be locally produced or released by enzymes or transporters. The vast majority of the discussed studies in this paper used the H_2S releasing drug sodium hydrosulfide (NaHS), which releases H_2S instantaneously in aqueous

solution. This is problematic because it results in a surge of H_2S, whereas endogenous H_2S release by cells is likely to occur in lesser amounts and a much slower rate. NaHS may therefore not mimic the biological effect of naturally produced H_2S. Treatment of neurodegeneration by NaHS might still be a possibility, but when studying the mechanisms induced by H_2S, these fast releases of high amounts of H_2S could influence findings on many levels. Fortunately, new methods of H_2S administration in vivo and in vitro are emerging. In 2008, sildenafil (ACS6) was described as an effective H_2S releasing substance in an intracellular, long-lasting controlled way[84]. Another interesting method of H_2S administration is the use of Tabiano's spa-waters (Italy) which are rich in H_2S. These spa-waters have been used in various neurodegenerative animal models with promising results. Caloric restriction has also been proposed as H_2S-increasing method. However, the potential of this method is limited, as it has only been reported to retain H_2S levels during aging. Novel methods of safe and slow H_2S administration are needed for both research and therapeutic ends.

The current understanding of H_2S and its potential neuroprotective functions is still in its infancy. However this paper summarizes convincing evidence of many H_2S-induced neuroprotective pathways, including anti-inflammation, anti-oxidation and anti-apoptosis. It may therefore be of substantial interest as a therapeutic target in neurodegenerative diseases.

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