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Invited review

Role of hydrogen sulfide in secondary neuronal injury

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A B S T R A C T

In acute neuronal insult events, such as stroke, traumatic brain injury, and spinal cord injury, pathological processes of secondary neuronal injury play a key role in the severity of insult and clinical prognosis. Along with nitric oxide (NO) and carbon monoxide (CO), hydrogen sulfide (H_2S) is regarded as the third gasotransmitter and endogenous neuromodulator and plays multiple roles in the central nervous system under physiological and pathological states, especially in secondary neuronal injury. The endogenous level of H_2S in the brain is significantly higher than that in peripheral tissues, and is mainly formed by cystathionine β-synthase (CBS) in astrocytes and released in response to neuronal excitation. The mechanism of secondary neuronal injury exacerbating the damage caused by the initial insult includes microcirculation failure, glutamate-mediated excitotoxicity, oxidative stress, inflammatory responses, neuronal apoptosis and calcium overload. H_2S dilates cerebral vessels by activating smooth muscle cell plasma membrane ATP-sensitive K channels (K_{ATP} channels). This modification occurs on specific cysteine residues of the K_{ATP} channel proteins which are S-sulfhydrated. H_2S counteracts glutamate-mediated excitotoxicity by inducing astrocytes to intake more glutamate from the extracellular space and thus increasing glutathione in neurons. In addition, H_2S protects neurons from secondary neuronal injury by functioning as an anti-oxidant, anti-inflammatory and anti-apoptotic mediator. However, there are still some reports suggest that H_2S elevates neuronal Ca^{2+} concentration and may contribute to the formation of calcium overload in secondary neuronal injury. H_2S also elicits calcium waves in primary cultures of astrocytes and may mediate signals between neurons and glia. Consequently, further exploration of the molecular mechanisms of H_2S in secondary neuronal injury will provide important insights into its potential therapeutic uses for the treatment of acute neuronal insult events.

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1. Introduction

The three most serious life-threatening pathological conditions of the central nervous system (CNS) are stroke, traumatic brain injury (TBI), and spinal cord injury (SCI), which are associated with significant financial and personal burdens. Although these acute neuronal insult events have different etiologies, they all involve both primary and secondary neuronal injury (Struffert et al., 2003). The initial injury is usually mechanical and secondary neuronal injury is triggered by an initial insult and occurs a few hours, several days or weeks following the initial insult (Borgens and Liu-Snyder, 2012). During secondary neuronal injury, healthy neurons around the injury site progressively degenerate, and eventually leading to more serious clinical symptoms. Therefore, secondary neuronal injury plays a key role in the severity of insult and subsequent clinical prognosis.

Along with nitric oxide (NO) and carbon monoxide (CO), hydrogen sulfide (H_2S) is regarded as an important gasotransmitter (Wang, 2002) and endogenous neuromodulator, drawing increasing attention in the literature. Traditional neurotransmitters bind

and activate membrane receptors, while gasotransmitters can freely diffuse to adjacent cells and directly bind to their target proteins to modify biological functions. Similar to NO which S-nitrosylates a diverse array of proteins, H_2S physiologically regulates protein functions by S-sulfhydration. However, S-nitrosylation typically inhibits enzymes, while S-sulfhydration activates them. Therefore, H_2S is a physiologic gasotransmitter as important as NO and CO (Gadalla and Snyder, 2010). In the past decade, increasing evidence shows that H_2S plays multiple roles in the CNS under physiological and pathological states. An examination of secondary neuronal injury has revealed a greater complexity of molecular processes than anticipated, and a further understanding of their relationships may provide insight into effective treatments (Fig. 1). Therefore, this article illustrates the role of H_2S in secondary neuronal injury in the context of cerebrovascular regulation, glutamate-mediated excitotoxicity, oxidative stress, inflammation, apoptosis and calcium concentrations (Fig. 2).

2. The synthesis and regulation of H_2S in the brain

H_2S is a small molecule that can freely pass through cell membranes. The concentration of endogenous H_2S in the rat brain reaches up to 50–160 µmol/L (Warenycia et al., 1989), significantly higher than peripheral blood (0–46 µmol/L) (Zhao et al., 2003), suggesting that H_2S may play an important physiological role in the brain.

However, recent studies have suggested that this may not be the case. Assuming that H_2S levels in the brain are maintained at the relative high tissue concentration of 50–160 µmol/L, the perfusion of blood with only 40 µmol/L H_2S would result in large fluctuations in H_2S levels. Catabolism of H_2S by mouse brain homogenates exceeds the rate of enzymatic release of H_2S, thus the whole tissue and blood concentrations of free H_2S are orders of magnitude less than conventionally accepted values (Furne et al., 2008; Ishigami et al., 2009).

H_2S can be formed from cysteine by pyridoxal-5′-phosphate (PLP)-dependent enzymes, including cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). However, CBS and CSE have different tissue distributions. CSE plays a major role in generating H_2S in the cardiovascular system (Hosoki et al., 1997) while the production of H_2S in the brain is suppressed by CBS inhibitors and enhanced by CBS activators (Abe and Kimura, 1996). The amount of H_2S in the brain of CBS gene knockout mice is significantly decreased compared to wild type mice (Eto and Kimura, 2002). These data suggest that CBS contributes to the production of endogenous H_2S in the brain (Fig. 2). In addition to the major PLP-dependent enzymes in the brain, H_2S can also be produced from cysteine by 3-mercaptopyruvate sulfurtransferase (3MST) in combination with cysteine aminotransferase (CAT) (Mikami et al., 2011; Shibuya et al., 2009, 2013).

The expression of CBS is mainly localised to astrocytes (Enokido et al., 2005; Ichinohe et al., 2005) and microglia (Hu et al., 2007), while 3MST is produced in neurons. By comparing the H_2S synthesis in different cell lines, Lee et al. found that the amount of H_2S produced by astrocytes was 7.9 times higher than microglia, 9.7 times higher than NT-2 cells and 11.5 times higher than SH-SY5Y cells, illustrating that astrocytes are the main cells that generate H_2S (Lee et al., 2009) (Fig. 2).

The synthesis of H_2S is regulated by changing CBS activity. The human CBS gene is localised to chromosome 21 at 21q22.3 (Munke et al., 1988). CBS exists as a homotetramer with a subunit molecular weight of 63 kDa. Each subunit can bind the cofactor PLP, S-adenosyl methionine (SAM) and heme (Banerjee and Zou, 2005; Miles and Kraus, 2004). SAM is an allosteric activator of CBS and activates CBS approximately 2-fold (Abe and Kimura,

1996; Shan and Kruger, 1998). CO binds to heme with high affinity and inhibits CBS activity (Dioum et al., 2002).

CBS inhibitors aminooxyacetate (AOAA) and hydroxylamine (HA) intensively suppress the synthesis of H_2S (Abe and Kimura, 1996). While H_2S production is greatly enhanced by L-glutamate and stimulation, the increase of CBS activity is regulated by a pathway involving Ca^{2+}/calmodulin and is enhanced in response to neuronal excitation (Eto and Kimura, 2002). Furthermore, digoxin increases H_2S concentration in the mouse brain (Wilinski et al., 2011b), while paracetamol plays the opposite role (Wilinski et al., 2011a).

3. H_2S mediates the dilation of cerebral vessels

In TBI and SCI, the initial mechanical insult and bone fragments may rupture blood vessels such that the blood supply to the injured site decreases. In addition, intracranial and intraspinal hematomas compress the nerve parenchyma and edema may also increase local pressure such that they prevent blood flow into tissue and exacerbate ischemia. In stroke, clots or hemorrhages deprive neurons and supporting cells of oxygen and glucose. Because of a low oxygen supply, their energy generation switches from oxidative phosphorylation to glycolysis leading to a significant reduction in ATP synthesis. ATP depletion appears to be the beginning of secondary neuronal injury (Fig. 1). In addition, the destruction of plasma or axonal membranes in TBI and SCI is the cause of the loss of function of various ion channels and transporters such as the Na^+–K^+ pump. With the destruction of the these membranes, ion homeostasis is disrupted and large amounts of Na^+ and Ca^{2+} accumulate, while abundant cytosolic K^+ is pumped out. Such a disruption in ion homeostasis can cause cell edema and intracellular calcium overload which may induce further lesions (Fig. 1). Consequently, the brain is particularly vulnerable to ischemia and these events are more likely to initiate secondary neuronal injury.

In the peripheral circulation, H_2S has been proven to dilate vessels by activating ATP-sensitive K channels (K_{ATP} channels) so that it triggers a cascade reaction in which intracellular potassium effluxes, membranes of vascular smooth muscle cells are hyperpolarised and voltage dependent calcium channels are closed. It has been shown that H_2S dilates rat aortic tissues in vitro in a K_{ATP} channel-dependent manner, which directly increases K_{ATP} channel currents and hyperpolarised cell membranes (Zhao et al., 2001).

However, there is little evidence to show indicate the role of H_2S in cerebrovascular regulation. Leffler et al. was the first to report that H_2S dilated cerebral cortical pial arterioles of newborn pigs in a concentration-dependent way, a process blocked by K_{ATP} channel inhibitor glibenclamide (Leffler et al., 2011). L-Cysteine increased H_2S in the cerebrospinal fluid, coincident with its vasodilation of pial arterioles and this increase can be blocked by the CSE inhibitor D,L-propargylglycine (PPG) but not by the CBS inhibitor AOAA. Furthermore, CSE was mainly expressed in cerebral vessels, while CBS was predominately located in neurons and astrocytes. These data showed that H_2S in the brain was produced by CSE and led to a decrease in vascular tone (Leffler et al., 2011). Morikawa et al. subsequently found that increased H_2S dilated vessels after cerebral ischemia, while CBS gene knock-out mice displayed an impaired vascular response due to cerebral ischemia and anoxia (Morikawa et al., 2012).

Liang et al. further found piglet cerebral arterioles expressed the inwardly rectifying K^+ 6.1 channel and sulfonylurea receptor 2B (SUR2B, K_{ATP} channel subunits). The K_{ATP} channel inhibitor glibenclamide can block vasodilation induced by H_2S and the vasodilatory effect of H_2S was attenuated in the SUR2 gene knockout mice compared to their wild type controls. Thus, the data indicated that