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Hydrogen sulfide prevents hypoxia-induced apoptosis via inhibition of an H_2O_2-activated calcium signaling pathway in mouse hippocampal neurons

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ABSTRACT

Hydrogen sulfide (H_2S), an endogenous gaseous mediator, has been shown to exert protective effects against damage to different organs in the human body caused by various stimuli. However, the potential effects of H_2S on hypoxia-induced neuronal apoptosis and its mechanisms remain unclear. Here, we exposed mouse hippocampal neurons to hypoxic conditions (2% O_2, 5% CO_2 and 93% N_2 at 37 °C) to establish a hypoxic cell model. We found that 4-h hypoxia treatment significantly increased intracellular reactive oxygen species (ROS) levels, and pretreatment with NaHS (a source of H_2S) for 30 min suppressed hypoxia-induced intracellular ROS elevation. The hypoxia treatment significantly increased cytosolic calcium ($[Ca^{2+}]_i$), and pretreatment with NaHS prevented the increase in $[Ca^{2+}]_i$. Additionally, polyethylene glycol (PEG)–catalase (a H_2O_2 scavenger) but not PEG–SOD (an O_2^- scavenger) conferred an inhibitory effect similar to H_2S on the hypoxia-induced increase in $[Ca^{2+}]_i$. Furthermore, we found that pretreatment with NaHS could significantly inhibit hypoxia-induced neuronal apoptosis, which was also inhibited by PEG-catalase or the inositol 1,4,5-triphosphate (IP_3) receptor blocker xestospongin C. Taken together, these findings suggest that H_2S inhibits hypoxia-induced apoptosis through inhibition of a ROS (mainly H_2O_2)-activated Ca^{2+} signaling pathway in mouse hippocampal neurons.

1. Introduction

Hydrogen sulfide (H_2S) is a well-known toxic gas with the characteristic foul odor of rotten eggs. The human body produces small amounts of H_2S as a signaling molecule with a number of biological signaling functions. H_2S is produced from cysteine by the enzymes cystathionine beta-synthase and cystathionine gamma-lyase. H_2S is now recognized as potentially protecting against cardiovascular disease [1] and Alzheimer's disease, where severely decreased H_2S concentrations are found in the brain [2]. Studies have demonstrated that H_2S serves as a novel neuromodulator/transmitter in the brain [3].

H_2S has been shown to exert protective effects against ischemia/reperfusion-induced cardiac injury [4], to regulate perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis [5], and to protect the kidney against ischemia/reperfusion injury [6]. Very recent studies have demonstrated that H_2S protects against chemical hypoxia-induced cytotoxicity in HaCaT and PC12 cells through inhibition of reactive oxygen species (ROS)-mediated signaling pathways [7,8]. Antioxidation is one of the main mechanisms underlying the protective action of H_2S, which exerts its effect not only by increasing glutathione (GSH) [9] but also by directly scavenging superoxide anions and hydrogen peroxide (H_2O_2) [10].

The brain only represents approximately 2% of the body's weight; however, it consumes approximately 20% of the body's oxygen. As a result, the brain is especially sensitive to hypoxia. Cerebral hypoxia is a common clinical event that mediates brain damage in many diseases, such as ischemic stroke [11], and neurodegenerative diseases such as Alzheimer's disease [12]. Persistent hypoxia causes overproduction of ROS, such as superoxide radicals (O_2^-), H_2O_2, and hydroxyl anions ($^{\bullet}OH$) [13], and it also causes intracellular calcium overload [14]; ROS overproduction and calcium overload are both leading mechanisms of brain injury. The neuroprotective effects of H_2S have been widely explored by many research groups. For example, H_2S has been shown to protect cells against cytotoxicity caused by peroxynitrite, β-amyloid, hypochlorous acid and H_2O_2 [15–18]. H_2S provides neuroprotection against MPTP-induced dopaminergic neuron degeneration [19]. However,

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the potential neuroprotective effects of H_2S against hypoxia-induced neurotoxicity and the mechanisms of protection remain to be clarified.

In the present study, we exposed mouse hippocampal neurons to hypoxic conditions (2% O_2, 5% CO_2 and 93% N_2) to establish a hypoxic cell model, and we explored the effects of H_2S on hypoxia-induced apoptosis and its mechanisms.

2. Materials and methods

2.1. Materials

Poly-L-lysine, 2′,7′-dichlorfluorescein-diacetate (DCFH-DA), sodium hydrosulfide (NaHS), polyethylene glycol (PEG)-catalase, PEG-superoxide dismutase (SOD), xestospongin C and propidium iodide (PI) were purchased from Sigma. Hoechst 33342 was purchased from Santa Cruz Biotechnology. Fura-2-acetoxymethyl ester (Fura-2/AM), trypsin and neurobasal/B27 medium were purchased from Invitrogen. DNase I and cytarabine were purchased from Roche. The β3-tubulin monoclonal antibody was purchased from Cell Signaling Technology. Fetal bovine serum (FBS) and horse serum were purchased from Hyclone.

2.2. Primary culture of mouse hippocampal neurons

BALB/c mice were purchased from the Experimental Animal Center of the Medical College of Sun Yat-Sen University. The mice were sacrificed by cervical dislocation after 16–18 days of pregnancy. The mice were immersed in 75% ethyl alcohol for 1–2 min and the fetuses were removed. The brain was dissociated, and the hippocampus was isolated from the brain and subjected to 0.25% trypsin digestion for 10 min at 37 °C. The digestion was terminated by addition of DMEM medium containing 10% FBS. The digestion product was centrifuged at 1000 g for 8 min, and the supernatants were removed. The cells were diluted to a density of 1×10^6/ml in medium containing 10% FBS, 10% horse serum, 25 μM l-glutamic acid and 0.5 mM l-glutamine and inoculated into confocal dishes coated with 0.04 mg/ml poly-L-lysine. After incubation in a 5% CO_2 incubator for 24 h at 37 °C, the culture medium was replaced with neurobasal/B27 medium. After 2 days of culture, 5 μM cytarabine was added to the medium to inhibit the growth of glial cells. Half of the culture medium was replaced every other day. The cells were used for subsequent experiments after 7 days of culture.

2.3. Hypoxic cell model establishment

The mouse hippocampal neurons were cultured in hypoxic conditions (2% O_2, 5% CO_2 and 93% N_2) at 37 °C to establish the hypoxic cell models.

2.4. Measurement of intracellular ROS content

The primary cultured mouse hippocampal neurons were grown on 25-mm diameter confocal dishes coated with 0.04 mg/ml poly-L-lysine. After being cultured for 7 days, the neurons were pretreated with 100 μM or 300 μM NaHS for 30 min followed by a 4-h hypoxia treatment. For the intracellular ROS content assay, DCFH-DA was added at a final concentration of 5 μM to neurobasal/B27 culture medium. After incubation at 37 °C for 50 min in the dark, the cells were washed three times with HEPES-buffered salt solution (HBSS) containing (in mM) 135 NaCl, 5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 5 HEPES, and 10 glucose at pH 7.4 (adjusted with NaOH). DCF fluorescence was recorded using a xenon arc lamp, interference filters, an electronic shutter (MT20, Germany), a 40× oil objective (Olympus) and a cooled CCD camera (Olympus, Japan). The mean DCF fluorescence intensity from three random fields was analyzed using Cell^R software (Germany).

2.5. $[Ca^{2+}]_i$ measurement

The primary cultured mouse hippocampal neurons were grown on 25-mm diameter confocal dishes coated with 0.04 mg/ml poly-L-lysine. After 7 days of culture, the neurons were pretreated with 100 μM or 300 μM NaHS for 30 min, or with 5000 U/ml PEG-catalase or 1000 U/ml PEG-SOD overnight, followed by a 4-h hypoxia treatment. The neurons were also treated with 1 μM of the inositol 1,4,5-triphosphate (IP_3) receptor blocker xestospongin C 2 min before the hypoxia treatment. For $[Ca^{2+}]_i$ measurement, Fura-2/AM (1 mM in anhydrous dimethyl sulfoxide) was diluted to 1 μM in neurobasal/B27 media before loading. Neurons were incubated in the medium for 40 min at room temperature in the dark. The cells were washed three times with HBSS. The confocal dish was mounted on the stage of an inverted fluorescence microscope (IX71, Olympus). Fura-2 fluorescence was recorded using a xenon arc lamp, interference filters, an electronic shutter (MT20, Germany), a 40× oil objective (Olympus) and a cooled CCD camera (Olympus, Japan). Data were collected online using Cell^R software (Germany). $[Ca^{2+}]_i$ was estimated from the F340/F380 ratio.

2.6. Apoptotic assay

Neuronal apoptosis was measured by flow cytometry using propidium iodide (PI) and Hoechst 33342 staining. For flow cytometric analysis after treatment, approximately 1×10^6 cells were collected by centrifugation (2000 rpm, 5 min) and washed with PBS. The cells were resuspended in binding buffer supplemented with 400 μl of PI (50 μg/ml) and incubated in the dark at room temperature for 30 min. The assay results were measured using a flow cytometer (FACSCalibur, USA). In the DNA histogram, the amplitude of the sub-G1 DNA peak, which is lower than the G1 DNA peak, represents the number of apoptotic cells. For Hoechst 33342 staining, the neurons were fixed for 30 min in fresh 4% paraformaldehyde at room temperature. After washing twice with PBS, a Hoechst 33342 solution (20 μg/ml) was added to the confocal laser dishes and incubated for 3 min. The apoptotic cells from three random fields were analyzed by fluorescence microscopy (Nikon Ti-U, Japan). The apoptotic cells were counted by two individuals, and the data are expressed as the fraction of apoptotic cells per 100 cells counted.

2.7. Statistical analysis

The data are expressed as the mean ± SD. The data were analyzed using the statistical program SigmaStat (Jandel Scientific, Chicago, IL, USA). The significance of differences among means was determined by one-way ANOVA followed by a Student Newman–Keuls post hoc test. The level of significance was set at $p < 0.05$.

3. Results

3.1. Morphology and purity of cultured mouse hippocampal neurons

The mouse hippocampal neurons displayed full cell bodies, a spindle shape and long dendrites forming a tight network under the microscope.

To determine the purity of the cultured neurons, we performed immunocytofluorescence analysis using an antibody against neuron-specific β3-tubulin protein to stain the neurons and Hoechst

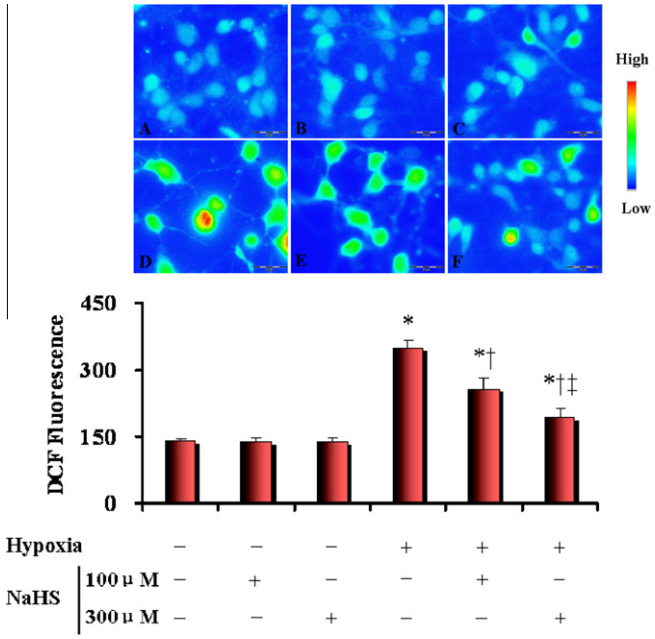

Fig. 1. The effect of H_2S on hypoxia-induced intracellular ROS content in cultured neurons. After neurons were cultured for 7 days, the neurobasal/B27 medium was changed and the ROS probe DCFH-DA (5 μM) was added to the cultures and incubated for 50 min. A: control; B: 100 μM NaHS; C: 300 μM NaHS; D: hypoxia; E: 100 μM NaHS + hypoxia; F: 300 μM NaHS + hypoxia. The ROS content was measured using the Cell^R-MT20 fluorescence imaging system. ∗: $p < 0.01$ vs. control; †: $p < 0.01$ vs. hypoxia; ‡: $p < 0.01$ vs. 100 μM NaHS + hypoxia. The quantitative data analysis was performed using 23–58 cells for each group, and the data are representative of data from at least 3 independent experiments.

33342 to stain neuronal nuclei (Supplementary Figure S1). The purity of neurons was calculated as approximately 91.1 ± 6.5%.

3.2. H_2S inhibits the hypoxia-induced increase in intracellular ROS content

To determine the potential effects of H_2S on hypoxia-induced intracellular ROS content, we performed DCF immunofluorescence intensity analysis. We found that a 4-h hypoxia treatment significantly increased the DCF fluorescence intensity of the cultured neurons. Pretreatment with NaHS (100 μM and 300 μM) attenuated the DCF fluorescence intensity during hypoxia; however, the intensity was still higher than the normal control (Fig. 1). These data indicate that hypoxia increases the intracellular ROS content and that H_2S can prevent hypoxia-induced ROS elevation.

3.3. ROS (H_2O_2)-mediated hypoxia-induced elevation of $[Ca^{2+}]_i$ in cultured neurons

To determine the role of ROS in increased $[Ca^{2+}]_i$ caused by hypoxia, we treated neurons with PEG-catalase (a H_2O_2 scavenger) or PEG-SOD (an O_2^- scavenger) during hypoxia. We used the F340/380 ratio as an indicator of cytosolic calcium concentration. We found that hypoxia significantly increased $[Ca^{2+}]_i$ in the cultured neurons, as suggested by an increased F340/380 ratio, and that PEG-catalase but not PEG-SOD prevented the hypoxia-induced $[Ca^{2+}]_i$ elevation. In addition, treatment with H_2O_2 (100 μM and 300 μM) affected the ratio of F340/380 in a manner similar to hypoxia (Fig. 2A). These data indicate that hypoxia increased the neuronal $[Ca^{2+}]_i$, an effect that could be mediated, at least in part, by intracellular ROS (mainly H_2O_2).

3.4. H_2S inhibits the hypoxia-induced increase in $[Ca^{2+}]_i$

We examined the effects of H_2S on $[Ca^{2+}]_i$ during hypoxia and found that, while pretreatment with NaHS attenuated the hypoxia-induced increase in $[Ca^{2+}]_i$, the $[Ca^{2+}]_i$ was still higher than the normal control in the cultured neurons (Fig. 2B). The IP_3 receptor blocker xestospongin C exerted similar inhibitory effects on hypoxia-induced $[Ca^{2+}]_i$ elevation. These data indicate that H_2S may inhibit the hypoxia-induced increase in neuronal $[Ca^{2+}]_i$ via IP_3 receptor-mediated calcium signaling activation.

3.5. Hypoxia-induced neuronal apoptosis is inhibited by H_2S, PEG-catalase or xestospongin C

Flow cytometric analysis revealed that hypoxia significantly increased (approx. 9.0-fold) the apoptotic ratio of the cultured neurons and that pretreatment with NaHS prevented the hypoxia-induced increase in apoptosis. The apoptotic rate in NaHS-pretreated neurons remained higher than in the normal control. Treatment with NaHS alone had no significant effect on the rate of apoptosis (Fig. 3A-E). Hoechst 33342 staining further confirmed the occurrence of hypoxia-induced neuronal apoptosis by reveal-

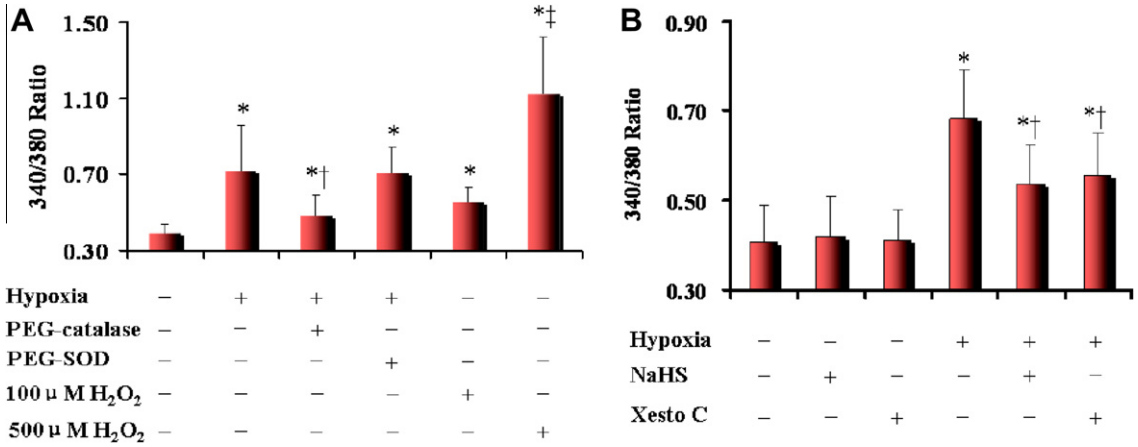

Fig. 2. The role of ROS in hypoxia-induced elevation of $[Ca^{2+}]_i$ and the effects of H_2S on $[Ca^{2+}]_i$ elevation in neurons. After neurons were cultured for 7 days, the neurobasal/B27 medium was changed, and Fura-2/AM (1 μM) was added to the cultures and incubated for 40 min. Following 3 washes with HBSS, the levels of $[Ca^{2+}]_i$ were measured using the Cell^R-MT20 fluorescence imaging system (A, B). ∗: $p < 0.01$ vs. control; †: $p < 0.01$ vs. hypoxia; ‡: $p < 0.01$, vs. 100 μM H_2O_2. The quantitative data analysis was performed using 41–55 cells for each group (A) and 52–64 cells for each group (B). The data are representative of data from at least 3 independent experiments.

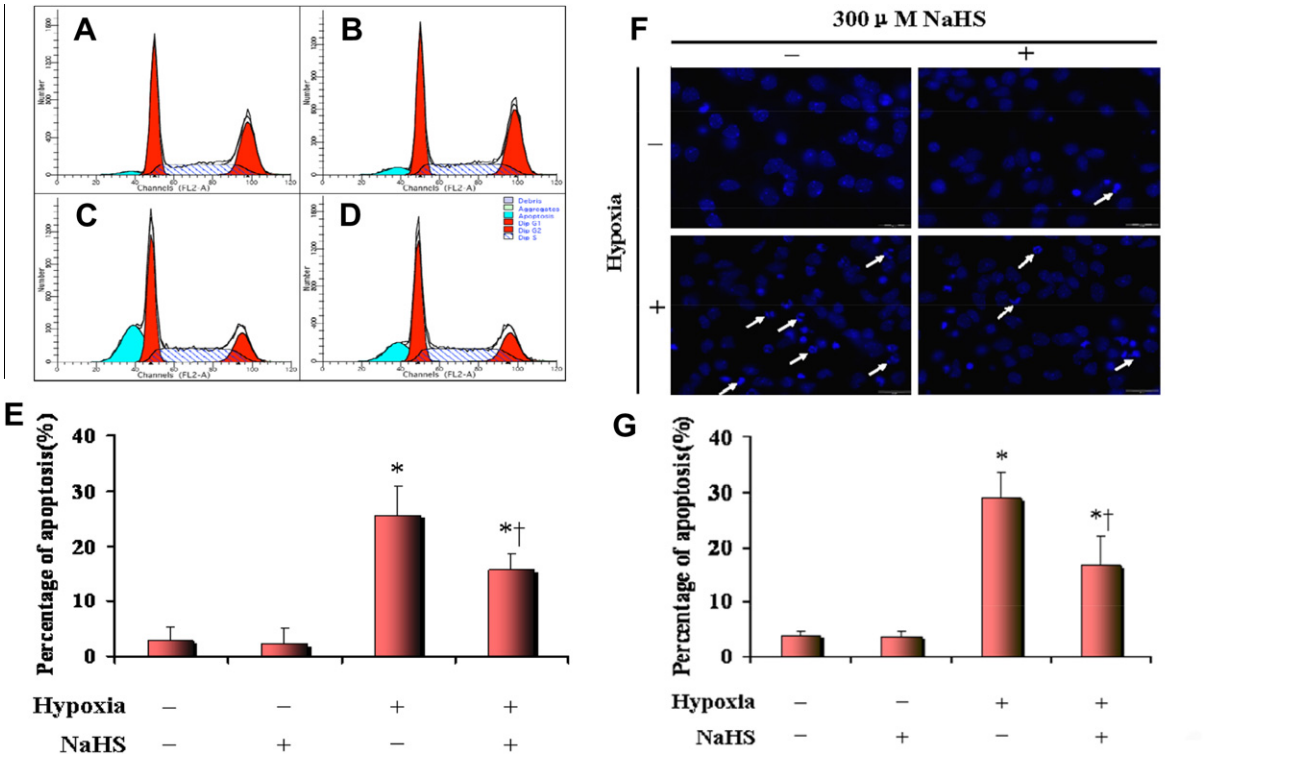

Fig. 3. The effects of H_2S on hypoxia-induced neuronal apoptosis. Changes in neuronal apoptosis were measured by flow cytometry using PI staining. A: control; B: 300 μM NaHS; C: hypoxia; D: 300 μM NaHS + hypoxia; E: quantitative analysis of neuronal apoptosis. Neuronal apoptosis was also measured by Hoechst 33342 staining (F). The arrow indicates the cells with apoptotic nuclear condensation and fragmentation. For the quantitative analysis of Hoechst 33342 staining, apoptotic cells were counted by two individuals, and the data are expressed as the fraction of apoptotic cells per 100 cells counted. Quantitative analysis revealed changes in the fraction of apoptotic cells among the groups (G). ∗: $p < 0.01$ vs. control; †: $p < 0.01$ vs. hypoxia. The data are representative of data from at least 3 independent experiments.

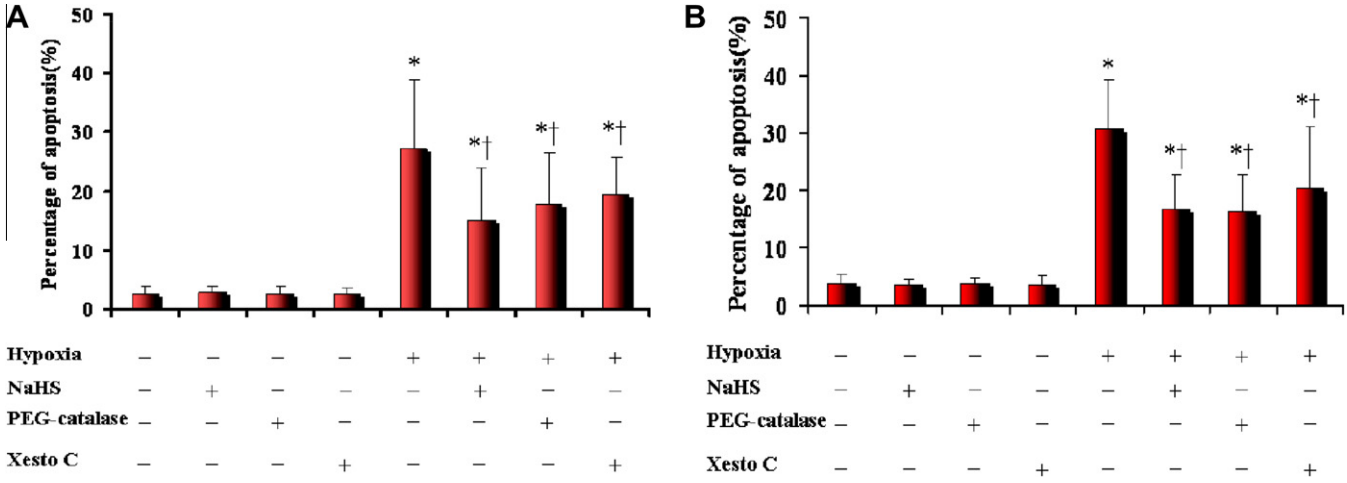

Fig. 4. The effects of ROS scavengers and an IP_3 receptor blocker on hypoxia-induced neuronal apoptosis. Quantitative analysis by flow cytometry showed the effects of ROS scavengers or an IP_3 receptor blocker on hypoxia-induced apoptosis (A). Quantitative analysis from Hoechst 33342 staining showed the effects of ROS scavengers or an IP_3 receptor blocker on hypoxia-induced apoptosis (B). ∗: $p < 0.01$ vs. control; †: $p < 0.01$ vs. hypoxia. The data are representative of data from at least 3 independent experiments.

ing apoptotic characteristics such as nuclear condensation and DNA fragmentation (Fig. 3F-G).

To determine the possible mechanisms underlying hypoxia-induced neuronal apoptosis, neurons were treated with NaHS, PEG-catalase or the IP_3 receptor blocker xestospongin C during hypoxia. The quantitative analysis of apoptosis by flow cytometry and Hoechst 33342 staining revealed that, similar to the action of H_2S, PEG-catalase or xestospongin C prevented hypoxia-induced neuronal apoptosis (Fig. 4A and B).

4. Discussion

In the present study, we found that H_2S suppressed both the elevation of intracellular ROS and the increase in cytosolic calcium induced by hypoxia in cultured neurons. Treatment with PEG-catalase but not PEG-SOD conferred inhibitory effects similar to H_2S on hypoxia-induced cytosolic calcium elevation. Moreover, we also found that pretreatment of neurons with NaHS, PEG-catalase or the IP_3 receptor blocker xestospongin C

suppressed hypoxia-induced mouse hippocampal neuronal apoptosis.

As H_2S is a reducing agent that readily reacts with H_2O_2 [20], it is possible that endogenous H_2S can scavenge ROS. The present data demonstrate that NaHS, a source of H_2S, lowers the hypoxia-induced intracellular ROS content by directly functioning as an antioxidant in the cultured neurons. A body of evidence has demonstrated that oxidative stress caused by increased ROS production is one of the main mechanisms leading to hypoxia-induced neuronal damage [21–23]. Overproduction of ROS can induce cytosolic calcium overload [14,24] and can therefore trigger neuronal apoptosis [25,26]. Our data show that the H_2O_2 scavenger PEG-catalase, but not the O_2^- scavenger PEG-SOD, exerted inhibitory effects similar to H_2S on hypoxia-induced cytosolic calcium elevation, indicating that the observed hypoxia-induced cytosolic calcium elevation is mainly caused by H_2O_2 rather than other ROS.

Neuronal apoptosis has been implicated in contributing to neurotoxin-induced neurotoxicity, and it plays an important role in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Studies have demonstrated that H_2S inhibits hypoxia-induced apoptosis of PC12 and SH-SY5Y cells [27,28] and protects hippocampal neurons against vascular dementia-induced injury via its anti-apoptotic function [29]. Our studies also show that H_2S, PEG-catalase or the IP_3 receptor blocker xestospongin C protect against neuronal apoptosis induced by hypoxia. These data indicate that IP_3 receptor-mediated calcium signaling activation is involved in hypoxia-induced neuronal apoptosis.

In conclusion, the present study demonstrates that H_2S inhibits hypoxia-induced apoptosis through a ROS (mainly H_2O_2)-activated Ca^{2+} signaling pathway in cultured hippocampal neurons.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.07.131.

References

[1] D.J. Lefer, A new gaseous signaling molecule emerges: cardioprotective role of hydrogen sulfide, Proc. Natl. Acad. Sci. USA 104 (2007) 17907–17908.
[2] K. Eto, T. Asada, K. Arima, T. Makifuchi, H. Kimura, Brain hydrogen sulfide is severely decreased in Alzheimer's disease, Biochem. Biophys. Res. Commun. 293 (2002) 1485–1488.
[3] H. Kimura, Hydrogen sulfide as a neuromodulator, Mol. Neurobiol. 26 (2002) 13–19.
[4] J.S. Bian, Q.C. Yong, T.T. Pan, Z.N. Feng, M.Y. Ali, S. Zhou, P.K. Moore, Role of hydrogen sulfide in the cardioprotection caused by ischemic preconditioning in the rat heart and cardiac myocytes, J. Pharmacol. Exp. Ther. 316 (2006) 670–678.
[5] S. Fiorucci, E. Antonelli, A. Mencarelli, S. Orlandi, B. Renga, G. Rizzo, E. Distrutti, V. Shah, A. Morelli, The third gas: H_2S regulates perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis, Hepatology 42 (2003) 539–548.
[6] P. Tripatara, N.S. Patel, V. Brancaleone, D. Renshaw, J. Rocha, B. Sepodes, H. Mota-Filipe, M. Perretti, C. Thiemermann, Characterisation of cystathionine gamma-lyase/hydrogen sulphide pathway in ischaemia/reperfusion injury of the mouse kidney: an in vivo study, Eur. J. Pharmacol. 606 (2009) 205–209.
[7] C. Yang, Z. Yang, M. Zhang, Q. Dong, X. Wang, A. Lan, F. Zeng, P. Chen, C. Wang, J. Feng, Hydrogen sulfide protects against chemical hypoxia-induced cytotoxicity and inflammation in HaCaT cells through inhibition of ROS/NF-κB/COX-2 pathway, PLoS One 6 (2011) e21971.
[8] A. Lan, X. Liao, L. Mo, C. Yang, Z. Yang, X. Wang, F. Hu, P. Chen, J. Feng, D. Zheng, L. Xiao, Hydrogen sulfide protects against chemical hypoxia-induced injury by inhibiting ROS-activated ERK1/2 and p38MAPK signaling pathways in PC12 cells, PLoS One 6 (2011) e25921.
[9] Y. Kimura, H. Kimura, Hydrogen sulfide protects neurons from oxidative stress, FASEB. J. 18 (2004) 1165–1167.
[10] B. Geng, L. Chang, C. Pan, Y. Qi, J. Zhao, Y. Pang, J. Du, C. Tang, Endogenous hydrogen sulfide regulation of myocardial injury induced by isoproterenol, Biochem. Biophys. Res. Commun. 318 (2004) 756–763.
[11] C. Roffe, Hypoxia and stroke, Age Ageing 31 (S2) (2002) 10–12.
[12] C. Peers, H.A. Pearson, J.P. Boyle, Hypoxia and Alzheimer's disease, Essays. Biochem. 43 (2007) 153–164.
[13] R.D. Guzy, B. Hoyos, E. Robin, H. Chen, L. Liu, K.D. Mansfield, M.C. Simon, U. Hammerling, P.T. Schumacker, Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing, Cell. Metab. 1 (2005) 401–408.
[14] P.M. Grinwald, C. Brosnahan, Sodium imbalance as a cause of calcium overload in post-hypoxic reoxygenation injury, J. Mol. Cell. Cardiol. 19 (1987) 487–495.
[15] M. Whiteman, J.S. Armstrong, S.H. Chu, S. Jia-Ling, B.S. Wong, N.S. Cheung, B. Halliwell, P.K. Moore, The novel neuromodulator hydrogen sulfide: an endogenous peroxynitrite 'scavenger', J. Neurochem. 90 (2004) 765–768.
[16] X.Q. Tang, C.T. Yang, J. Chen, W.L. Yin, S.W. Tian, B. Hu, J.Q. Feng, Y.J. Li, Effect of hydrogen sulphide on beta-amyloid-induced damage in PC12 cells, Clin. Exp. Pharmacol. Physiol. 35 (2008) 180–186.
[17] M. Whiteman, N.S. Cheung, Y.Z. Zhu, S.H. Chu, J.L. Siau, B.S. Wong, J.S. Armstrong, P.K. Moore, Hydrogen sulphide: a novel inhibitor of hypochlorous acid-mediated oxidative damage in the brain, Biochem. Biophys. Res. Commun. 326 (2005) 794–798.
[18] M. Lu, L.F. Hu, G. Hu, J.S. Bian, Hydrogen sulfide protects astrocytes against H(2)O(2)-induced neural injury via enhancing glutamate uptake, Free. Radic. Biol. Med. 45 (2008) 1705–1713.
[19] M. Lu, F.F. Zhao, J.J. Tang, C.J. Su, Y. Fan, J.H. Ding, J.S. Bian, G. Hu, The Neuroprotection of Hydrogen Sulfide Against MPTP-Induced Dopaminergic Neuron Degeneration Involves Uncoupling Protein 2 Rather Than ATP-Sensitive Potassium Channels, Antioxid. Redox. Signal. 2012 Apr 20.
[20] I. Devai, R.D. Delaune, Effectiveness of selected chemicals for controlling emission of malodorous sulfur gases in sewage sludge, Environ. Technol. 23 (2002) 319–329.
[21] A. Kumar, R. Goyal, Gabapentin attenuates acute hypoxic stress-induced behavioral alterations and oxidative damage in mice. possible involvement of GABAergic mechanism, Indian. J. Exp. Biol. 46 (2008) 159–163.
[22] J.A. Jefferson, J. Simoni, E. Escudero, M.E. Hurtado, E.R. Swenson, D.E. Wesson, G.F. Schreiner, R.B. Schoene, R.J. Johnson, A. Hurtado, Increased oxidative stress following acute and chronic high altitude exposure, High. Alt. Med. Biol. 5 (2004) 61–69.
[23] S. Love, Oxidative stress in brain ischemia, Brain. Pathol. 9 (1999) 119–131.
[24] N.S. Dhalla, L. Golfman, S. Takeda, N. Takeda, M. Nagano, Evidence for the role of oxidative stress in acute ischemic heart disease: a brief review, Can. J. Cardiol. 15 (1999) 587–593.
[25] I.I. Kruman, A. Nath, M.P. Mattson, HIV-1 protein Tat induces apoptosis of hippocampal neurons by a mechanism involving caspase activation, calcium overload, and oxidative stress, Exp. Neurol. 154 (1998) 276–288.
[26] P. Nicotera, S. Orrenius, The role of calcium in apoptosis, Cell Calcium. 23 (1998) 173–180.
[27] J.L. Meng, W.Y. Mei, Y.F. Dong, J.H. Wang, C.M. Zhao, A.P. Lan, C.T. Yang, P.X. Chen, J.Q. Feng, C.H. Hu, Heat shock protein 90 mediates cytoprotection by H_2S against chemical hypoxia-induced injury in PC12 cells, Clin. Exp. Pharmacol. Physiol. 38 (2011) 42–49.
[28] A.S. Tay, L.F. Hu, M. Lu, P.T. Wong, J.S. Bian, Hydrogen sulfide protects neurons against hypoxic injury via stimulation of ATP-sensitive potassium channel/protein kinase C/extracellular signal-regulated kinase/heat shock protein 90 pathway, Neuroscience 167 (2010) 277–286.
[29] L.M. Zhang, C.X. Jiang, D.W. Liu, Hydrogen sulfide attenuates neuronal injury induced by vascular dementia via inhibiting apoptosis in rats, Neurochem. Res. 34 (2009) 1984–1992.