Neurochemistry International 56 (2010) 508–515

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/neuint

Effect of hydrogen sulfide on intracellular calcium homeostasis in neuronal cells☆

Qian Chen Yong [a], Chooi Hoong Choo [a], Boon Hian Tan [a], Chian-Ming Low [a,b], Jin-Song Bian [a,*]

[a] Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, 10 Medical Drive, Singapore 117597, Singapore
[b] Department of Anaesthesia, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

ARTICLE INFO

Article history:
Received 17 September 2009
Received in revised form 8 December 2009
Accepted 16 December 2009
Available online 22 December 2009

Keywords:
SH-SY5Y
NMDA receptor
L-type calcium channel
T-type calcium channel
PKA
PKC

ABSTRACT

Hydrogen sulfide (H_2S) is now known as a new biological mediator. In the present study, the effects of H_2S on intracellular calcium ($[Ca^{2+}]_i$) in neuronal SH-SY5Y cells was investigated. In SH-SY5Y neuronal cells, NaHS, a H_2S donor, concentration-dependently increased $[Ca^{2+}]_i$. The H_2S-induced Ca^{2+} elevation was significantly attenuated by EGTA-treated calcium-free Krebs' solution. This elevation was also reduced by antagonists of L-type (verapamil and nifedipine), T-type (mibefradil) calcium channels and N-methyl-D-aspartate receptor (MK-801, AP-5 and ifenprodil). A 90% reduction in H_2S-induced $[Ca^{2+}]_i$ elevation was found in cells pretreated with combination of all three kinds of inhibitors. Depletion of intracellular Ca^{2+} store with thapsigargin or cyclopiazonic acid or blockade of ryanodine receptor with ruthenium red significantly attenuated the effect of H_2S on $[Ca^{2+}]_i$. Inhibition of protein kinase A (PKA), phospholipase C (PLC) and protein kinase C (PKC) suppressed the H_2S-elevated $[Ca^{2+}]_i$, suggesting that H_2S may regulate $[Ca^{2+}]_i$ via both PKA and PLC/PKC pathways. In conclusion, it was found in this study that H_2S increased $[Ca^{2+}]_i$ in SH-SY5Y neuronal cells by increasing Ca^{2+} influx via plasma membrane and in turn releasing calcium from intracellular calcium store. The findings in the present study provide the direct evidence that H_2S may serve as a neuromodulator.

1. Introduction

H_2S has recently been defined as a new gaseous mediator alongside with nitric oxide and carbon monoxide. As a biologically active gas, H_2S exerts different functions at physiological relevant concentrations in different systems such as nervous (Han et al., 2005; Hu et al., 2007; Kimura, 2000) and cardiovascular systems (Bian et al., 2006; Pan et al., 2008; Yong et al., 2008). Endogenous H_2S is generated by at least two pyridoxal-dependent enzymes; explicitly cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). The expression of both enzymes is tissue specific. CSE is mainly expressed in cardiovascular system while CBS activity is much greater in brain (Wang, 2002). More recently, Shibuya et al. (2009) discovered another novel source of H_2S in brain, 3-mercaptopyruvate sulfurtransferase, which was shown to produce H_2S more efficiently than CBS does. The physiological concentration of H_2S in brain tissue is detected to be 50–160 μM, which is higher than the concentration of H_2S in rat serum (approximately 46 μM) (Wang, 2002). Physiological concentration of H_2S has been reported to increase the sensitivity of N-methyl-D-aspartate (NMDA) receptor and facilitate the induction of long-term potentiation (LTP), a synaptic model in learning and memory (Abe and Kimura, 1996). It was also found that H_2S may protect neurons from oxidative stress by increasing glutathione levels (Kimura and Kimura, 2004). Meanwhile, Whiteman et al. (2004) demonstrated H_2S inhibited peroxynitrite ($ONOO^-$)-mediated cytotoxicity to a similar extent as GSH. The potential anti-oxidant action of H_2S was attributed to the functional thiol group which can scavenge $ONOO^-$ (Halliwell et al., 1999). In our laboratory, we recently demonstrated that H_2S produces anti-neuroinflammatory, anti-oxidant and anti-apoptotic effects in neuron and glial cells (Hu et al., 2009, 2007; Lu et al., 2008). These findings may have important implications in the treatment of neurodegenerative diseases.

Calcium is an essential second messenger for neural functions, such as release of neurotransmitters, synaptic plasticity, neuronal excitation and gene transcription. Changes in intracellular calcium induced by modifying the activity of membrane ion channels may directly alter neuronal excitability. Elevation of $[Ca^{2+}]_i$ is also important in modifying synaptic plasticity such as LTP (Kimura, 2000). We recently found that H_2S regulates calcium homeostasis in microglial cells (Lee et al., 2006). Our findings support the possibility that H_2S may serve as a neuromodulator to facilitate signaling between neurons and microglial cells. Nagai et al. (2004) also demonstrated that H_2S can increase $[Ca^{2+}]_i$ and induce calcium waves in astrocytes but not in neurons. However, a recent study showed that prolonged treatment with H_2S may induce cell death via increasing cytosolic calcium level (Garcia-Bereguiain et al., 2008). The present study is therefore designed to determine

☆ Supporting Grant: National Medical Research Council (NMRC, 1183/2008).
* Corresponding author. Tel.: +65 6516 5502; fax: +65 6873 7690.
E-mail address: phcbjs@nus.edu.sg (J.-S. Bian).

0197-0186/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuint.2009.12.011

whether H_2S can dynamically regulate intracellular calcium in neurons, which may provide direct evidence for the neuromodulatory effect of H_2S.

2. Materials and methods

2.1. Chemicals

Sodium hydrosulfide (NaHS), (5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK-801), 2-amino-5-phosphonopentanoic acid (AP-5), thapsigargin, cyclopiazonic acid, nifedipine, ifenprodil, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride (H-89), N,N,N′,N′,-tetraacetic acid (EGTA), 5-[N-(3,4-dimethoxyphenylethyl)-methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvalero-nitrile hydrochloride (verapamil), mibefradil and U-73122 hydrate were obtained from Sigma–Aldrich (St. Louis, MO). Chelerythrine chloride was purchased from Calbiochem (Darmstadt, Germany). Fura-2 pentakis(acetoxymethyl) ester (Fura-2/AM) were obtained from Molecular Probes, Eugene, OR, USA. All chemicals were dissolved in water, apart from thapsigargin, nifedipine, verapamil and Fura-2/AM, which were dissolved in dimethylsulphoxide (DMSO).

Like numerous previous researchers, we have used NaHS as a donor for H_2S. When NaHS is dissolved in water, HS^- is released and forms H_2S with H^+. This provides a solution of H_2S with better defined concentration both in terms of accuracy and reproducibility when compared to a solution obtained by bubbling H_2S gas into water (Abe and Kimura, 1996; Nagai et al., 2004).

2.2. Cell cultures of SH-SY5Y neuroblastoma cell line

The human neuroblastoma SH-SY5Y cell line was used as cell model to neuronal cells. Cells were purchased from the American Tissue Culture Collection (ATCC, Rockville, MD, USA) and cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (streptomycin and penicillin). The medium was changed every day. The cells were cultured on sterilized glass coverslips one day prior calcium measurements.

2.3. Measurements of intracellular calcium level ($[Ca^{2+}]_i$) in neurons

Adherent cells cultured on sterilized coverslips were incubated with 4 μM fura-2/AM for 30 min in DMEM. The unincorporated dye was removed by washing the cells twice in fresh incubation solution. Loaded cells were maintained at room temperature for 30 min before measurements of $[Ca^{2+}]_i$ in order to allow any fura-2/AM in the cytosol to de-esterify. Cells loaded with fura-2/AM were transferred to the stage of an inverted microscope (Nikon Ltd., Japan) in a superfusion chamber at room temperature. The inverted microscope was coupled with a dual-wavelength excitation spectrofluorometer (Intracellular Imaging Inc, USA). Cells were perfused with Krebs' bicarbonate buffer (KB buffer, mM; 117 NaCl, 5 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 1.25 $CaCl_2$, 25 $NaHCO_3$, 11 glucose, pH 7.4) or EGTA-treated calcium-free Kreb's bicarbonate buffer (mM; 2 EGTA, 117 NaCl, 5 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 11 glucose, pH 7.4). Drugs were added directly into the bathing solution during calcium measurement and the change in fluorescent intensity was monitored.

The ratio of fluorescent signals obtained at 340 nm (F_{340}) and 380 nm (F_{380}) excitation wavelengths were recorded. F_{340}/F_{380} ratio (R) was used to represent the $[Ca^{2+}]_i$ in the cells. R/R_0 was employed to assess the change in $[Ca^{2+}]_i$, where R_0 represents the fluorescent signal before drug treatment and R represents the fluorescent signal after drug treatment.

2.4. Cytotoxicity assay

Toxicity of NaHS on SH-SY5Y cells was determined using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoleum (MTT) assay (Sigma–Aldrich). Briefly, cells were seeded in 96-well plates, allowed to attach overnight. Cells were later treated with 200 μM NaHS or drug-free medium for 1.5 h. At the end of each treatment, MTT was added to each well at a final concentration of 0.5 mg/ml and the cells were further incubated at 37 °C for 4 h. Then, the insoluble formazan was dissolved with dimethyl sulfoxide (DMSO); colorimetric determination of MTT reduction was measured at 570 nm with a reference wavelength at 630 nm. Control cells treated with vehicle (DMEM) were taken as 100% viability.

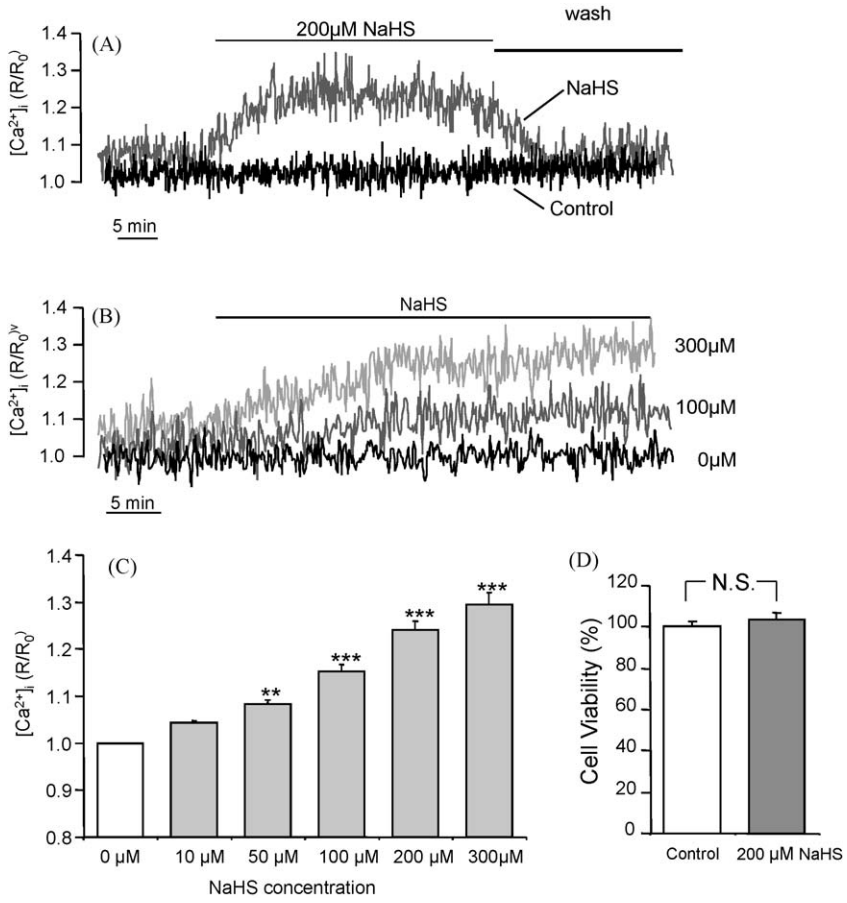

Fig. 1. NaHS increased $[Ca^{2+}]_i$ in SH-SY5Y neuronal cells. (A) Representative tracing showing that application of NaHS (200 μM) increased $[Ca^{2+}]_i$ and washout of NaHS reversed $[Ca^{2+}]_i$ back to baseline. (B) Representative tracing showing the effect of different concentrations of NaHS (100 and 300 μM) on $[Ca^{2+}]_i$. (C) Group data showing the concentration-dependent effect of NaHS on $[Ca^{2+}]_i$. $^{**}p < 0.01$, $^{***}p < 0.001$ vs control, n = 38–56. (D) MTT assay showing that NaHS at 200 μM had no effect on cell viability of SH-SY5Y cells, N.S.: not significant, n = 10 in each group.