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Hydrogen Sulfide Inhibits Chronic Unpredictable Mild Stress-Induced Depressive-Like Behavior by Upregulation of Sirt-1: Involvement in Suppression of Hippocampal Endoplasmic Reticulum Stress

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Abstract

Background: Hydrogen sulfide (H_2S) is a crucial signaling molecule with a wide range of physiological functions. Previously, we confirmed that stress-induced depression is accompanied with disturbance of H_2S generation in hippocampus. The present work attempted to investigate the inhibitory effect of H_2S on chronic unpredictable mild stress-induced depressive-like behaviors and the underlying mechanism.
Methods: We established the rat model of chronic unpredictable mild stress to simulate depression. Open field test, forced swim test, and tail suspension test were used to assess depressive-like behaviors. The expression of Sirt-1 and three marked proteins related to endoplasmic reticulum stress (GRP-78, CHOP, and cleaved caspase-12) were detected by western blot.
Results: We found that chronic unpredictable mild stress-exposed rats exhibit depression-like behavior responses, including significantly increased immobility time in the forced swim test and tail suspension test, and decreased climbing time and swimming time in the forced swim test. In parallel, chronic unpredictable mild stress-exposed rats showed elevated levels of hippocampal endoplasmic reticulum stress and reduced levels of Sirt-1. However, NaHS (a donor of H_2S) not only alleviated chronic unpredictable mild stress-induced depressive-like behaviors and hippocampal endoplasmic reticulum stress, but it also increased the expression of hippocampal Sirt-1 in chronic unpredictable mild stress-exposed rats. Furthermore, Sirtinol, an inhibitor of Sirt-1, reversed the protective effects of H_2S against chronic unpredictable mild stress-induced depression-like behaviors and hippocampal endoplasmic reticulum stress.

Significance Statement

Depression is a chronic and recurrent serious mental disorder that affects about 10% of the world population and imposes a substantial societal burden. Therefore, understanding the prevention bases and looking for a future therapeutic target on depression are extremely urgent. The present work is to investigate the inhibitory action of H_2S in CUMS-induced depression-like behaviors and the underlying mechanisms. In our study, we found that H_2S ameliorated CUMS-induced depression-like behaviors, involving inhibition of hippocampal ER stress, by upregulation of hippocampal Sirt-1. Findings from our present work identify H_2S as a potential target for therapeutic intervention in depression.

Conclusion: These results demonstrated that H_2S has an antidepressant potential, and the underlying mechanism is involved in the inhibition of hippocampal endoplasmic reticulum stress by upregulation of Sirt-1 in hippocampus. These findings identify H_2S as a novel therapeutic target for depression.

Keywords: chronic unpredictable mild stress, depression, endoplasmic reticulum stress, hydrogen sulfide, silent mating type information regulation 2 homolog 1

Introduction

Depression, a chronic and recurrent serious mental disorder characterized by loss of pleasure, mood disturbance, and suicidal tendencies (Tanti and Belzung, 2010), affects over 10% of the world's population and imposes a substantial societal burden (Ferrari et al., 2014). Moreover, the treatment of depression has attracted great attention around the world (Ferrari et al., 2013). Unfortunately, the existing treatments are too often ineffective and fail to resolve symptoms completely (Liu et al., 2011). Therefore, novel therapeutic targets on depression still need to be addressed.

Hydrogen sulfide (H_2S) is recognized as a crucial signaling molecule with a wide range of roles in the development of normal physiological conditions and many kinds of diseases (Kuksis et al., 2014; Sikora et al., 2014; Zhang and Bian, 2014; Wallace and Wang, 2015). Interestingly, H_2S has been reported to enhance synaptic transmission and facilitate the induction of long-term potentiation (Abe and Kimura, 1996). It is widely accepted that dysfunction of synaptic plasticity contributes to the pathophysiology of depression (Duman, 2002; Duman and Aghajanian, 2012). Furthermore, our recent results have demonstrated that stress-induced depression is accompanied with disturbance of H_2S generation in hippocampus (Tan et al., 2015). Taken together, we hypothesize that H_2S might serve as a novel antidepressant gaseous mediator. Thus, in the present work, the antidepressant-like potential of H_2S was evaluated by behavioral alterations caused by chronic unpredictable mild stress (CUMS).

Endoplasmic reticulum (ER) stress, a state in which the unfolded and misfolded protein accumulation affects the normal physiological function of cells, refers to the excessive stress caused by dysfunction of the endoplasmic reticulum (Boyce and Yuan, 2006; Kim et al., 2008). Previous studies attested that ER stress is a critical step in the pathology of neurodegeneration disease (Stefani et al., 2012; Roussel et al., 2013) as well as in the pathogenesis of CUMS-elicited depression (Pavlovsky et al., 2013; Zhang et al., 2014). In fact, the potential anti-ER stress effects of H_2S has been a focus in the research (Xie et al., 2012; Li et al., 2014; Wei et al., 2014). These results prompted us to investigate whether H_2S reserved CUMS-provided ER stress to understand the mechanisms underlying the antidepressant-like action of H_2S.

Silent mating type information regulation 2 homolog 1 (Sirt-1), an NAD^+-dependent histone deacetylase, is an essential metabolic regulatory transcription factor (Nemoto et al., 2005; Rodgers et al., 2005; Lagouge et al., 2006; Canto et al., 2009) that regulates physiological processes by the acetylation and deacetylation cycle (Yamamoto et al., 2007; Pillarisetti, 2008). In addition to cellular regulation, emerging evidence supported that Sirt-1 has neuroprotective properties (Parker et al., 2005; Kim et al., 2007; Avraham et al., 2010; Ho et al., 2010). Extensive studies have focused on the essential role of Sirt-1 in synaptic plasticity (Gao et al., 2010; Herranz et al., 2010; Michan et al., 2010). Moreover, recent studies demonstrated that chronic stress-induced depression contributes to the reduction of hippocampal Sirt-1 activity (Abe-Higuchi et al., 2016), which indicated that Sirt-1is an anti-depressant-like factor. Additionally, our previous work has proved that H_2S inhibits formaldehyde-induced ER stress in PC12 cells via upregulating Sirt-1 expression (Li et al., 2014). Therefore, we assumed that Sirt-1 is indispensable for the protective effect of H_2S against CUMS-induced ER stress and depressive-like behaviors.

Therefore, the present study was undertaken to elucidate whether H_2S ameliorates depressive-like behaviors in CUMS-exposed rats and whether Sirt-1 mediates the antidepressant-like potential of H_2S by suppressing hippocampal ER stress. In this study, we showed that H_2S inhibits CUMS-induced depressive-like behaviors and hippocampal ER stress, while it upregulates the expression of hippocampal Sirt-1. We further demonstrated that blocking Sirt-1 reverses the protective effects of H_2S in depression-like behaviors and hippocampal ER stress. Our results identified H_2S as a potential antidepressant molecule, which is involved in inhibition of hippocampal ER stress via upregulating hippocampal Sirt-1.

MATERIALS AND METHODS

Animals

Healthy adult male Wistar rats, weighing 200 to 220 g (n = 180) (SJA Laboratory Animal Co., Ltd) were housed in a temperature-controlled room under a 12-h-light/12-h-dark cycle (lights on at 7:00 AM) with free access to food and water. We made efforts to reduce the number and suffering of the animals. Additionally, all experimental operations were carried out in accordance with the National Animal Health and Management Regulations of the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Use and Protection Committee of the University of South China.

Antibodies and Reagents

NaHS (a donor of H_2S), Imipramine, dimethyl sulfoxide, specific monoclonal anti-CHOP antibody, and Sirtinol were purchased from Sigma. Imipramine was dissolved in phosphate buffered saline (PBS) as solution and was administered i.p. of 15 mg/kg. Specific monoclonal anti-GRP-78 antibody and anti-Sirt-1 antibody were purchased from Epitomics. Specific monoclonal anti-cleaved caspase-12 antibody was produced by Santa Cruz Biotechnology. Beta-actin antibody, goat anti-mouse antibody, and goat anti-rabbit antibody were purchased from Proteintech.

Drug Treatments and Experimental Design

Rats were randomly divided into 8 treatment groups. In the control group (20 rats), rats were handled without stress for 4 weeks and injected with PBS (i.p.) and artificial CSF (ACSF) (intracerebroventricularly [i.c.v.]) in the last 2 weeks. In the CUMS group (24 rats), CUMS rats were exposed to a stressor daily for 2 weeks and then received 2 weeks of PBS i.p. injections during continuous 2-week CUMS treatment. In the CUMS + NaHS (30 μmol/kg or 100 μmol/kg) group (24 rats/group), CUMS rats were exposed to a stressor for 4 weeks and injected i.p. with 30 μmol/kg/d or 100 μmol/kg/d NaHS for the last 2 weeks. In the CUMS + imipramine (15 mg/kg) group (24 rats), CUMS rats were injected i.p. with 15 mg/kg/d imipramine for the last 2 weeks of the 4-week CUMS exposure. In the CUMS + NaHS (100 μmol/kg) + Sirtinol (10 nmol) group (24 rats), CUMS rats were cotreated with 100 μmol/kg/d NaHS (i.p.) and 10 nmol Sirtinol (i.c.v.) for the last 2 weeks of the 4-week CUMS procedure. In the 100 μmol/kg/d NaHS-alone group and 10 nmol Sirtinol alone-treated group (20 rats/group), rats received conventional treatment for 2 weeks and were injected with NaHS (100 μmol/kg/d, 14 days, i.p.) and Sirtinol (10 nmol, 14 days, i.c.v.), respectively. Sirtinol was diluted in sterilized ACSF/50% dimethyl sulfoxide and i.c.v. injected at a dose of 10 nmol. The depression-like behavioral tests were performed 24 hours after the last injection. Only rats with depression-like behaviors were used for subsequent studies; about 10% of rats were excluded. Then, 1 day after the behavioral tests, the hippocampus region tissues were rapidly collected and stored at –80°C for analysis (Figure 1). Data collection and analysis in the experiments were performed in a blinded fashion.

CUMS Procedure

The CUMS procedure was carried out as previously reported (Nollet et al., 2012) with minor modifications. In brief, rats in the CUMS group were exposed to a variety of mild stressors: (1) cold swim at 5°C for 5 minutes, (2) food deprivation for 24 hours, (3) water deprivation for 24 hours, (4) soiled cage (200 mL water in 100 g sawdust bedding) for 12 hours, (5) alteration of light and dark cycles for 24 hours, (6) tail pinching for 1 minute (7) stroboscope (120 flashes/min) for 2 hours; (8) tail suspension for 5 minutes; (9) unpredictable foot shocks (0.4 mA, one shock/0.5 s, 10-second duration) and restricted movement for 10 to 11 seconds. Rats randomly received 3 of these stressors per day in the sequence shown above for 4 weeks. However, the same stressor was not applied continuously to avoid being predicted by rats.

Implantation of the i.c.v. Cannula

Rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p., Sigma) and then mounted on a stereotaxic apparatus for operation. Trimming the incision along the midline of the head and exposing the bregma to determine the lateral ventricle. An aseptically cannula was implanted into the right side of the lateral ventricle for drug administration using the coordinates: AP, -1.0 mm; ML, -1.5 mm; DV, -3.0 mm (Paxinos). To ensure the drug had been completely delivered, the injection needle should be slowly pulled out and kept in position for an additional 2 minutes before being removed. The rats were kept warm under a lamp until they recovered from anesthesia. After the surgery, all rats received antiphlogistic penicillin once per day for 3 days. All the experiments were randomized and observed by an experimenter who was unaware of the treatment.

Behavioral Test

Open Field Test (OFT)

To assess the possible effects of drug treatment on spontaneous locomotor activity, the animals were exposed to the open-field paradigm, as previously described (Cai et al., 2010). The rat was placed in a black box (270 mm × 270 mm × 380 mm) individually into the center of the arena and was permitted free exploration after 12 minutes of adaption. The number of crossings and the total distance were recorded during a 5-minute test. Meanwhile,
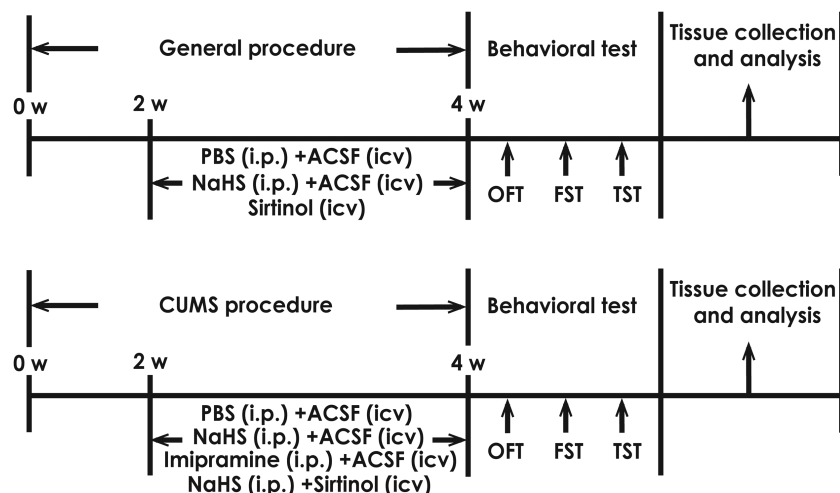
Figure 1. Schematic diagram of the experimental schedule. Rats were given the corresponding drug treatment in the last 2 weeks of the 4-week general handling or chronic unpredictable mild stress (CUMS) exposure. ACSF, artificial CSF; OFT, open field test; TST, tail suspension test; FST, forced swimming test; i.c.v., intracerebroventricular injection.

the box was cleansed with medical alcohol after occupancy by each rat to eliminate the effect of odors. The activities of the rats were analyzed by using spontaneous activity video analysis software.

Forced Swimming Test (FST)

The FST was established according to the protocol described in detail in Porsolt et al. (1977) with some modifications. Rats were individually forced to swim for 15 minutes in a transparent plastic vessel (diameter 18 cm, height 50 cm) filled with 30 cm of water (24 ± 1°C). Twenty-four hours later, an official test was carried out in the same condition to determine the immobility time in 5 minutes. Each rat was forced to swim for 5 minutes, and the duration of immobility, climbing, and swimming was measured during the 5 minutes. The definition of immobility was the absence of all movements except motions required to maintain the rat's head above the water.

Tail Suspension Test (TST)

The TST was conducted as previously described (Steru et al., 1985). Briefly, each rat was individually suspended 50 cm above the floor by adhesive tape placed approximately 2 cm from the tip of the tail for 5 minutes hanging on a hook. Rats were considered as immobile when they were passively suspended and remained completely motionless. The immobility represents a failure of persistence in escape-directed behavior and was considered to be a struggling helpless state response (Moretti et al., 2014).

Western-Blot Analysis

Rats were sacrificed 1 day after the behavioral test. Hippocampus tissue of the brain was dissected and then homogenized to collect the supernatant. Subsequently, the protein content was assayed by BCA protein assay kit (Beyotime) and then transferred to the polyvinylidene fluoride membrane (Merck Millipore Ltd.) after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The protein membranes were blocked for 2 hours at room temperature with 5% skim milk in Tris Buffered Saline containing Tween 20. Membranes were incubated with primary antibody (anti-Beta actin, 1:2000; anti-CHOP, 1:500; anti-GRP-78, 1:1000; and anti-cleaved caspase-12, 1:1000) in 5% bovine serum albumin, 1× Tris Buffered Saline, and 0.05% Tween-20 at 4°C with gentle shaking overnight. After that, membranes were washed and then incubated with secondary antibody for 2 hours. Finally, the membranes were visualized by an image analysis system equipped with a software BIO-ID (Vilber Lourmat) and protein band was calculated with the integrated optical density by AlphaImager 2200 software.

Statistical Analysis

Statistical analysis was carried out using SPSS 16.0 software for Windows (SPSS Inc.). All values are expressed as means ± SEM. Data was analyzed by 2-way ANOVA. The level of significance was set at $P < .05$.

RESULTS

H_2S Inhibits CUMS-Induced Depression-Like Behaviors

To determine whether H_2S inhibits CUMS-induced depression-like behaviors, we evaluated TST and FST. The effects for stress ($F_{1, 60} = 95.81$, $P<.001$; $F_{1, 50} = 24.17$, $P < .001$; $F_{1, 49} = 1.62$, $P = .209$), treatment ($F_{4, 60} = 10.16$, $P < .001$; $F_{4, 50} = 4.57$, $P = .003$; $F_{4, 49} = 1.42$, $P = .242$), and stress × treatment interaction ($F_{4, 60} = 1.87$, $P = .127$; $F_{4, 50} = 2.31$, $P=.071$; $F_{4, 49}=2.39$, $P=.064$) (Figure 2A–C) in immobility, swimming, and climbing time of the FST were revealed by 2-way ANOVA, respectively. In the FST, the duration of immobility in CUMS-exposed rats was increased (Figure 2A; $P < .01$) and the swimming time (Figure 2B; $P < .01$) was decreased compared with control rats, while the climbing time (Figure 2C, $P > .05$) was not changed. However, treatment with NaHS (30, 100 μmol/
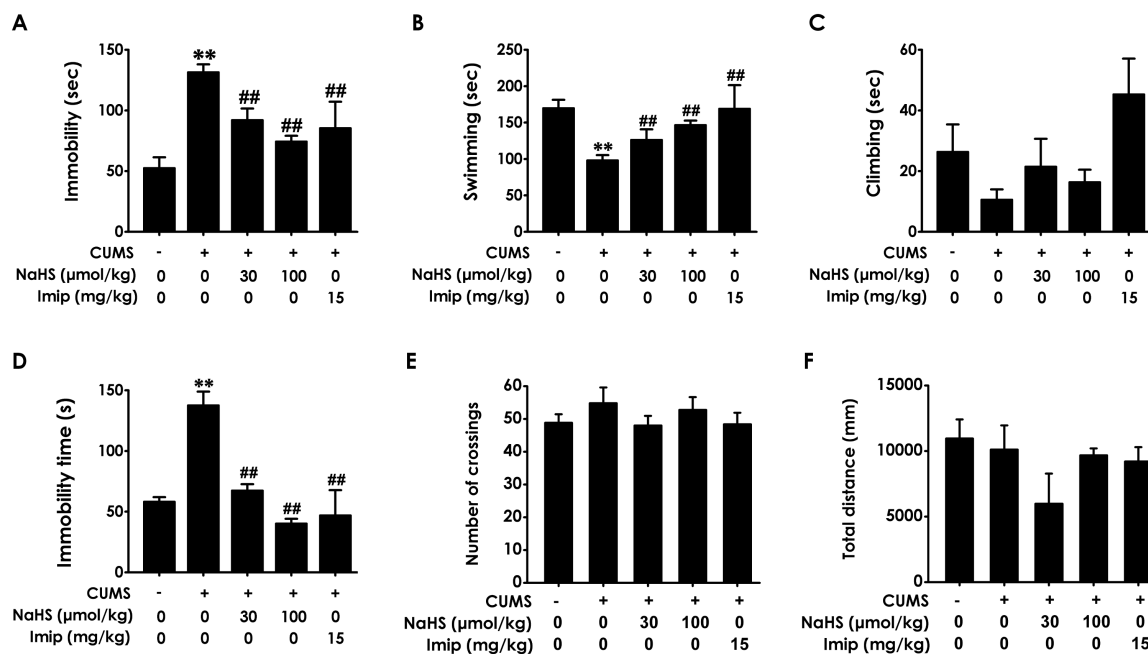
Figure 2. Effects of H_2S on the depressive-like behaviors of chronic unpredictable mild stress (CUMS)-exposed rats. After 2-week CUMS exposure, the rats were further treated with CUMS in the absence or presence of NaHS (30, 100 μmol/kg/d, i.p.) or imipramine (15 mg/kg/d, i.p.) for 2 weeks. Rats performed the forced swimming test (FST) (A-C), tail suspension test (TST) (D), and open field test (OFT) (E-F). Values are the mean ± SEM (n = 8-10/group). **$P < .01$ vs control group; ##$P < .01$ vs CUMS group.

kg/d, 2 weeks) significantly reduced immobility time ($P < .01$) and increased the swimming time ($P < .01$) in CUMS-exposed rats as well as treatment with imipramine, indicating that H_2S exerts an antidepressant-like effect. In the TST, the 2-way ANOVA revealed significant effects for stress ($F_{1, 64} = 51.58$, $P < .001$) and treatment ($F_{4, 64} = 11.80$, $P < .001$), but there is no significant stress × treatment interaction ($F_{4, 64} = 1.16$, $P = .338$) (Figure 2D). NaHS (30, 100 µmol/kg/d, 2 weeks) and imipramine obviously reduced the immobility time in CUMS-exposed rats (Figure 2D; $P < .01$), which presents evidence to further confirm the antidepressant-like effect of H_2S.

To exclude the possible effect of H_2S on spontaneous locomotor activity, which may contribute to the decreased immobility in the FST and TST, rats were exposed to the open field apparatus for 5 minutes (Zhou et al., 2011). Two-way ANOVA showed no significant effects for stress, treatment, and stress × treatment interaction on either the number of crossing ($F_{1, 64} = 1.18$, $P = .282$; $F_{4, 64} = 0.33$, $P = .855$; $F_{4, 64} = 1.96$, $P = .111$; Figure 2E) or the total distance ($F_{1, 58} = 3.48$, $P = .067$; $F_{4, 58} = 0.87$, $P = .487$; $F_{4, 58} = 1.79$, $P = 0.144$; Figure 2F) in the OFT, which confirmed that antidepressant-like effects of both H_2S and imipramine were not a nonspecific consequence of hypolocomotion. Additionally, the original design using H_2S alone does not differ from the behavioral test (data not shown).

H_2S Prevents the Hippocampal ER Stress in CUMS-Exposed Rats

Three marker proteins indicating the ER stress level, GRP-78, CHOP, and cleaved caspase-12, were monitored by western blot to explore the effects of H_2S on CUMS-induced hippocampal ER stress. For hippocampal GRP-78, CHOP, and cleaved caspase-12 levels, 2-way ANOVA showed significant effects for stress ($F_{1, 20} = 52.47$, $P < .001$; $F_{1, 20} = 91.16$, $P < .001$; $F_{1, 20} = 30.21$, $P < .001$), treatment ($F_{4, 20} = 11.92$, $P < .001$; $F_{4, 20} = 12.74$, $P < .001$; $F_{4, 20} = 23.82$, $P < .001$), and stress × treatment interaction ($F_{4, 20} = 1.33$, $P = .294$; $F_{4, 20} = 2.28$, $P = .096$; $F_{4, 20} = 0.19$, $P = .94$) (Figure 3), respectively. The GRP-78 (Figure 3A), CHOP (Figure 3B), and cleaved caspase-12 (Figure 3C) expressions in the hippocampus of rats exposed to CUMS for 4 weeks were significantly increased ($P < .01$), while treatment with NaHS (30, 100 µmol/kg/d, 2 weeks, i.p.) markedly decreased the expressions of GRP-78, CHOP, and cleaved caspase-12 ($P < .01$); moreover, treatment with NaHS (100 µmol/kg/d, 2 weeks, i.p.) alone did not change the expressions of GRP-78 ($P > .05$), CHOP ($P > .05$), and cleaved caspase-12 ($P > .05$) in control rats, which indicated that H_2S could inhibit hippocampal ER stress induced by CUMS.

H_2S Upregulates Sirt-1 Expression in the Hippocampus of CUMS-Exposed Rats

To explore the role of Sirt-1 in the antidepressant-like actions of H_2S, we first investigated the effect of H_2S on the expression of Sirt-1 in the hippocampus of rats. As shown in Figure 4, 2-way ANOVA revealed significant effects for stress ($F_{1, 20} = 33.47$, $P < .001$) and treatment ($F_{4, 20} = 22.33$, $P < .001$), but not for stress × treatment interaction ($F_{4, 20} = 1.19$, $P = .346$) in the hippocampus. After 4-week exposure of CUMS, the expression of Sirt-1 in the hippocampus was significantly
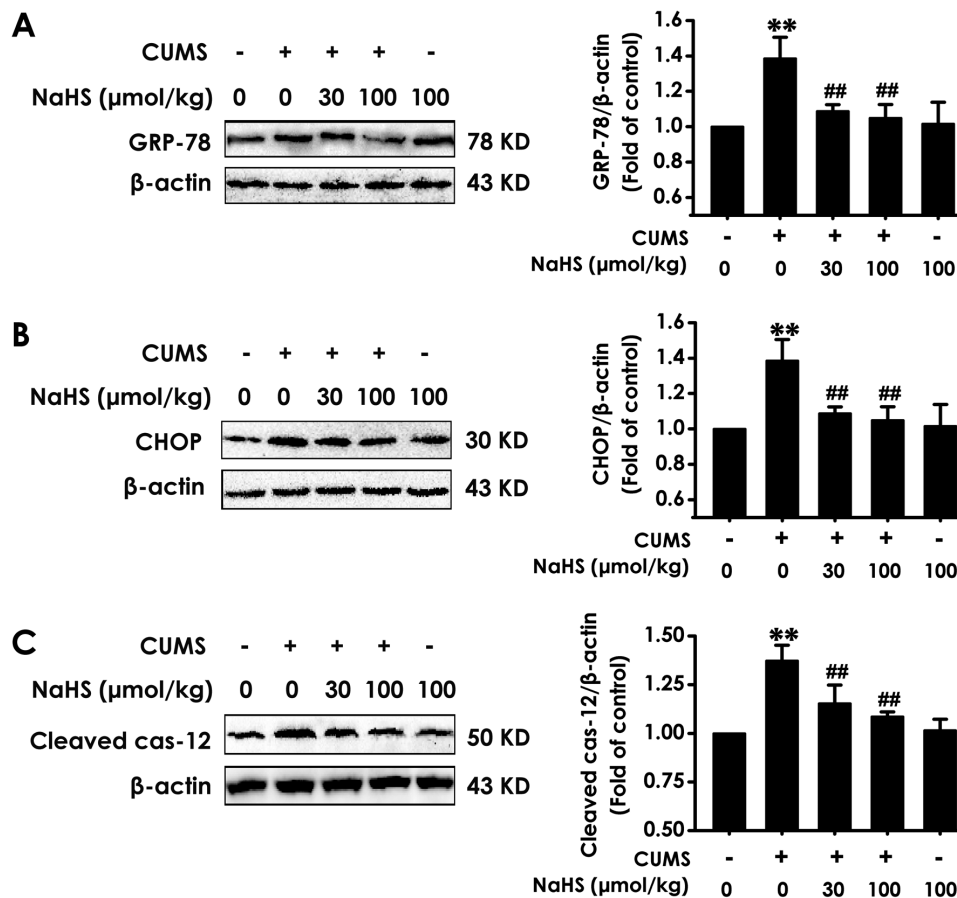

Figure 3. Effects of H_2S on expressions of CHOP, GRP-78, and cleaved caspase-12 in the hippocampus of chronic unpredictable mild stress (CUMS)-exposed rats. After treatment with CUMS for 2 weeks, the rats were further treated with CUMS in the absence or presence of NaHS (30, 100 µmol/kg/d, i.p.) for 2 weeks. GRP-78 (A), CHOP (B), and cleaved caspase-12 (C) in hippocapus of rats were detected by western blot. Values are the mean ± SEM (n = 3/group). **$P < .01$ vs control group; ##$P < .01$ vs CUMS group.

decreased ($P < .01$), while treatment with NaHS (30, 100 µmol/kg/d, 2 wekks, i.p.) reversed the downregulation of Sirt-1 ($P < .01$) induced by CUMS, indicating that H_2S upregulates Sirt-1 expression in the hippocampus of CUMS-exposed rats. In addition, treatment with NaHS (100 µmol/kg/d, 2 weeks, i.p.) did not change the levels of Sirt1 ($P > .05$) in control rats. These data suggested H_2S upregulates the hippocampal Sirt1 in CUMS-exposed rats.

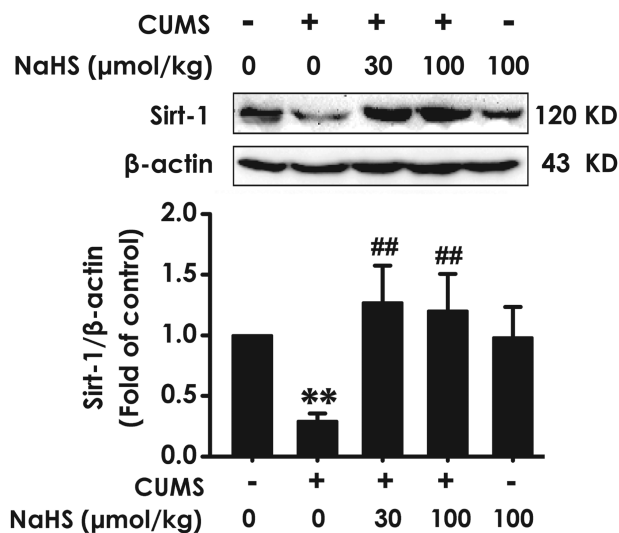**Figure 4.** Effects of H_2S on the hippocampal Sirt-1 expression in chronic unpredictable mild stress (CUMS)-exposed rats. After treatment with CUMS for 2 weeks, the rats were further treated with CUMS in the absence or presence of NaHS (30, 100 µmol/kg/d, i.p.) for 2 weeks. Sirt-1 expression in hippocampus was detected by western blot. Values are the mean ± SEM (n = 3/group). $^{**}P < .01$ vs control group; $^{##}P < .01$ vs CUMS group.

Inhibition of Sirt-1 by Sirtinol Reverses the Antidepressant-Like Effects of H_2S

To further confirm the mediatory role of Sirt-1 in the antidepressant-like effects of H_2S, we investigated whether Sirtinol, an inhibitor of Sirt-1, reverses the antidepressant-like effects of H_2S. Rats were exposed to CUMS for 4 weeks and treated with NaHS (100 µmol/kg/d, i.p.) in the absence or presence of Sirtinol (10 nmol/d, i.c.v.) for the last 2 weeks of 4-week CUMS. For immobility, swimming, and climbing time in the FST, 2-way ANOVA showed significant effects for Sirtinol ($F_{1, 28} = 11.45$, $P = .026$; $F_{1, 33} = 6.05$, $P = .019$; $F_{1, 32} = 0.70$, $P = .409$), H_2S ($F_{1, 28} = 7.12$, $P = .013$; $F_{1, 33} = 11.01$, $P = .002$; $F_{1, 32} = 2.74$, $P = .108$), and Sirtinol × H_2S interaction ($F_{1, 28} = 11.45$, $P = .002$; $F_{1, 33} = 15.58$, $P < .001$; $F_{1, 32} = 0.001$, $P = .975$) (Figure 3), respectively (Figure 5A–C). Sirtinol (10 nmol/d, i.c.v.) dramatically prevented H_2S from shortening immobility (Figure 5A; $P < .01$) and increasing the duration of swimming (Figure 5B; $P < .01$) in the FST as well as shortening immobility in the TST (Fig. 5D; $P < .01$), whereas it did not change the climbing time (Figure 5C; $P > .05$) in the FST. For the TST, 2-way ANOVA revealed significant main effects for Sirtinol ($F_{1, 31} = 7.98$, $P = .008$) and H_2S ($F_{1, 31} = 2.91$, $P = .098$) with a significant Sirtinol × H_2S interaction ($F_{1, 31} = 29.27$, $P < .001$) (Figure 5D). Additionally, Sirtinol (10 nmol/d, 2 weeks, i.c.v.) treatment alone did not affect both the immobility, swimming, and climbing time in FST and the immobility time in the TST, indicating that the inhibitory role of Sirtinol on the antidepressive action of NaHS was not due to the possibility that Sirtinol affected the normal ability of swimming and climbing. These data indicated that inhibition of Sirt-1 by Sirtinol reverses the antidepressant-like effects of H_2S. Moreover, treatment with Sirtinol did not affect the total distance of spontaneous activity and the number of crossing in the OFT (Figure 5E,F; $P > .05$), which further rule out nonspecific motoric effects of Sirtinol that influence activity in the FST and TST.
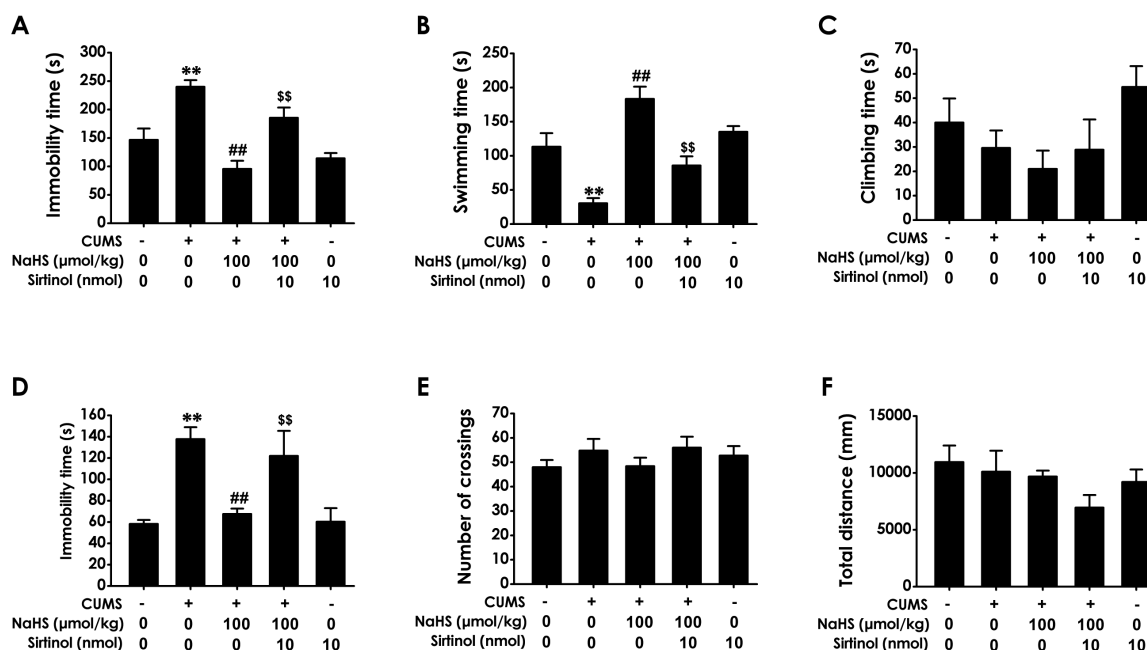

Figure 5. Effects of Sirtinol on the antidepressant-like actions of H_2S in chronic unpredictable mild stress (CUMS)-exposed rats. Rats were exposed to CUMS for 4 weeks and treated with NaHS (100 µmol/kg/d, i.p.) in the absence or presence of Sirtinol (10 nmol/d, i.c.v.) for the last 2 weeks of 4-week CUMS. The forced swimming test (FST) (A-C), tail suspension test (TST) (D), and open field test (OFT) (E-F) were used to assess the depressive-like behaviors of rats. Values are the mean ± SEM (n = 8–10/group). $^{**}P < .01$ vs control group; $^{##}P < .01$ vs CUMS group; $^{$$}P < .01$ vs cotreatment with CUMS and NaHS group.
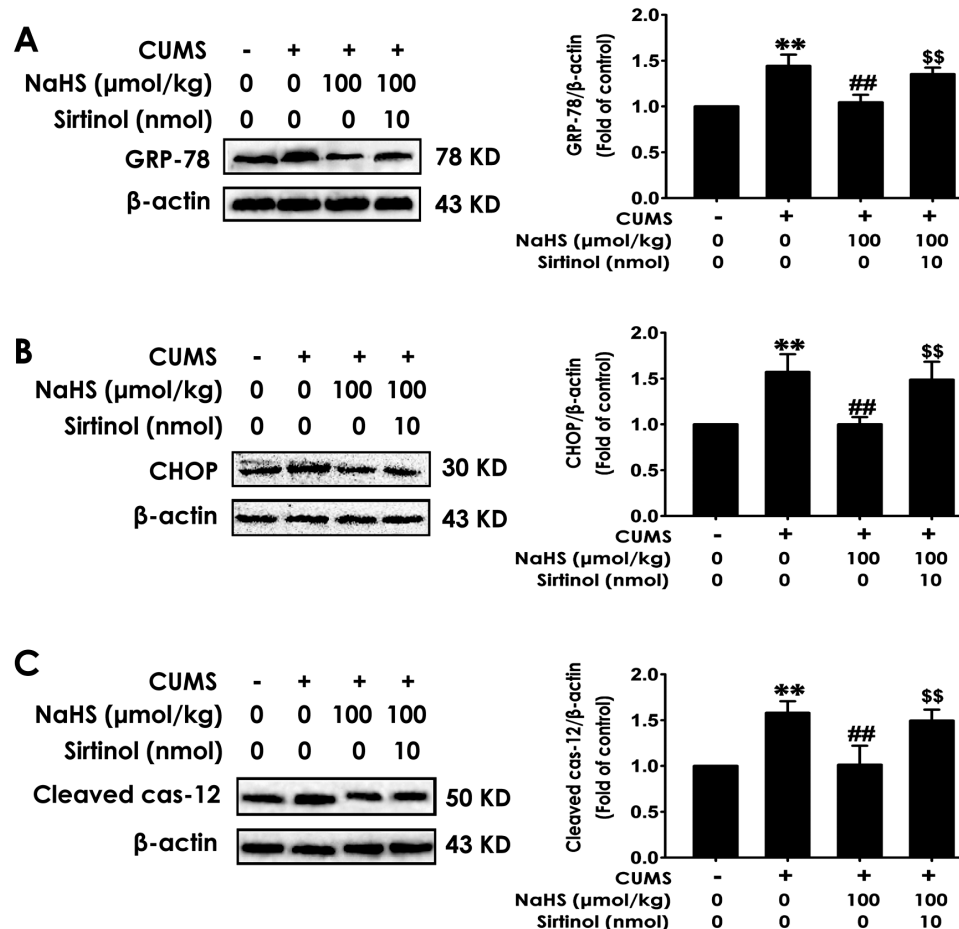
Inhibition of Sirt-1 by Sirtinol Attenuates the Protective Effect of H_2S against CUMS-Induced ER Stress

To elaborate the mediatory role of Sirt-1 in the protection of H_2S against CUMS-induced hippocampal ER stress, we detected whether Sirtinol, an inhibitor of Sirt-1, abolishes the protective role of H_2S against hippocampal ER stress in CUMS-exposed rats. After exposing to CUMS for 2 weeks, rats were cotreated with CUMS and NaHS (100 µmol/kg/d, i.p.) in the absence or presence of Sirtinol (10 nmol/d, i.c.v.) for 2 weeks. For ER stress-related proteins, GRP78, CHOP, and cleaved caspase-12, 2-way ANOVA showed significant effects for Sirtinol ($F_{1, 11} = 13.63$, $P = .004$; $F_{1, 11} = 10.78$, $P = .007$; $F_{1, 11} = 20.74$, $P = .001$), H_2S ($F_{1, 11} = 5.94$, $P = .033$; $F_{1, 11} = 20.33$, $P = .001$; $F_{1, 11} = 13.12$, $P = .004$), and Sirtinol × H_2S interaction ($F_{1, 11} = 8.75$, $P = .013$; $F_{1, 11} = 44.37$, $P < .001$; $F_{1, 11} = 50.27$, $P < .001$) (Figure 6), respectively. Sirtinol (10 nmol/d, 2 weeks, i.c.v.) significantly eliminated NaHS (100 µmol/kg, 2 weeks, i.p.), reduced the expressions of GRP-78 (Figure 6A, $P < .01$) and CHOP (Figure 6B, $P < .01$), and cleaved caspase-12 (Figure 6C, $P < .01$) in hippocampus of CUMS-exposed rats, while treatment with Sirtinol (10 nmol/d, 2 weeks, i.c.v.) alone did not affect the GRP-78 (Figure 6A, $P > .05$), CHOP (Figure 6B, $P > .05$), and cleaved caspase-12 (Figure 6C, $P > .05$) levels in control rats, which documented that inhibition of Sirt-1 reverses the antidepressive effect of H_2S on CUMS-induced hippocampal ER stress.

Discussion

The limited efficacy and serious side effects of current antidepressant drugs emphasize the demand for better approaches to the treatment of depression (Tanti and Belzung, 2010; Ferrari et al., 2014). The present study establishes the antidepressant potential action of H_2S in CUMS-induced depressive-like behaviors. Our findings are as follows: (1) H_2S attenuates CUMS-induced depressive-like behaviors; (2) H_2S prevents the level of ER stress in the hippocampus of CUMS-exposed rats; (3) H_2S upregulates the expression of Sirt-1 in the hippocampus of CUMS-exposed rats; and (4) inhibited Sirt-1 interrupts H_2S-offered antidepressant and anti-ER stress effects in CUMS-exposed rats. These results identify H_2S is a potential inhibitor of CUMS-induced depressive-like behaviors as a result of attenuating hippocampal ER stress via upregulation of hippocampal Sirt-1.

H_2S involves a range of biological and physiological functions, especially in the neurodegenerative diseases (Jia et al., 2013; Liu et al., 2013; Kuksis et al., 2014; Sikora et al., 2014; Wei et al., 2014; Zhang and Bian, 2014). In the present work, we performed a rat model of CUMS to confirm the antidepressant-like properties of H_2S and the underling mechanism. We found that exposure of rats to CUMS significantly increased the immobility time in FST and TST, and decreased the climbing time and swimming time in FST, which indicated that CUMS causes depressive-like

Figure 6. Effects of Sirtinol on the inhibitory role of H_2S on chronic unpredictable mild stress (CUMS)-induced hippocampal endoplasmic reticulum (ER) stress. After treatment with CUMS for 2 weeks, rats were cotreated with CUMS and NaHS (100 µmol/kg/d, i.p.) in the absence or presence of Sirtinol (10 µmol/d, i.c.v.) for 2 weeks. The expressions of GRP-78 (A), CHOP (B), and cleaved caspase-12 (C) in hippocampus were detected by western blot. Values are the mean ± SEM (n = 3/group). ** $P < .01$ vs control group; ## $P < .01$ vs CUMS group; \$\$$P < .01$ vs cotreatment with CUMS and NaHS group.

behaviors. Novelty, NaHS, a donor of H_2S, suppressed these depressive-like behaviors in CUMS-exposed rats, which verified the antidepressant-like activities of H_2S. Our results strengthen and expand the previously established links between H_2S and depression (Chen et al., 2013; Tang et al., 2015).

It has been confirmed that there are significant relationships between ER stress response and neuropsychiatric disorders such as major depressive disorder (Bown et al., 2000) and bipolar disorder (So et al., 2007; Hayashi et al., 2009). Recently, evidence demonstrated that ER stress-related proteins are also increased in the hippocampus of chronic social defeat stress- (Huang et al., 2013) and CUMS- (Tan et al., 2015) treated animal depression models. These previous findings suggest the hippocampal ER stress is involved in the pathological process of exogenous stress-induced depression. Furthermore, Ishisaka et al., 2011) reported that the antidepressant-like role of luteolin is via suppressing ER stress in hippocampus (Ishisaka et al., 2011). Interestingly, increasing evidence suggested that H_2S is a protective gaseous signaling molecule by preventing ER stress (Wei et al., 2010; Chen et al., 2011; Wang et al., 2012). Therefore, regulation of ER stress is critical for understanding the contribution of H_2S to the inhibition in CUMS-induced depressive-like behaviors. We detected the influence of H_2S on the expression of ER stress-marked proteins, such as GRP-78, CHOP, and cleaved caspase-12, in CUMS rat model. We found that H_2S alleviates the upregulation of CHOP, GRP-78, and cleaved caspase-12 in the hippocampus of rats exposed to CUMS. Our outcomes indicated that the antidepressant potential action of H_2S is endowed with its significant neuroprotective properties antagonistic CUMS-offered hippocampal ER stress.

Recently, Sirt-1 has been identified to act as a defender of ER stress (Li et al., 2011; Jung et al., 2012) and function as a neuroprotectant (Han, 2009; Zhang et al., 2011; Donmez, 2012). Additionally, overexpression of Sirt-1 prevents axonal degeneration (Araki et al., 2004), but the suppression of Sirt-1 significantly exacerbates neuronal damage (Chen et al., 2005) and disturbs the information of synaptic plasticity (Michan et al., 2010). Moreover, it has been suggested that dysfunction of Sirt-1 may be implicated in the pathophysiology of psychiatric disorders (Herskovits and Guarente, 2014). Interestingly, the recent findings from Abe-Higuchi et al. (2016) indicated that inhibition of hippocampal Sirt-1 function leads to an increase in depression-like behaviors. Thus, we speculate that upregulation of Sirt-1 might be a potential target for an antidepressant drug. In our study, we found that H_2S markedly upregulates the expression of Sirt-1 in the hippocampus of CUMS-exposed rats. Moreover, blocking Sirt-1 pathway with Sirtinol, a specific inhibitor of Sirt-1, reverses H_2S-elicited protective effect against CUMS-induced hippocampal ER stress and inhibitory role in depression-like behavior. These results indicated that the antidepressant-like properties of H_2S is via upregulation of hippocampal Sirt-1. It has been demonstrated that resveratrol, a Sirt-1 activator, has an antidepressant-like effect (Ge et al., 2013; Hurley et al., 2014; Liu et al., 2014). These previous findings provide a reasonable interpretation for our present results. Therefore, our present work not only suggests the essential mediatory role of Sirt-1 in the antidepressant-like properties of H_2S but also extends the previous observations on the importance of the Sirt-1 pathway as a key target for antidepressant (Abe-Higuchi et al., 2016).

To summarize, our present study demonstrated that H_2S attenuates CUMS-induced depression-like behaviors and ER stress. H_2S upregulates Sirt1 expression in CUMS-exposed rats. Moreover, inhibition of Sirt1 reverses H_2S-elicited protection against the ER stress and depression-like behaviors induced by CUMS. These findings suggest that H_2S produces an antidepressive effect by inhibition of hippocampal ER stress as a result of upregulation of Sirt1 in hippocampus. Our findings provide a comprehensive view on the antidepressive effect of H_2S that partially identify a new approach for the treatment of depression. Additionally, to enhance and expand our findings, S-sulfhydration would no doubt be a promising strategy for the important and significant role of H_2S with regard to the regulation of Sirt1 in our further study, given the evidence that H_2S elicits its biological functions by S-sulfhydration (Mustafa et al., 2009, 2011; Krishnan et al., 2011; Paul and Snyder, 2012).

Statement of Interest

None.

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