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Original Article

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H_2S protects PC12 cells against toxicity of corticosterone by modulation of BDNF-TrkB pathway

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Abstract

Corticosterone, one of the glucocorticoids, is toxic to neurons and plays an important role in depressive-like behavior and depression. We previously showed that hydrogen sulfide (H_2S), a novel physiological mediator, plays an inhibitory role in depression. However, the mechanism underlying H_2S-triggered antidepressant-like role is not clearly known. Brain-derived neurotrophic factor (BDNF), a neurotrophic factor, plays a neuroprotective role that is mediated by its high-affinity tropomysin-related kinase B (TrkB) receptor. In this study, to investigate the underlying mechanism of H_2S-induced antidepressant-like role, we explored whether H_2S could protect neurons against corticosterone-mediated cyctotoxicity and whether this protective role of H_2S was involved in the regulation of BDNF-TrkB pathway. Our data demonstrated that sodium hydrosulfide (NaHS), the donor of H_2S, could prevent corticosterone-induced cytotoxicity, apoptosis, accumulation of intracellular reactive oxygen species (ROS) and loss of mitochondrial membrane potential (MMP) in PC12 cells. NaHS not only induced the up-regulation of BDNF but also prevented the down-regulation of BDNF by corticosterone. It was also found that blocking BDNF-TrkB pathway by K252a, an inhibitor of TrkB, abolished the protection of H_2S against corticosterone-induced cytotoxicity, apoptosis, accumulation of ROS, and loss of MMP. These results suggest that H_2S protects against the neurotoxicity of corticosterone by modulation of the BDNF-TrkB pathway.

Key words: brain-derived neurotrophic factor, tyrosine kinase receptor B, corticosterone, hydrogen sulfide, neurotoxicity

Introduction

Depression is a common debilitating disease which affects up to about 350 million people in the world, resulting in serious personal suffering and economic loss [1,2]. The pathogenesis of depression is very complicated. It has been reported that stress is a strong risk factor for depression [3,4]. Hypothalamic–pituitary–adrenal (HPA) axis was found to respond to stress, and particularly its hyperactivity is a key feature of depression [5]. The durable increases in glucocorticoids (corticosterone in rodents, cortisol in humans) which are the endpoint of HPA axis activation mainly mediate the influence of repeated or chronic stress in animal models as well as in humans [6,7]. The elevation of corticosterone induced by stressors may be closely associated with the development of depression [8]. Acute corticosterone administration can result in anxiety and amygdaloid dendritic hypertrophy

[9]. Moreover, chronic corticosterone exposure induces neuronal damage in hippocampus [10] and depressive-like behavior in animal model [11], apoptosis and injury of PC12 cells *in vitro* [12,13]. Interestingly, corticosterone can cause synaptic degeneration and exert toxic effects on hippocampal neurons, and finally induces depression [14]. It is also well-known that corticosterone exposure induces a decrease in dendritic spine density in hippocampus, which might be one of the pathophysiological mechanisms underlying depression progression [15]. These studies have confirmed that corticosterone is closely related to depression. Based on these observations, we speculate that the drug that can block corticosterone-induced neurotoxicity may have a hopeful therapeutic potential in preventing and treating depression.

Hydrogen sulfide (H_2S), the third endogenous gaseous mediator identified after nitric oxide (NO) and carbon monoxide (CO) [16–18], is found to be a neuroprotectant [19–23]. A recent report has documented that the endogenous H_2S level is decreased in chronic unpredictable mild stress (CUMS) [24]. In addition, our recent studies showed that H_2S could prevent depressive-like actions in the behavioral models of depression [25,26]. These data imply that H_2S may be a potential therapeutic target for the treatment of depression. However, the mechanism underlying the anti-depressive effects of H_2S remains unknown. Thus, we wonder whether the anti-depression activity of H_2S is associated with its ability to diminish the corticosterone-induced neurotoxicity.

Brain-derived neurotrophic factor (BDNF), an important neurotrophin, and its single transmembrane receptor, tropomysin-related kinase B (TrkB) have been shown to have antidepressant-like effects [27]. Accumulating evidence has demonstrated that serum BDNF levels are low in depressed patients, which are restored to near normal levels by antidepressant treatment [28–30]. Additionally, the levels of BDNF in hippocampus are reduced in corticosterone-induced mouse depression model [31]. Increasing data have shown that BDNF has neuroprotective activity [32–34]. BDNF is responsible for the structure and function of plasticity in the brain [35], prevents damages to neurons in the brain [36], and plays a key role in neural development and maintenance of the central and peripheral neurons [37]. Therefore, we hypothesize that BDNF-TrkB pathway may mediate the protective activity of H_2S against corticosterone-induced neurotoxicity.

The purpose of the present study was to explore whether H_2S protects neurons against corticosterone-induced toxicity as well as the possible regulatory role of BDNF-TrkB pathway in this protection. Our results demonstrats that H_2S has protective activity against corticosterone-induced neurotoxicity in PC12 cells, and that the underlying mechanism may involve the up-regulation of BDNF-TrkB pathway.

Materials and Methods

Reagents

Corticosterone, Sodium hydrosulfide (NaHS), and K252a were obtained from Sigma (St Louis, USA). Cell counting kit-8 (CCK-8) was supplied by Dojindo Molecular Technologies, Inc. (Rockvile, USA). Specific monoclonal antibody to BDNF was purchased from Epitomic Inc. (Burlingame, UK). Anti-β-actin antibody was purchased from Proteintech (Danvers, USA). Mitochondrial membrane potential (MMP) assay kit with JC-1 was purchased from Beyotime Biotechnology (Shanghai, China).

Cell culture

PC12 cells were supplied by the Experimental Animal Center of Sun Yat-sen University (Guangzhou, China) and were routinely grown in Dulbecco's modified Eagle's Medium (DMEM; GibicoBRL, Ground Island, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin, and incubated at the temperature of 37°C in a humidified incubator consisting of 95% air and 5% CO_2. The cell culture media was refreshed every other day.

Evaluation of cell viability

CCK-8 assay was used to evaluate the viability of PC12 cells. Cells were seeded in 96-well culture plate at a concentration of 1×10^5 cells per well. After treatment, 5 μl CCK-8 solutions were added to each well and cells were incubated at 37°C for another 3 h. The optical density of each well was measured at 450 nm using a microplate reader (Molecular Devices, Sunnyvale, USA). Optical density is directly proportional to the number of living cells in the culture. The data obtained were expressed as percentage of viable cells relative to the untreated control group.

Analysis of apoptosis by flow cytometry

Apoptosis was evaluated by propidium iodide (PI) staining. PC12 cells were seeded into 6-well plates at 1×10^6 cells per well. After treatment, PC12 cells were detached with trypsin (2.5 g/l), centrifuged at 500 *g* for 10 min and then washed twice with PBS. The collected cells were fixed with 70% ethanol for 24 h at −20°C. After being washed with PBS twice, PC12 cells were incubated with 1 mg/ml RNase (Sigma) at 37°C for 30 min. Finally, cells were stained with PI at a final concentration of 50 mg/l in the dark at 4°C for 30 min, and then subject to flow cytometry analysis (Beckman-Coulter, Pasadena, USA). In the DNA histogram, the amplitude of the sub-G1 DNA peak was taken as the number of apoptotic cells.

Assessment of intracellular reactive oxygen species generation

The formation of intracellular reactive oxygen species (ROS) is detected by nitro blue tetrazolium (NBT) assay based on NBT reduction. That is, NBT is converted to purple formazan by superoxide anion in the assay [38]. PC12 cells were seeded in 96-well plates at a density of 1×10^5 cells/well. At the end of treatment period, 100 μl of NBT solutions (1.0 mg/ml in DMEM) was added into each well and then the plates were cultured for 2 h at 37°C. After being washed with PBS twice, PC12 cells were dissolved in 0.1 ml of 2 M KOH and 0.1 ml of dimethyl sulfoxide (DMSO). Finally, the absorption of each well was detected at the wavelength of 570 nm using a microplate reader.

Measurement of MMP

The MMP was measured using the JC-1 assay kit according to the manufacturer's instructions. PC12 cells were seeded in 35 mm dish. When cells reached 70% confluence, cells were cultured with the indicated conditioned-media for 24 h. After the medium was removed, the cells were rinsed with PBS. Each dish was added with 1 ml cell suspension and 1 ml diluted JC-1 solution, and then maintained in a cell culture incubator. Twenty minutes later, the supernatant was removed and cells were rinsed twice with JC-1 staining washing buffer (1×), and then fluorescence was immediately determined by laser scanning confocal microscopy (LSCM) and the mean fluorescence intensity (MFI) was quantitative analyzed. The MFI of red or green was measured at an excitation of 490 nm and emission of 530 nm (green florescent monomers) or 590 nm (red florescent aggregates), respectively [39]. The ratio MFI of red fluorescence to MFI of green fluorescence is an index of the level of MMP in the positive cells.

Western blot analysis for BDNF expression

After drug treatment, PC12 cells were harvested and lysed with cell lysis solution [20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulphonyl fluoride (PMSF), 1 mM Na_3VO_4, leupeptin, and EDTA] for 30 min at 4°C. The supernatant was collected after centrifugation at 12,000 *g* for 10 min at 4°C and stored at –20°C. Protein concentration was quantified using a BCA Protein Assay Kit (Solarbio, Beijing, China). After denaturation at 100°C for 5 min in loading buffer, the same amounts of supernatant from each sample were seperated by 10 or 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) with molecular weight ladders, then electrophoretically transferred onto actived polyvinylidene fluoride (PVDF) membrane, and blocked with Tris-buffered saline with Tween 20 (TBST, 50 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.1% Tween-20) containing 5% Bovine Serum Albumin (BSA, Sigma) for 2 h. Subsequently, membranes were incubated with the following primary antibodies: anti-BDNF monoclonal antibody (1 : 1000) and anti-β-actin antibody (1 : 2000), overnight at 4°C. Duplicate blots probed with mouse anti-β-actin antibody were used as loading controls. Blots were washed with TBST for three times, and then incubated with appropriate horseradish peroxidase-conjugated secondary antibodies (1 : 5000) in blocking solution for 2 h. After further washing, the blots were visualized using the enhanced chemiluminescence kit (Beyotime Biotechnology, Shanghai, China) under an image analysis system equipped with a software BIO-ID (Vilber Lourmat, Marne la Vallee, France). The experiment was carried out three times.

Statistical analysis

Data were expressed as the mean ± standard error of means (S.E.M.). The significance of inter-group differences was evaluated using one-way analysis of variance (ANOVA). Differences were considered significant at $P < 0.05$.

Results

H_2S prevented corticosterone-induced cytotoxicity in PC12 cells

The effect of H_2S on the inhibition of cell viability caused by corticosterone was first investigated in PC12 cells. The inhibition of cell

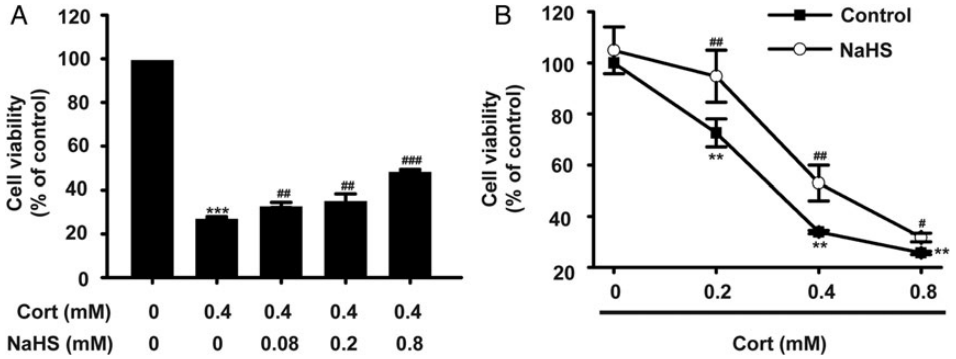

Figure 1. H_2S attenuated the cytotoxicity of corticosterone in PC12 cells (A) PC12 cells were pretreated with different concentrations (0.08, 0.2 or 0.8 mM) of NaHS for 30 min prior to 24 h-exposure to corticosterone (0.4 mM). (B) PC12 cells were pre-incubated with NaHS at a concentration of 0.2 mM for 30 min and then co-treated with 0.2, 0.4, or 0.8 mM of corticosterone for 24 h. Cell viability was measured by CCK-8 assay. Data were presented as the mean ± SEM ($n = 3$). $^{**}P < 0.01$, $^{***}P < 0.01$ vs. control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, vs. corticosterone-treated alone group.

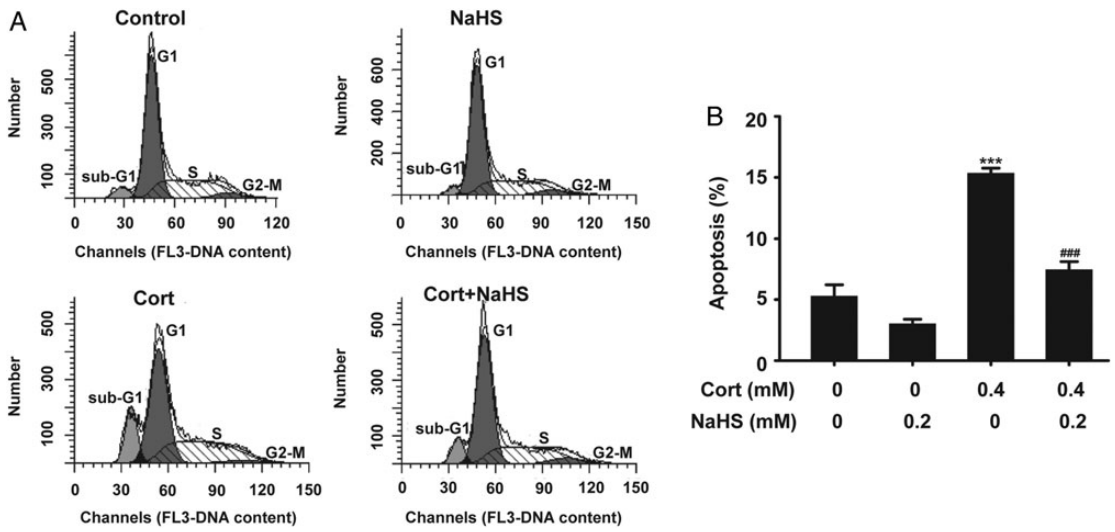

Figure 2. H_2S repressed corticosterone-induced apoptosis in PC12 cells After pretreatment with 0.2 mM NaHS for 30 min, PC12 cells were exposed to 0.4 mM corticosterone for 24 h. (A) The apoptosis of PC12 cells was detected by FCM after PI staining. (B) Quantitative analysis of the apoptosis rate. Data were presented as the mean ± SEM ($n = 3$). $^{***}P < 0.001$, vs. control group. $^{\#\#\#}P < 0.001$, vs. corticosterone-treated alone group.

viability in PC12 cells induced by 24 h of incubation with 0.4 mM corticosterone was significantly reversed by 30 min of pretreatment with 0.08, 0.2 or 0.8 mM NaHS (**Fig. 1A**). Furthermore, pretreatment for 30 min with 0.2 mM NaHS attenuated the decrease in the viability of PC12 cells induced by 24 h of incubation with 0.2 or 0.4 mM corticosterone, but did not affect the inhibition of cell viability of PC12 cells induced by 24 h of incubation with 0.8 mM corticosterone (**Fig. 1B**). These data indicated that H_2S produced protective effects against corticosterone-induced cytotoxicity.

H_2S antagonized corticosterone-induced apoptosis in PC12 cells

The effect of H_2S on corticosterone-induced apoptosis was investigated in PC12 cells. As shown in **Fig. 2**, after 24 h of treatment, 0.4 mM corticosterone triggered considerable apoptosis in PC12 cells. And this apoptotic effect was blocked by preincubation with 0.2 mM NaHS for 30 min. However, 0.2 mM NaHS alone did not influence the apoptosis of PC12 cells. Taken together, these data suggested that H_2S played a protective role against corticosterone-induced apoptosis.

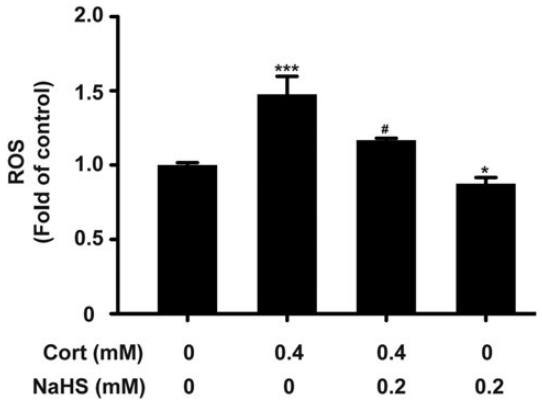

Figure 3. H_2S abolished corticosterone-induced accumulation of intracellular ROS in PC12 cells PC12 cells were pretreated with 0.2 mM NaHS for 30 min and then co-treated with 0.4 mM corticosterone for 24 h. The level of ROS in PC12 cells was detected by NBT reduction assay. Data were presented as the mean ± SEM ($n = 3$). $^{*}P < 0.05$, $^{***}P < 0.001$, vs. control group. $^{\#}P < 0.05$, vs. corticosterone-treated alone group.

H_2S reduced corticosterone-induced accumulation of intracellular ROS in PC12 cells

To further determine the protective effect of H_2S against corticosterone-induced neurotoxicity, we explored whether H_2S inhibited intracellular ROS accumulation caused by corticosterone in PC12 cells. As illustrated in **Fig. 3**, pretreatment with 0.2 mM NaHS for 30 min remarkably reversed the increase in the level of intracellular ROS induced by treatment with 0.4 mM of corticosterone for 24 h. Moreover, 0.2 mM NaHS alone also decreased the basal level of intracellular ROS in PC12 cells. These data suggested that H_2S could protect PC12 cells from corticosterone-induced oxidative stress.

H_2S prevented corticosterone-induced dissipation of MMP in PC12 cells

Dissipation of MMP plays a key role in apoptosis [40], so we measured MMP in PC12 cells with JC-1 staining. The reduced ratio of red fluorescence to green fluorescence indicates the loss of MPP [41]. As shown in **Fig. 4**, the ratio of red fluorescence to green fluorescence in PC12 cells was decreased after 24 h of treatment with 0.4 mM corticosterone. However, 30 min of preincubation with 0.2 mM NaHS significantly reversed the decrease in the ratio of red fluorescence to green fluorescence in PC12 cells caused by 24 h of incubation with 0.4 mM corticosterone. Furthermore, 0.2 mM NaHS alone had no effect on the ratio of red fluorescence to green fluorescence in PC12 cells. These data indicated a protective role of H_2S against corticosterone-induced MMP loss.

H_2S up-regulated BDNF expression and abolished corticosterone-induced down-regulation of BDNF expression in PC12 cells

To investigate whether BDNF mediated the mechanism underlying the protective effect of H_2S against corticosterone-triggered neurotoxicity, we first explored whether H_2S regulated the level of BDNF expression in PC12 cells. After 24 h of incubation with 0.08, 0.2 or 0.8 mM

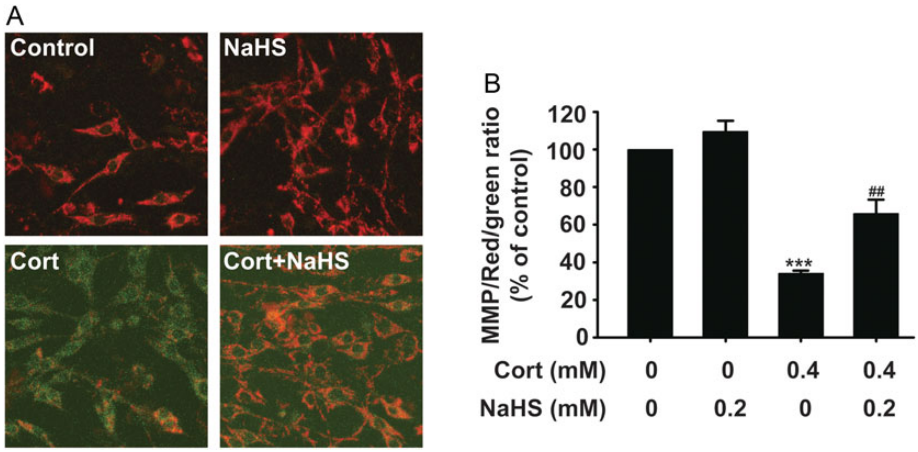

Figure 4. H_2S prevented the dissipation of MMP induced by corticosterone in PC12 cells After 30 min exposure to 0.2 mM NaHS, PC12 cells were co-treated with 0.4 mM corticosterone for 24 h. The level of MMP was determined by JC-1 staining. (A) The fluorescence of JC-1 in PC12 cells is observed under a confocal microscope (200×). (B) The MMP in PC12 cells was quantitatively measured as the ratio MFI of red fluorescence to MFI of green fluorescence. Data were presented as the mean ± SEM ($n = 3$). $^{***}P < 0.001$, vs. control group. $^{\#\#}P < 0.01$, vs. corticosterone-treated alone group.

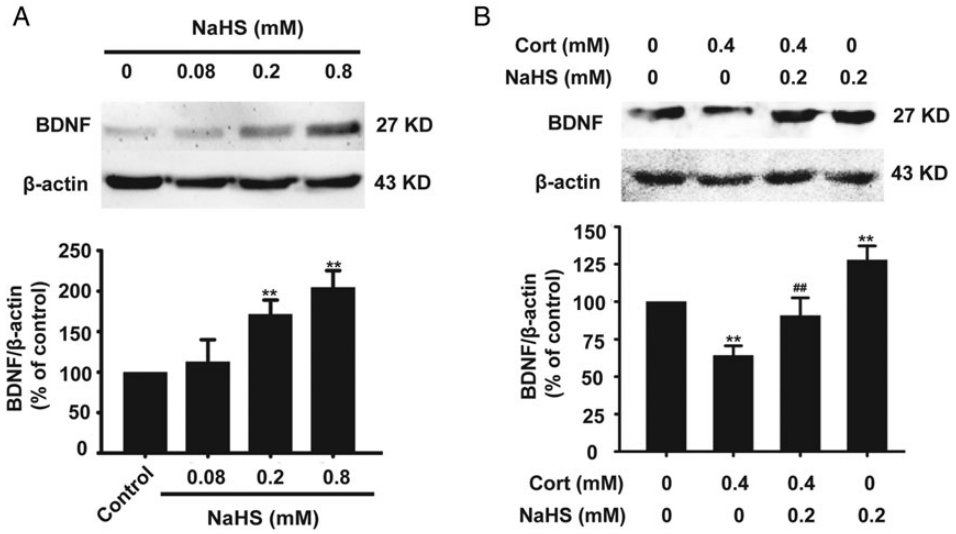

Figure 5. H_2S augmented BDNF expression in PC12 cells (A) PC12 cells were exposed to 0.08, 0.2 or 0.8 mM NaHS for 24 h. (B) PC12 cells were pretreated with 0.2 mM NaHS for 30 min and then co-treated with 0.4 mM corticosterone for 24 h. The expression of BDNF was measured by western blot analysis. Data were presented as the mean ± SEM ($n = 3$). $^{**}P < 0.01$, vs. control group. $^{\#}P < 0.05$, vs. corticosterone-treated alone group.

NaHS, the expression of BDNF in PC12 cells was significantly increased in a concentration-dependent manner (**Fig. 5A**). Moreover, the suppressed expression of BDNF by 24 h of treatment with 0.4 mM corticosterone was remarkably abolished by 30 min of pretreatment with 0.2 mM NaHS (**Fig. 5B**). These data indicated an up-regulatory role of H_2S in BDNF expression.

Blocking BDNF-TrkB pathway attenuated the protective effect of H_2S against corticosterone-induced cytotoxicity in PC12 cells

To determine the mediatory role of BDNF-TrkB pathway in the protective effect of H_2S against corticosterone-elicited cytotoxicity in PC12 cells, we further investigated whether K252a, a specific inhibitor of BDNF-TrkB pathway, could abolish this protective effect of H_2S against corticosterone-elicited inhibition of cell viability in PC12 cells. As shown in **Fig. 6**, 10 or 20 nM K252a remarkably prevented the increase in cell viability induced by treatment with NaHS, which suggested that inhibition of BDNF-TrkB pathway reversed the protective effect of H_2S against corticosterone-elicited cytotoxicity in PC12 cells. K252a at the concentration of 20 nM did not influence the cell viability of PC12 cells. These data indicated that BDNF-TrkB pathway may mediate the protective effect of H_2S against corticosterone-induced cytotoxicity.

Inhibition of BDNF-TrkB pathway suppressed the protective effect of H_2S against corticosterone-induced apoptosis in PC12 cells

We further investigated whether the inhibition of BDNF-TrkB pathway by K252a antagonized the protective effect of H_2S against corticosterone-induced apoptosis in PC12 cells. As illustrated in **Fig. 7**, 10 nM K252a abolished NaHS-induced inhibition of PC12 cell apoptosis caused by 24 h incubation with 0.4 mM corticosterone. Neither NaHS (0.2 mM) nor K252a (10 nM) alone significantly affected the apoptosis of PC12 cells. Taken together, these data

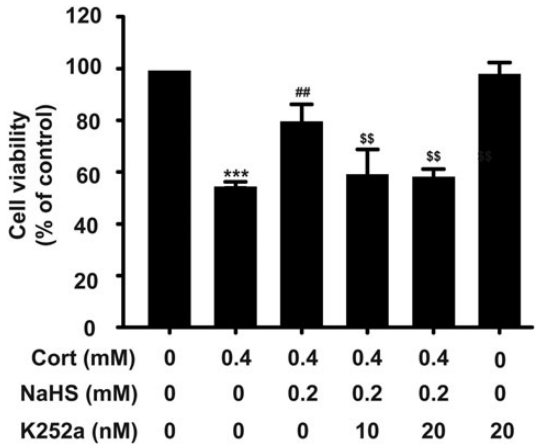

Figure 6. K252a abolished the protective effect of H_2S against the cytotoxicity of corticosterone to PC12 cells PC12 cells were pretreated with 10 or 20 nM K252a for 30 min before pretreatment with 0.2 mM NaHS for 30 min prior to 24-h exposure to 0.4 mM corticosterone. Cell viability was detected by CCK-8 assay. Data were expressed as the mean ± SEM ($n = 3$). $^{***}P < 0.001$, vs. control group. $^{\#\#}P < 0.01$, vs. corticosterone-treated alone group, $^{\$\$}P < 0.01$, vs. NaHS and corticosterone co-treatment group.

demonstrated that BDNF-TrkB pathway could mediate the protective role of H_2S against corticosterone-induced apoptosis.

Blocking BDNF-TrkB pathway prevented the protective effect of H_2S against corticosterone-induced oxidative stress in PC12 cells

The effect of K252a on the protective role of H_2S against corticosterone-induced oxidative stress was also investigated in PC12. As shown in **Fig. 8**, pretreatment of PC12 cells with 10 nM K252a for 30 min significantly reversed the NaHS-exerted inhibition of accumulation of ROS caused by 24 h of incubation with 0.4 mM corticosterone. NaHS (0.2 mM) or K252a (10 nM) alone did not

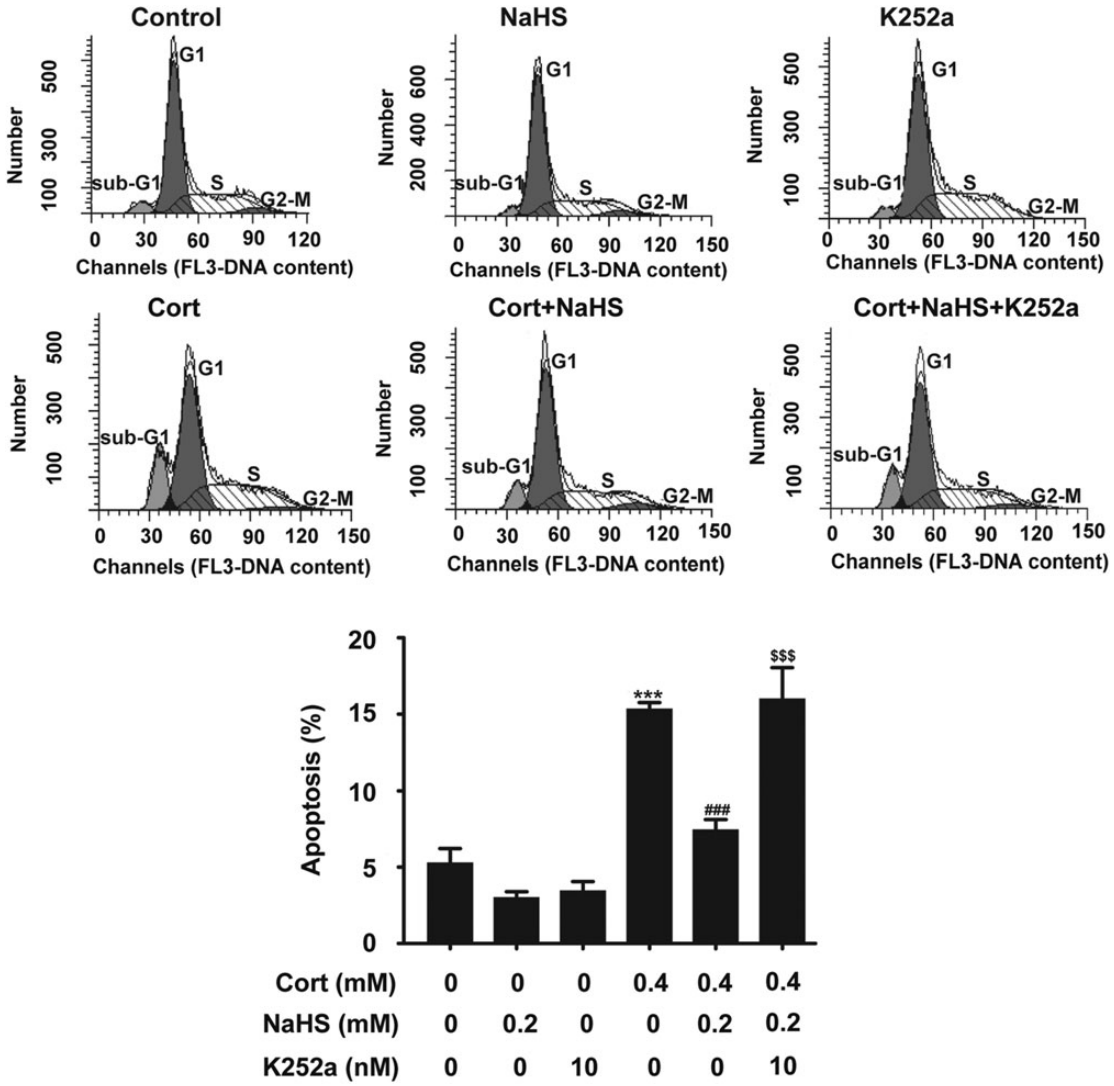

Figure 7. K252a abolished the protective effect of H_2S against corticosterone-induced apoptosis in PC12 cells PC12 cells were pre-incubated with 10 nM K252a for 30 min before treatment with 0.2 mM NaHS for 30 min, and then exposed to 0.4 mM corticosterone for 24 h. The apoptosis of PC12 cells was assayed by FCM after PI staining. Data were expressed as the mean ± SEM ($n = 3$). $^{***}P < 0.001$, vs. control group. $^{\#\#\#}P < 0.001$, vs. corticosterone-treated alone group, $^{\$\$\$}P < 0.001$, vs. NaHS and corticosterone co-treatment group.

affect the level of ROS. These data indicated that BDNF-TrkB pathway may mediate the protective action of H_2S against corticosterone-induced oxidative stress.

Inhibition of BDNF-TrkB pathway abolished the protective effect of H_2S against corticosterone-induced dissipation of MMP in PC12 cells

Finally, we investigated the effect of K252a on the H_2S-exerted protection against the dissipation of MMP caused by corticosterone in PC12 cells. As shown in **Fig. 9**, pretreatment with 10 nM K252a remarkably reversed the protective effect of NaHS against the dissipation of MMP triggered by 24 h of incubation with 0.4 mM corticosterone in PC12 cells, suggesting that inhibition of BDNF-TrkB pathway could abolish the protective effect of H_2S against corticosterone-induced disspation of MMP. NaHS (0.2 mM) or K252a (10 nM) alone had no effect on the level of MMP.

Discussion

In the present study, we demonstrated that administration of PC12 cells with NaHS, a donor of H_2S, significantly protected PC12 cells against corticosterone-elicited cytotoxicity, apoptosis, accumulation of intracellular ROS, and loss of MMP. In addition, our results showed that H_2S up-regulated BDNF expression and blocked corticosterone-induced down-regulation of BDNF expression. Furthermore, K252a, the inhibitor of TrkB receptor, abolished the protective effect of H_2S against the corticosterone-induced neurotoxicity. Taken together, these findings reveal that BDNF-TrkB pathway mediates the protective effect of H_2S against the corticosterone-induced neurotoxicity.

Corticosterone, a principal corticosteroid, has been shown to elicit toxic effects on neurons [13,42,43]. Increasing data revealed that increase in brain corticosterone concentration is associated with depression [44–46]. Blocking the neurotoxicity of corticosterone

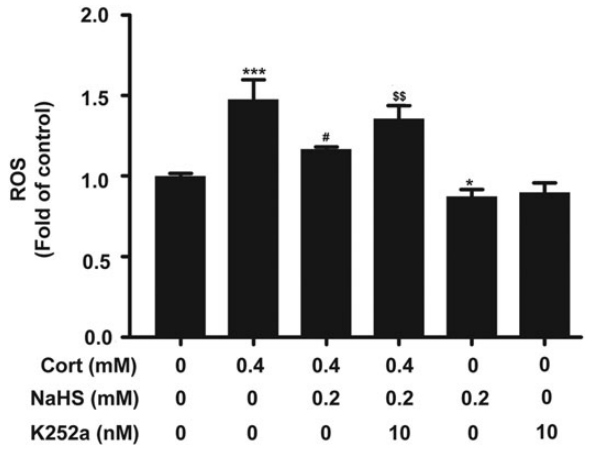

Figure 8. K252a abolished the protective effect of H_2S against corticosterone-elicited oxidative stress in PC12 cells PC12 cells were pre-incubated with 10 nM K252a for 30 min before treatment with 0.2 mM NaHS for 30 min. PC12 cells were pre-incubated with 0.2 mM NaHS for 30 min and then co-treated with 0.4 mM corticosterone for 24 h. The level of intracellular ROS was determined by NBT reduction assay. Data were expressed as the mean ± SEM ($n = 3$). $^{*}P < 0.05$, $^{***}P < 0.001$, vs. control group. $^{\#}P < 0.05$, vs. corticosterone-treated alone group. $^{\$\$}P < 0.01$, vs. NaHS and corticosterone co-treatment group.

could be a new therapeutic approach for the treatment of depression. H_2S is a novel physiological mediator. We have previously found the antidepressant-like action of H_2S [25]. However, the mechanisms underlying H_2S-mediated antidepressant-like role are not clear. H_2S has been reported to have neuroprotection activity [47–49]. We previously showed that H_2S prevented the neurotoxicity induced by homocysteine [50], formaldehyde [51], and 1-methy-4-phenylpyridinium ion [52]. Thus, the aim of present work is to investigate whether H_2S prevents the neurotoxicity of corticosterone.

Numerous findings have demonstrated that corticosterone causes neurotoxicity, apoptosis [13,42,53], and oxidative stress [54,55]. Based on these findings, our present study was designed to detect the toxicity of corticosterone to PC12 cells. PC12 cells were derived from a tumor found in the rat adrenal medulla with catecholaminergic neuronal properties [56]. PC12 cells have been extensively used as a model to study the neurotoxicity of numerous stimulants *in vitro* [57–59]. Our data demonstrated that treatment of PC12 cells with corticosterone obviously decreased the cell viability, indicating the neurotoxic effect of corticosterone on PC12 cells. We also demonstrated that treatment of PC12 cells with NaHS, the H_2S donor, significantly antagonized corticosterone-induced cytotoxicity in PC12 cells. Apoptosis is essential to the maintainance of physiologic balance between cell death and cell growth, participates in various biological processes, including the removal of unwanted cells, developmental sculpturing and tissue homeostasis [60,61]. We also showed that treatment of PC12 cells with NaHS attenuated the apoptosis of PC12 cells caused by corticosterone. Oxidative stress has been reported to mediate corticosterone-induced apoptosis in PC12 cells [13]. Mitochondria are important organelles in a variety of cells, particularly in the nervous system. Mitochondria play a crucial role in energy production [62]. Mitochondrial damage, an early event during apoptosis, is a crucial factor related to cell death and some models of apoptosis [40]. Mitochondrial damage is concomitant with the alteration of MMP and generation of intracellular ROS in apoptosis [63]. We further detected the influences of H_2S on the changes of ROS and MMP resulted from corticosterone treatment. It was found that NaHS prevented corticosterone-induced accumulation of ROS and loss of MMP in PC12 cells. Taken together, our data demonstrated the protective role of H_2S against the neurotoxicity of corticosterone. It was reported that the neurotoxicity of corticosterone plays an important role in the development of depression [14,15]. In addition, our recent studies have shown that H_2S has antidepressant-like effect [25,26]. Thus, we suggest that the anti-depressive effect of H_2S may be related to its inhibitory effect against the corticosterone-induced neurotoxicity

We further explored the possible mechanisms underlying the protective effect of H_2S against the corticosterone-induced neurotoxicity. BDNF is a member of the neurotrophin family and the activity of BDNF is mediated by the high-affinity TrkB receptor [64]. Accumulated observations have demonstrated that BDNF induces neuroprotective effects [65–67]. Therefore, we focused on the role of BDNF in the protective effect of H_2S against the corticosterone-induced neurotoxicity. Our present observations showed that NaHS increased BDNF level and reversed the inhibition of BDNF level induced by corticosterone in PC12 cells. Furthermore, we also found that K252a, the inhibitor of BDNF-TrkB pathway, significantly reversed the protective role of H_2S against corticosterone-induced cytotoxicity, apoptosis, accumulation of ROS, and loss of MMP. In the present study, we use low concentration (20 nM) of K252a, which does not cause apoptosis. If K252a alone causes apoptosis, it will be difficult to identify whether the apoptosis induced by co-treatment with K252a (10 nM), NaHS (0.2 mM), and corticosterone (0.4 mM) is resulted from the inhibition of BDNF-TrkB pathway-induced prevention against the protective effect of H_2S on the corticosterone toxicity or from the damange of K252a itself. A previous study from Wang *et al.* [68] has revealed that BDNF/TrkB signaling pathway might mediate the neuroprotective effect of curcumin against glutamate excitotoxicity. Moreover, our recent studies have demonstrated that up-regulation of the BDNF-TrkB pathway is responsible for the H_2S-exerted inhibitory effect on homocysteine-induced endoplasmic reticulum stress and neuronal apoptosis in rat hippocampus [67] and that BDNF-TrkB pathway is involved in the H_2S-induced neuroprotective effect against the neurotoxicity of formaldehyde [57]. According to these observations, we think that up-regulation of BDNF-TrkB is sufficient and necessary for neuronal protection. In addition, Jiang *et al.* [69] have shown that BDNF-TrkB pathway mediates the antidepressant actions of SKF83959 in a chronic social defeat stress model of depression. Koike *et al.* [27] have revealed that BDNF/TrkB signaling may be involved in the sustained antidepressant-like role of LY341495. Thus, up-regulating BDNF-TrkB is sufficient and necessary for anti-depression. Therefore, we conclude that BDNF-TrkB pathway mediates H_2S-induced protection against the neurotoxicity of corticosterone.

In conclusion, our present work confirmed that H_2S attenuated corticosterone-exerted neurotoxicity to PC12 cells. We further found that blocking BDNF-TrkB pathway could prevent the protective role of H_2S against the neurotoxicity of corticosterone to PC12 cells. These data reveal that BDNF-TrkB pathway is responsible for the protective effect of H_2S against corticosterone-mediated neurotoxicity. Our findings provide a novel insight into the mechanism underlying H_2S-mediated antidepressant-like action.

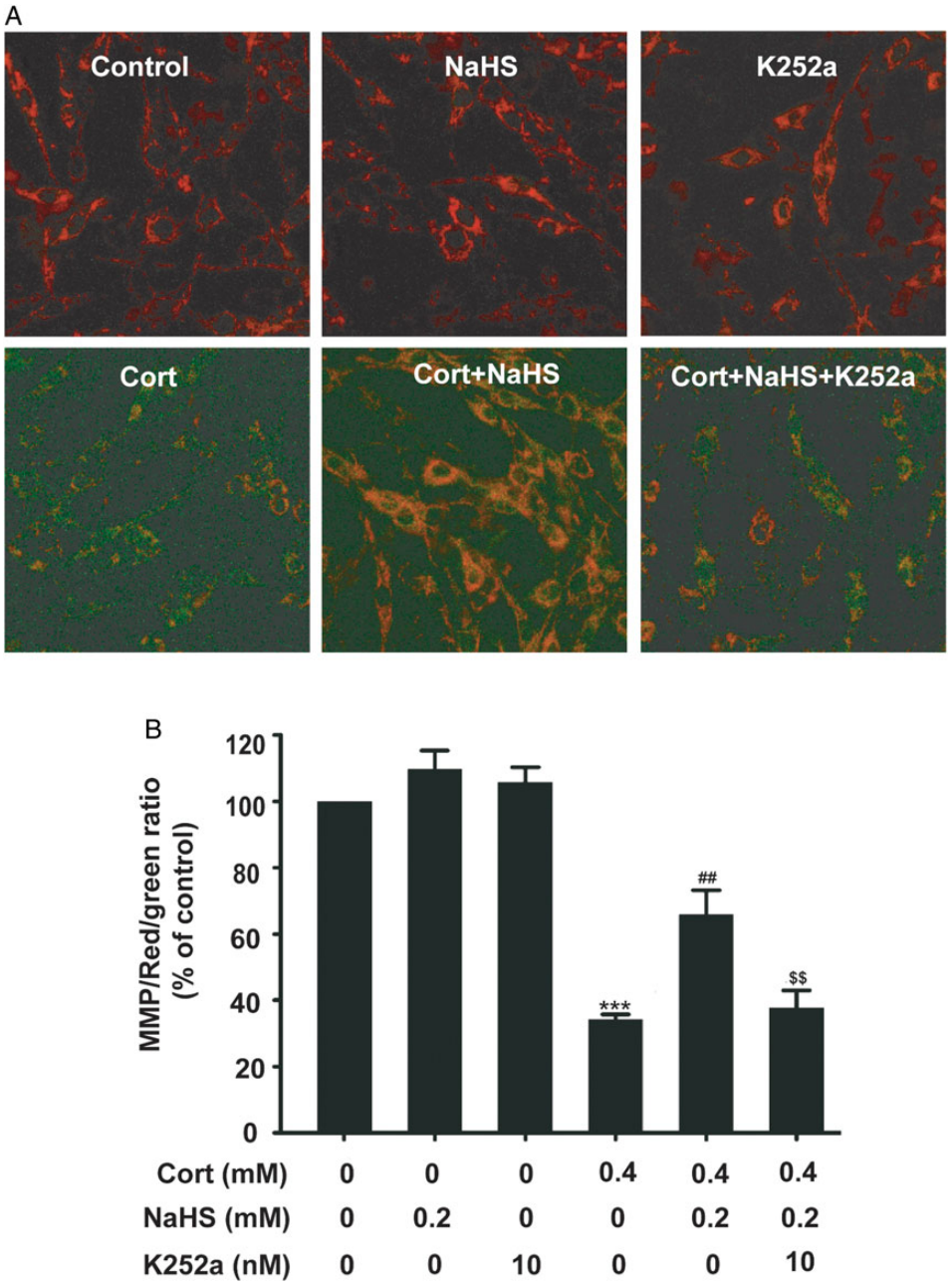

Figure 9. K252a abolished the protective effect of H_2S against the corticosterone-triggered MMP loss in PC12 cells PC12 cells were pretreated with 10 nM K252a for 30 min before incubation with 0.2 mM NaHS for 30 min. Then cells were exposed to 0.4 mM corticosterone for 24-h. (A) The fluorescence of JC-1 in PC12 cells was observed under a confocal microscope (200×). (B) The MMP in PC12 cells was quantitatively measured as the ratio MFI of red fluorescence to MFI of green fluorescence. Data were expressed as the mean ± SEM ($n = 3$). $^{***}P < 0.001$, vs. control group. $^{\#\#}P < 0.01$, vs. corticosterone-treated alone group. $^{\$\$}P < 0.01$, vs. NaHS and corticosterone co-treatment group.

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