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Research report

The endoplasmic reticulum-related events in *S*-nitrosoglutathione-induced neurotoxicity in cerebellar granule cells

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Abstract

Nitric oxide (NO)-induced neurotoxicities are involved in the pathogenesis of several neurodegenerative disorders featured by misfolded proteins. However, the details remain to be investigated. In the present work, we focus on the study of some endoplasmic reticulum-related events in *S*-nitrosoglutathione (GSNO)-induced neurotoxicity in cerebellar granule cells (CGCs) and we demonstrated that: (1) GSNO caused sustained elevation of intracellular calcium; (2) This calcium elevation resulted partially from the depletion of endoplasmic reticulum (ER) calcium stores; (3) There was ER stress which was indicated by the incomplete splicing of X-box binding protein (XBP-1) mRNA by 8-polysialyltransferase (Pst1); (4) GSNO upregulated the expression of the proapoptotic growth arrest and DNA damage-inducible gene (Gadd153) and caused the depletion of intracellular glutathione (GSH) pools. At the same time, GSNO downregulated the expression of the antiapoptotic gene Sarco/endoplasmic reticulum calcium-ATPase (SERCA2b) in parallel with the downregulation of the antiapoptotic ER chaperones—glucose-regulated protein genes (Grp78 and Grp94). These effects indicate that ER is one of the NO targets in GSNO-induced neurotoxicity in cerebellar granule cells besides mitochondria.

Theme: Development and regeneration
Topic: Neuronal death

Keywords: *S*-Nitrosoglutathione; Endoplasmic reticulum stress; Neurotoxicity; Calcium; Cerebellar granule cell

1. Introduction

Nitric oxide (NO), as a putative messenger, is implicated in the physiological and pathological processes in CNS and mitochondria are the primary targets for NO toxicity in the brain [3,5,31]. However, recently more attention has been focused on the endoplasmic/sarcoplasmic reticulum (ER/SR) as another possible target of NO toxicity [10].

ER is an organelle that regulates the folding and assembling of secretory proteins and membrane proteins, the cellular response to stress, and the balance of intracellular calcium [27,37]. Conditions that could disturb the homeostasis in the ER are referred to as ER stress [33], including the loss of the ER intraluminal oxidative environment, the depletion of intracellular calcium stores, and the accumulation of misfolded proteins in the ER. ER stress triggers at least two distinct cellular responses [20]. One involves the upregulation of genes encoding ER chaperones, such as glucose-regulated protein genes (Grp78 and Grp94), which promote protein folding [22], as well as genes encoding sarco/endoplasmic reticulum

Abbreviations: CGCs, cerebellar granule cells; CREB, cAMP-responsive element binding protein; ER, endoplasmic reticulum; Gadd gene, growth arrest and DNA damage-inducible gene; GluR5, ionotropic glutamate receptor subunit 5; Grp, glucose-regulated protein; GSNO, *S*-nitrosoglutathione; IRE1, ER to nucleus signaling 1 (ERN1, ERN1 encodes the ER to nucleus signaling 1 protein, a human homologue of the yeast Ire1 gene product); MMP, mitochondrial membrane permeabilization; MTT, 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide; NO, nitric oxide; Pst1, sialyltransferase 8D (alpha-2, 8-polysialyltransferase); SERCA, sarco/endoplasmic reticulum calcium-ATPase; UPR, unfolded protein response; XBP-1, X-box binding protein 1

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calcium-ATPase (SERCA2) [8], which is a pump translocating calcium from the cytosol to the ER to maintain the intracellular calcium homeostasis. The other is apoptosis caused by severe ER stress mediated by the transcriptional activation of Gadd153 and the activation of caspase-12 [32,52]. This cellular response is probably linked to the pathogenesis of several different neurodegenerative disorders featured by misfolded proteins such as Alzheimer's disease and Parkinson's disease [34,35]. ER dysfunction has also been reported to be a death-initiating pathway, which may be upstream of the "central executioner" mitochondria pathway [14]. However, the involvement of ER stress-related events in NO-induced neurotoxicity in cerebellar granule cells (CGCs) is poorly understood up to now.

S-nitrosothiols have garnered considerable interests due to their abilities to act as NO donors. Recently, a metabolic enzyme for *S*-nitrosoglutathione (GSNO) was reported to be conserved from bacteria to humans [25]. In particular, endogenous GSNO, which is most likely generated in the endothelial cells and astroglial cells during oxidative stress, has been identified in the rat cerebellum [21]. All this evidence suggests that GSNO is probably the endogenous NO reservoir [17] and plays potential roles in the brain. Furthermore, the intrinsic NO/NO+ characteristics [1,41,42] of GSNO endow it accessible to modify the reactive protein sulfhydryls by a redox-based *S*-nitrosylation mechanism [43]. Therefore, the details of GSNO in neurotoxicity will be valuable for understanding the roles of NO in the pathogenesis of neurodegenerative diseases.

In the present work, we focus on studying whether there exists ER stress in GSNO-induced neurotoxicity in cerebellar granule cells. Furthermore, we investigate the change of a serial of ER-related genes (Gadd153, SERCA2b, Grp78, and Grp94) expression at the transcriptional level.

2. Material and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM) and Newborn calf serum (NCS) were purchased from Hyclone. Uniq-10 total RNA Minpreps SuperKit was purchased from Sangon (Shanghai, China). Access Quick™ RT-PCR system and Pst1 were purchased from Promega. GSH, HEPES, Poly-L-lysine, cytosine arabino-furanoside, Penicillin, Streptomycin, MTT, Fura-2/AM were purchased from Sigma (St. Louis, MO, USA). Monochlorobimane was purchased from Fluka. All other chemicals were analytical grade.

2.2. Preparation of GSNO

GSNO was prepared as described previously [40] with some modification. Briefly, glutathione in 0.1 M HCl was incubated with an equal concentration of sodium nitrite at 4 for 45 min, then the solution was neutralized with 5 M NaOH to pH 7.4. The yield of GSNO was quantified by the characteristic absorbance of its nitrosothiol moiety at 335 nm ($\varepsilon_{335} = 902\ M^{-1}\ cm^{-1}$). Fresh solutions of GSNO were prepared just before each experiment.

2.3. Cell culture and treatment

Primary cultures of rat cerebellar granule cells were prepared as described previously [16]. Briefly, cerebella were dissected out from 6-day-old Sprague–Dawley rats. After the removal of meninges and blood vessels, cerebella were minced and dissociated by mild trypsinization. Cells were plated in a six-well multidish at a density of 1×10^6 cells/ml (2.5 ml/well) precoated with ploy-L-lysine (500 μg/ml) and maintained in DMEM supplemented with D-glucose (33 mM), KCl (25 mM), glutamine (2 mM), HEPES (10 mM), 10% NCS, 50 units/ml penicillin and 50 μg/ml streptomycin at 37 °C in humidified air with 5% CO_2 atmosphere. Cytosine arabino-furanoside (10 μM) was added in 18–20 h after plating to prevent nonneuronal cells proliferation. The culture medium was renewed in 24 h. CGCs were treated by GSNO on the 3rd–5th day when they were not fully differentiated to avoid the effects of ion channels and neuronal nitric oxide synthase (nNOS) expression [36].

2.4. Neuronal viability

GSNO-induced cell toxicity was evaluated by the neuronal viability with an MTT assay. After exposure to GSNO, cells were incubated with 0.5 mg/ml MTT at 37 °C for 3.5 h. The medium was removed followed by addition of dimethyl sulfoxide (DMSO) to dissolve the formazan product. After 30 min of incubation, the product formed was analyzed at 595 nm using an automatic microtiter reader (Bio-Rad). The optical density of the control sample was defined as 100% of the neuronal viability.

2.5. Quantification of intracellular calcium levels

The intracellular calcium concentrations were measured as described previously [6,26]. CGCs were cultured in specialized dishes with a quartz glass on the bottom. Briefly, culture medium was removed and CGCs were loaded with 5 μM fura-2/AM at 37 °C for 60 min in buffered salt solution (BSS, pH 7.4): NaCl (130 mM), KCl (5.4 mM), $CaCl_2$ (1.8 mM), $MgCl_2$ (0.8 mM), D-glucose (15 mM), HEPES (5 mM). And then CGCs were rinsed with BSS to remove Fura-2/AM. The mobilization of intracellular calcium was measured immediately after the treatment of 1 mM GSNO expressed by the ratio of the fluorescent density at 340/380 nm with a Nikon Dia-

phot300 inverted epifluorescent microscope and digital camera (HAMAMATSU, Japan).

2.6. Analysis of ER-induced X-box binding protein 1 (XBP-1) mRNA cleavage

To investigate whether GSNO caused ER stress in CGCs, we evaluated the ER to nucleus signaling 1 (IRE1)-dependent cleavage of X-box binding protein 1 (XBP-1) mRNA using RT-PCR [7]. Total RNA from treated or untreated CGCs was isolated and was reverse transcribed to cDNA. The PCR reactions amplified a 600-bp cDNA product encompassing the IRE1 cleavage sites with upstream primer 5′AAACAGAGTAGCAGCGCAGACTGC3′ and downstream primer 5′GGATCTCTAAAACTAGAGGCTTGGGATCTCTAAAACTAGAGGCTTGGTG3′. The products of RT-PCR were incubated with sialyltransferase 8D (Pst1) at 37 °C for 4 h, and the restriction fragments were resolved in a 2% agarose gel.

2.7. Measurement of intracellular GSH contents

To estimate the change of intracellular redox status, GSH levels were measured using a method described previously [13] with some modification. After exposure to 1 mM GSNO, CGCs were washed twice with phosphate-buffer saline (PBS) followed with incubation with 50 μM monochlorobimane at 37 °C for 15 min. Cells were then washed twice with PBS and lysed in 0.02% Triton X-100 in PBS. The lysates were sedimented at 12,000 × *g* for 10 min to remove insoluble debris. The concentrations of protein were determined using Bradford method and the intensities of fluorescence were measured with the emission at 480 nm (excitation at 400 nm) with spectrofluorimeter. The contents of intracellular GSH were expressed as fluorescent density per mg protein and the GSH contents of the control sample were defined as 100%. The relationship between GSH and the fluorescence at 480 nm was linear in the experimental cellular system.

2.8. RT-PCR assay

Total RNA was isolated from CGCs using the Uniq-10 total RNA Minpreps SuperKit. RT-PCR reactions were performed by Access Quick™ RT-PCR system, and PCR reactions were conducted with the Mastercycler personal (Eppendorf) for 30 cycles using the following conditions: denaturation 1 min at 94 °C, annealing 1 min at 55 or 60 °C, and primer extension of 2 min at 72 °C. The PCR products were separated in 2% agarose gels supplemented with ethidium bromide, and bands were visualized by UV transillumination and were photographed. GSNO-induced changes of genes expression levels were calculated with relative optical density of bands compared with marker band evaluated by image analysis.

The primers for gene-specific RT-PCR were as follows:

GluR5	upstream primer	5′-GTTTGTGATTGCGAGGTTCACA-3′
	downstream primer	5′-CAGGTTGGCCGTGTAGGATGA-3′;
Gadd153	upstream primer	5′-TCAGATGAAATTGGGGGCAC-3′
	downstream primer	5′-TTTCCTCGTTGAGCCGCTCG-3′;
Grp78	upstream primer	5′-GTTCTGCTTGATGTGTGTCC-3′
	downstream primer	5′-TTTGGTCATTGGTGATGGTG-3′;
Grp94	upstream primer	5′-TCCCCCTTAATGTTTCCCGTG-3′
	downstream primer	5′-TAGCCCTTCTTCAGAAGCCTC-3′;
SERCA2b	upstream primer	5′-GGGAGTGGGGCAGTGGCAGC-3′
	downstream primer	5′-CGTCTCTCTGGGCTGAGGGG-3′;
β-Actin:	upstream primer	5′-GAAGCATTTGCGGTGGACCA-3′
	downstream primer	5′-TCCTGTGGCATCCACCAAAC-3′.

GSNO-induced change in Gadd153 mRNA level was evaluated by quantitative PCR essentially described previously [18]. Quantitative PCR was performed as follows. All PCR reactions were conducted together with an internal standard which had a nonmatched sequence derived from rat Iontropic glutamate receptor subunit 5 (GluR5) in the middle and the sequence of the interest at each end. The internal standard could therefore be amplified together with the same set of primers as Gadd153, but yielded a smaller PCR amplification product. The PCR reactions of other ER-related genes (Grp78, Grp94, and SERCA2b) were conducted with the external standard β-actin [30].

2.9. Statistical analysis

All results are expressed as means ± S.E.M. of three or more experiments. Statistical analysis of the results was determined by the student *t*-test. The difference between means was considered statistically significant at $p < 0.05$.

3. Results

3.1. The neurotoxic effect of GSNO on CGCs

To elucidate the effect of GSNO on the CGCs, the cell viability was estimated by the MTT assay. First, we exposed the cells to 1 mM GSNO for different time and found that the cell viability start to decrease and the change becomes significant after 8 h. The GSNO-induced neurotoxicity was positively correlated with the duration of treatment (Fig. 1a). Second, we exposed the cells to different concentrations of GSNO for 24 h. The data showed that the cell viability of the groups treated with GSNO decreased significantly as compared to the control group, and the toxic effect was positively correlated with the GSNO concentration. That is, the cell viability dropped from about 80–40% after the exposure with GSNO from 100 μM to 2 mM (Fig. 1b). Furthermore, to eliminate the possible effects of the other factors in this system, we mimicked the above experiment with 1 mM $NaNO_2$, 1 mM GSH, and 1 mM exhausted GSNO solution in which GSNO had decomposed completely. The results

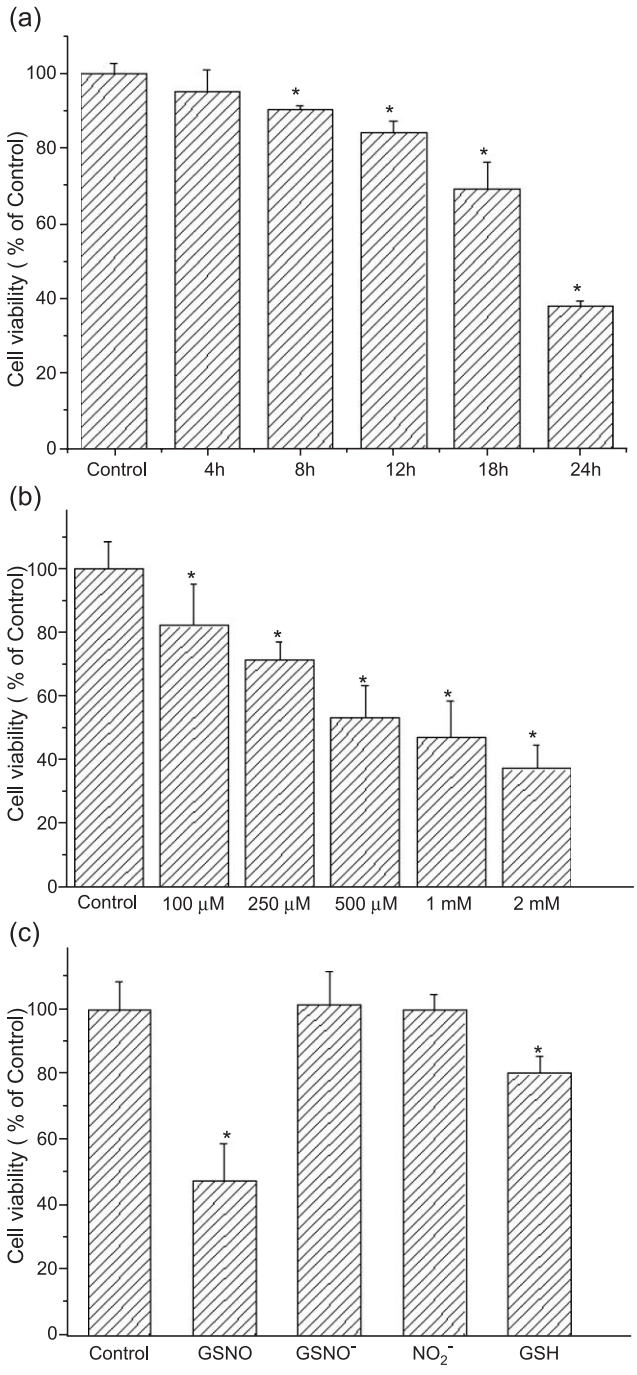

Fig. 1. Neurotoxicity indicated by the decreased cell viabilities induced by GSNO in CGCs. (a) Treated by 1 mM GSNO for different time. (b) Treated by different concentrations of GSNO for 24 h. (c) The cell viability of CGCs treated by 1 mM GSNO, 1 mM exhausted GSNO (GSNO-), 1 mM NaNO2, 1 mM GSH. *$p < 0.05$, as compared with the control.

showed that these factors did not significantly affect the cell viability (Fig. 1c). The neurotoxic effect of GSNO on the CGCs is therefore conclusive. In addition, the cell death is apoptotic rather than necrotic because there is DNA ladder in electrophorosis results and there is no significant release of lactate dehydrogenase (LDH) in our experimental conditions (data is not shown).

3.2. GSNO disturbs ER calcium homeostasis

To answer the question of whether GSNO disturbs the intracellular calcium homeostasis, we measured the changes of the intracellular calcium levels in the calcium-rich and the calcium-free Lock's solution respectively. In the calcium-rich solution, the level of the intracellular calcium elevated to 172 ± 7% of the control group (Fig. 2a), whereas the intracellular calcium level in the calcium-free system was 139 ± 4% of the control group (Fig. 2b). These results indicated that the elevation of the intracellular calcium in the GSNO-induced neurotoxicity was partially contributed by the intracellular calcium stores.

The next question is whether the increase of intracellular calcium was related to the ER. It is known that caffeine can cause the calcium release from ER calcium store through the activation of the ryanodine receptor-sensitive calcium channel. Fig. 2c showed that the release of the ER calcium store induced by 40 mM caffeine was significantly lower (102 ± 1% of the baseline) in GSNO-pretreated cells than that without GSNO-pretreated cells (121 ± 2% of the baseline). The lower release of calcium from ER indicated that the pretreatment of GSNO caused the depletion of ER calcium stores.

3.3. The existence of ER stress

ER calcium homeostasis is essential for the formation and the maintenance of the calcium-dependent structural protein complex and functional protein complex [9]. To investigate whether the depletion of ER calcium stores triggers ER stress in CGCs or not, we evaluated the IRE1-dependent cleavage of XBP-1 mRNA. It has been reported that the unfolded protein response (UPR) activated by ER stress could activate the transcription factor IRE1. The activated IRE1 can remove one small intron containing a Pst1 cleavage site from the XBP-1 mRNA and lead to the incomplete splicing of XBP-1 mRNA when treated with Pst1 [7,19]. Our results showed that the XBP-1 mRNA from GSNO-treated CGCs was incompletely cleaved by Pst1 (Fig. 3). This implied that GSNO did cause ER stress.

3.4. Upregulation of Gadd153 expression and the decrease of intracellular GSH

What are the downstream events of ER stress in the neurotoxicity? It has been reported that induction of Gadd153 gene expression correlates with the onset of apoptosis, Gadd153 gene might be one key factor in this event [11,15,52]. As shown in Fig. 4a, Gadd153 expression was upregulated and this change becomes significant after 8 h: mRNA levels were 105 ± 20% (4 h), 394 ± 60 % (8 h), 415 ± 70% (12 h) of the control, respectively. The elevation of Gadd153 expression in perturbation of the ER

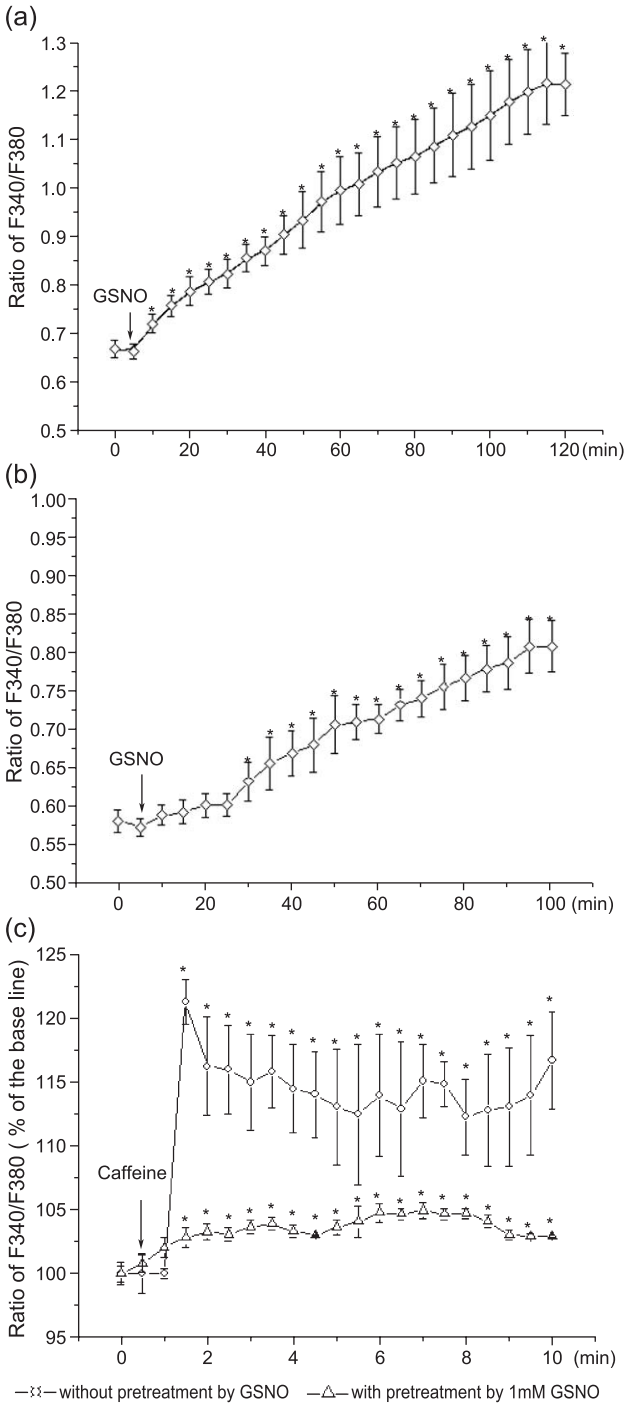

Fig. 2. GSNO disturbed the ER calcium stores in CGCs. (a) The mobilization of intracellular calcium induced by 1 mM GSNO in calcium-containing BBS. (b) The mobilization of intracellular calcium induced by 1 mM GSNO in calcium-free BBS. (c) The calcium mobilization induced by 40 mM caffeine in CGCs with or without pretreatment of 1 mM GSNO. All pots were based on separated neurons (n>10). The result shown is a representative of three independent experiments *$p < 0.05$, as compared with the baseline before the treatment by 1 mM GSNO.

homeostasis may result in the depletion of cellular glutathione [29]. This was further supported by the change of intracellular GSH levels in CGCs treated with 1 mM

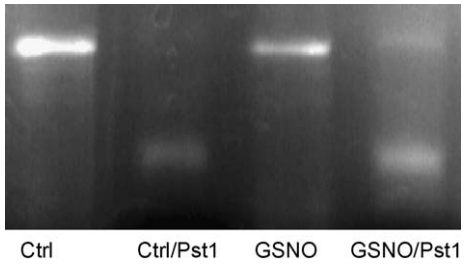

Fig. 3. Incomplete splicing of XBP-1 mRNA by Pst1 indicated ER stress in CGCs treated by GSNO. The result shown is a representative of three independent experiments.

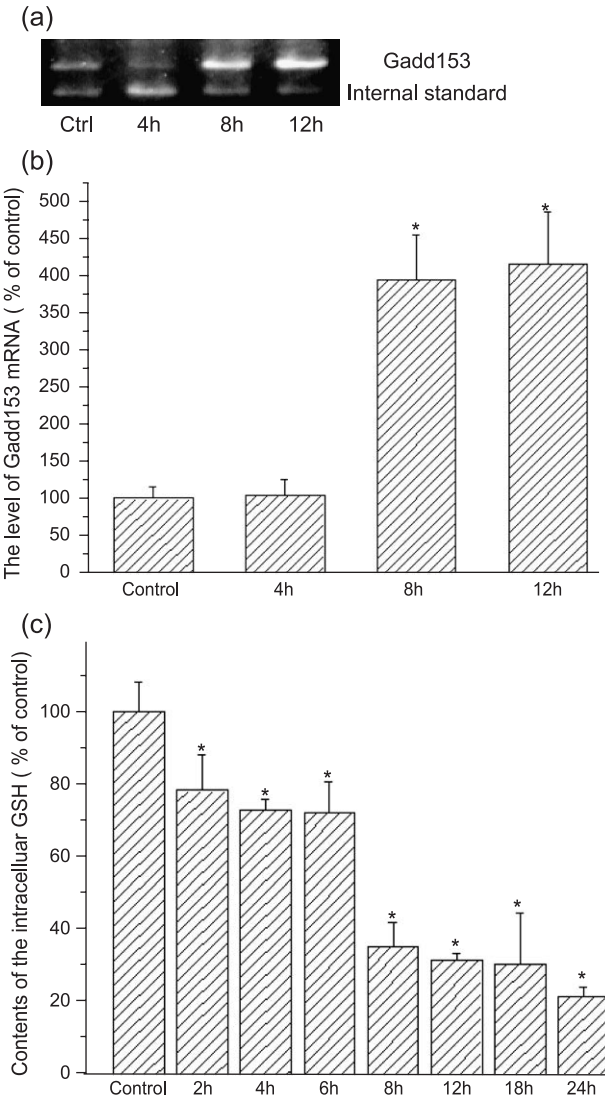

Fig. 4. GSNO upregulated the expression of Gadd153 and decreased the intracellular GSH levels. (a) and (b) The expression of Gadd153 in CGCs induced by 1 mM GSNO for different period (0, 4, 8, 12 h). The levels of Gadd153 expression were upregulated and the change becomes significant after 8 h. The result shown is a representative of three independent experiments. GluR5 was used as internal standard. (c) The changes of intracellular GSH levels in CGCs treated by 1 mM GSNO for different period (0, 2, 4, 6, 8, 12, 18, and 24 h). The decrease of the intracellular GSH contents was time-dependent. All data were represented as mean ± S.E. (n = 4). *$p < 0.05$ as compared with the control group.

GSNO. As shown in Fig. 4c, the contents of intracellular GSH were time-dependently decreased. (The GSH level decreased to $72 \pm 2\%$, $73 \pm 2\%$, $67 \pm 2\%$, $35 \pm 6\%$, $32 \pm 2\%$, $23 \pm 2\%$, $22 \pm 2\%$ of the control group following 2, 4, 6, 8, 12, 18, 24 h GSNO treatment, respectively). The changes of Gadd153 expression and the intracellular GSH level attribute to the neurotoxicity in CGCs.

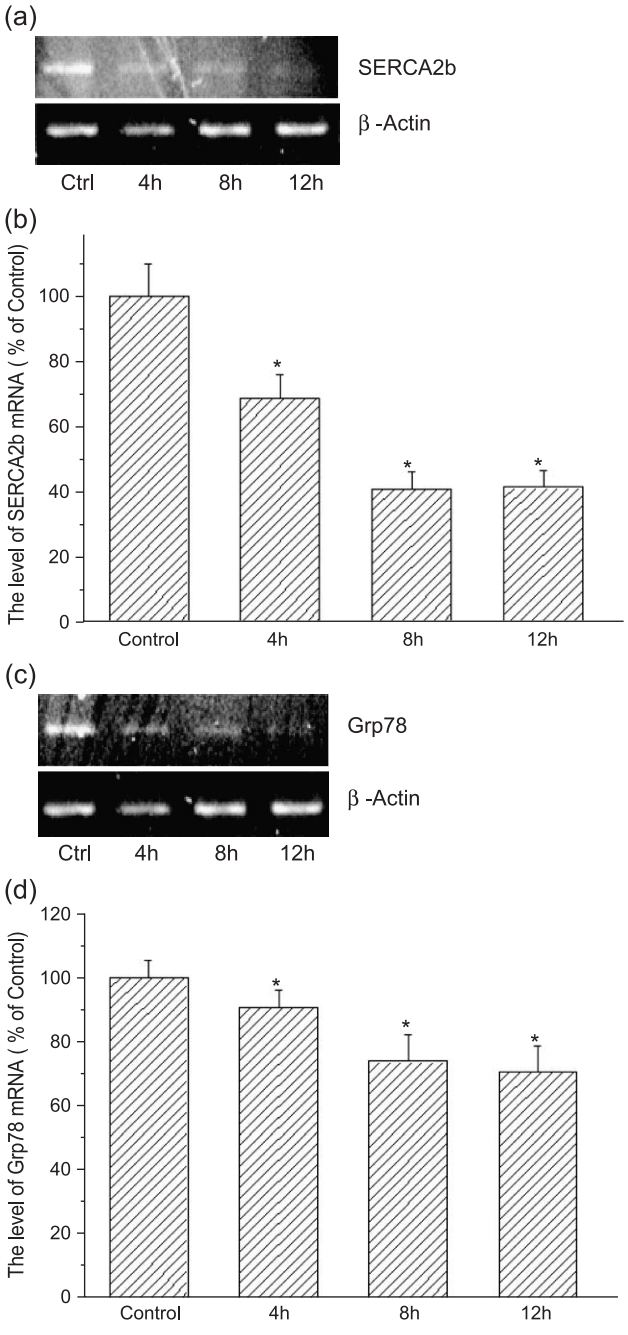

Fig. 5. GSNO suppressed the expression of SERCA2b, Grp78, Grp94 in CGCs induced by 1 mM GSNO for different period (0, 4, 8, and 12 h). (a) and (b) SERCA2b. (c) and (d) Grp78. (e) and (f) Grp94. β-Actin was used as an external standard in all above experiments. The result shown is a representative of three independent experiments. $*p < 0.05$ as compared with the control group.

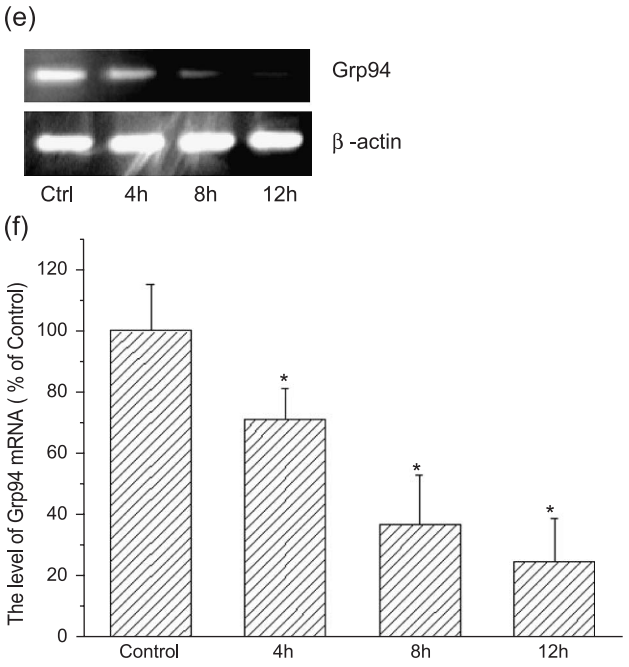

Fig. 5 (*continued*).

3.5. Downregulation of SERCA2b, Grp78 and Grp94 expression

Furthermore, we attempted to know the effects of GSNO on ER calcium pump ER Ca^{2+}-ATPase (SERCAs) and ER chaperones such as Grp78 and Grp94. Although it has been reported that NO inhibited the SERCA activity [10], whether GSNO affects the SERCA-encoding genes at the transcriptional level remains to be investigated. Fig. 5a and b showed that GSNO downregulated the expression of SERCA2b (mRNA levels were $69 \pm 7\%$ (4 h), $41 \pm 5\%$ (8 h), $41 \pm 5\%$ (12 h) of the control, respectively). This result implied that the suppression of SERCA2b mRNA might contribute to the prolonged disturbance of ER calcium stores. At the same time, we measured the mRNA expression of Grp78 and Grp94. As shown in Fig. 5c, d, e and f, Grp78 and Grp94 were all suppressed significantly (Grp78: $90 \pm 9\%$ (4 h), $74 \pm 8\%$ (8 h), $71 \pm 7\%$ (12 h) of the control group, respectively; Grp94: $91 \pm 10\%$ (4 h), $85 \pm 8\%$ (8 h), $71 \pm 9\%$ (12 h) of the control group, respectively). These results are corroborated with the earlier data of NO-dependent suppression of gene expression in some other experimental systems [2,10,46].

4. Discussion

In this study, we showed that ER stress existed in the GSNO-induced neurotoxicity in CGCs and there was an upregulation of Gadd153 and the depletion of the GSH pool. Gadd153 is a proapoptotic gene encoding a tran-

scription factor which is recognized as an inhibitor of gene expression through interacting with other transcription factors [12,39] and cAMP-responsive element binding protein (CREB) families [44,49]. However, the phosphorylation of CREB can upregulate bcl-2, which is an important regulator of intracellular GSH [29,47]. Therefore, the depletion of intracellular GSH pool may be related to the suppression of proapoptotic bcl-2 due to the interaction between Gadd153 and CREB [23]. It is also reported that GSH depletion switched nitric oxide neurotrophic effects to cell death [24]. All these evidence suggested that the upregulation of Gadd153 and the disturbance of cell redox status play crucial roles in the neurotoxicity induced by GSNO.

SERCAs are encoded by three homologous genes (SERCA1, SERCA2, SERCA3), in which SERCA2b isoform is expressed ubiquitously in all nonmuscle tissues. Previous evidence indicated that SERCA2b could be upregulated to protect cells from stress [8]. Grp78 and Grp94 are also reported to be upregulated as a response to unfolded protein in ER stress [5]. However, in the present study, we found these genes (SERCA2b, Grp78 and Grp94) were all significantly downregulated. The downregulation was possibly processed via a common pathway. It has been reported that cell viability diminished greatly following the repression of Grp78 induction [14]. Our results suggested the repressed transcription of all these protective genes caused by GSNO possibly contributed to the sensitization of the neurons to stress, switching neurons from survivable stress to death. The expression of SERCA2b, Grp78 and Grp94 was also closely related to the IRE1-dependent XBP-1 signaling pathway via the following possibility: the translation of XBP-1 mRNA, the posttranslational modification of XBP-1, and the interaction of XBP-1 with specific gene promoters [8,45,48]. The results of the downregulated expression of SERCA2b, Grp78 and Grp94 implied that the above pathway was affected by GSNO. The detail remains to be further investigated.

NO is reported to deplete ER calcium stores by inhibiting SERCA activity [10,46] or by activating ryanodine receptors via protein nitration [51]. In our experimental model, we found that SERCA2b was downregulated at the transcriptional level, and we deduced that SERCA2b dysfunction might be a mechanism of the depletion of ER calcium stores because (1) SERCAs contain the active thiols, which is possibly sensitive to nitrosylating species [28]. (2) In addition, NO inhibits the activity of SERCA2a by tyrosine nitration [46], SERCA2b differs from SERCA2a only in its C-terminal regions [33]. (3) And, our results showed that GSNO depleted the intracellular GSH pool, which may provide an oxidative environment to facilitate the S-nitrosylation of SERCA2b.

Although we have elucidated a serial of ER stress-related events in GSNO-induced neurotoxicity in CGC, there is the crosstalk between the ER stress pathway and the mitochondrial pathway. There are at least three major aspects concerned in the crosstalk between the two organelles. (1) Energy metabolites: GSNO was potent in inhibiting energy metabolites [4] and inhibiting creatine kinase by *S*-nitrosoglutathione [50]. The effect of GSNO on mitochondria ATP likely affects the energy supply of ER, which might cause some ER response; that is, ATP depletion resulted from the inhibition of mitochondrial ATP synthesis may be the main reason for the disruption of ER calcium stores [6]. A contradictory conclusion has also been reported in which the depletion of ER calcium stores was not due to ATP depletion [10]. (2) Bcl-2: Bcl-2 is an ER-resident protein regulating the cellular redox status and may translocate to mitochondria to regulate mitochondrial membrane permeabilization (MMP). (3) Calcium signals: which may act as mediator of the crosstalk due to the close spatial contact of IP3R in ER with the mitochondrial surface [38].

In conclusion, we established the experimental model of neurotoxicity induced by GSNO in CGCs. Our results elucidated the following: (1) GSNO could cause the sustained elevation of intracellular calcium in CGCs and this elevation was partially the result of the depletion of ER calcium stores. (2) There existed ER stress in the GSNO-induced neurotoxicity indicated by the IRE1-dependent cleavage of XBP-1 mRNA. (3) GSNO caused the upregulation of Gadd153 and the depletion of intracellular GSH pools, which were a death signal-induced by severe ER stress. At the same time, we found that GSNO downregulated the expression of SERCA2b at the transcriptional level in parallel with the downregulation of ER chaperones Grp78 and Grp94, which might imply an alternative mechanism involved in NO neurotoxicity. Our results elucidated ER stress aspects in GSNO-induced neurotoxicity in cerebellar granule cells, which could deepen our understanding of the detail of NO in the pathogenesis of several neurodegenerative disorders featured by misfolded proteins.

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