Endoplasmic reticulum stress contributes to CRH-induced hippocampal neuron apoptosis

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ABSTRACT

The hypothalamic–pituitary–adrenal (HPA) axis is critical to mediating the body’s response to stress. Corticotropin releasing hormone (CRH) plays a central role in controlling the stress response and regulating the HPA axis. Recent findings support CRH participates in the stress-induced hippocampal neuron apoptosis, but the underlying mechanisms are not fully understood. Our present study demonstrates that CRH can independently decrease hippocampal neuron cell viability \textit{in vitro} in a concentration- and time-dependent manner. CRH receptor 1 (CRHR1) is involved in CRH-induced neuron apoptosis. Endoplasmic reticulum (ER) stress response marker, glucose-regulated protein 78 (GRP78), either protein or mRNA, is significantly elevated after treatment of CRH, and decreased when co-treated with salubrinal, ER stress inhibitor. The ER stress associated proapoptotic transcription factor C/EBP homologous protein (CHOP) and cleavage of caspase-12 protein expression are also increased following CRH treatment. Furthermore, we investigate which ER stress cascades are affected by CRH. CRH activates inositol-requiring enzyme 1 (IRE1), apoptosis signal regulating kinase 1 (ASK1), and c-jun kinase (JNK). Neuron apoptotic rate, examined by flow cytometry, is increased when CRH treatment and attenuated by salubrinal, thioredoxin (ASK1 inhibitor) and SP600125 (JNK inhibitor). Therefore, current data indicate that ER stress, through activating the IRE1/ASK1/JNK cascade, plays an important role in CRH-induced neuron apoptosis.

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Introduction

Activation of the hypothalamic–pituitary–adrenal (HPA) axis is recognized as a defining endocrine feature of the stress response. This activation is primarily mediated by corticotropin releasing hormone (CRH). Recently, several lines of evidence suggest that CRH may play a role in stress-induced hippocampal dysfunction [1]. For instance, CRH contributes to hippocampal ischemic injury, an effect prevented by the CRH antagonist \textalpha -helical CRH (\textalpha hCRH) [2]. Similarly, astressin, a potent CRH antagonist, exerts a considerable neuroprotective effect on hippocampal cell damage following kainic acid-induced excitotoxic seizures [3]. In hippocampal organotypic cultures, exogenous synthetic CRF blunts the dendritic growth and influences the function of the immature hippocampus [4]. Furthermore, administration of CRH to the brain of immature rats induces hippocampal CA3 neuron apoptosis.

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independent of glucocorticoids [5]. These phenomena all involve cell apoptosis [6]. However, the effects of CRH on the apoptotic machinery have not been fully elucidated.

The endoplasmic reticulum (ER) is an important subcellular organelle that is responsible for the proper folding and sorting of proteins. It is susceptible to various stresses that provoke the accumulation of unfolded proteins in the ER lumen. ER stress response, also known as the unfolded protein response (UPR), is triggered by accumulation of unfolded protein in the ER lumen. At least three ER transmembrane receptors are important in the initial signaling of ER stress to the cell by UPR. These proteins are: inositol-requiring enzyme-1 (IRE1), activating transcription factor 6 (ATF6) and protein kinase RNA (PKR)-like ER kinase (PERK) [7]. GRP78 (glucose-regulated protein 78 kDa), the best-characterized ER chaperone protein, associates with the intraluminal regions of IRE1, ATF6 and PERK in their inactive state and upon ER stress it is dissociated followed by their activation leading to UPR. So it has served as a classical marker for UPR activation [8]. UPR signaling can protect cells from such ER stress by expanding the amount of ER in the cell, enhancing the degradation of misfolded proteins, and reducing the synthesis of new proteins. If homeostasis cannot be reestablished, however, UPR signaling eventually induces cell death by apoptosis.

IRE1 is first identified as an ER membrane-spanning receptor protein kinase required for inositol phototrophy [9,10]. Jonathan et al. has shown that IRE1 signaling affects cell fate during the unfolded protein response, in three parallel branches of the UPR [11]. The IRE1 pathway can activate apoptosis by recruiting the adaptor protein TRAF2 (TNF-receptor-associated factor 2), which recruits the apoptosis signaling kinase-1 (ASK1), which, in turn, activates c-jun N-terminal kinase (JNK).

Based on these findings, in the present study, we explored the effects of CRH on cell viability of hippocampal neurons in vitro, and the relation between neuron cell apoptosis and ER stress. Furthermore, the IRE1/ASK1/JNK cascade pathways were examined. Our results indicate that CRH can decrease the cell viability of primary cultured hippocampal neurons in a concentration- and time-dependent manner. The marker chaperon of ER stress, GRP78, either protein or mRNA levels, is elevated in CRH-induced neuron cell apoptosis. In addition to the UPR, induction of CHOP and cleavage of pro-caspase-12 are also detected. The IRE1/ASK1/JNK pathways are activated, which might participate in the CRH-induced apoptosis.

**Materials and methods**

**Reagents and antibodies**

Reagents for primary hippocampal neuron culture, including Dulbecco’s modified Eagle’s medium (DMEM), neurobasal medium and B27 were purchased from Gibco-BRL (Gaithersburg, MD, USA). CRH was purchased from Anaspec (Fremont, CA, USA). CP154526, a CRH type 1 receptor (CRHR1) antagonist, was obtained from Tocris Bioscience (Bristol, UK). Tunicamycin, thioridoxin, poly-L-lysine and MTT were from Sigma-Aldrich (USA). SP600125 was from Enzo Life Science (Farmingdale, NY, USA). Salubrinal and XBP-1 antibody were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies targeted towards MAP2 and CHOP were purchased from Cell Signaling Technology (Beverly, MA, USA). GRP78, caspase-12, total- and phosphorylated (p)-IRE1, total- and phosphorylated (p)-ASK1 were obtained from Abcam (Cambridge, UK). Total- and phosphorylated (p)-JNK were from Epitomics (Burlingame, CA, USA).

**Primary hippocampal neuron culture and treatment**

Pregnant Sprague–Dawley rats were supplied by the Experimental Animal Center of Hebei Medical University (Shijiazhuang, Hebei, China). Hippocampal neurons prepared from embryonic day 18 rats were cultured for 8 d on poly-l-lysine (0.1 mg/ml)-coated dishes in neurobasal medium with 2% B27 supplement as described previously [12,13]. Cultures were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. The cultured neurons were identified by immunocytochemistry with antibody against microtubule associated protein-2 (MAP2), which is marker for neurons. The culture with more than 90% neurons was used in the following experiments.

On the eighth day in vitro, different concentrations of CRH, from 1 × 10⁻⁸ M to 1 × 10⁻⁵ M, were added to the cells for various periods of time to investigate the cell viability. CP154526 (CP, 5 × 10⁻⁴ M, CRHR1 antagonist), co-treated with CRH, was used to examined the pathway of CRH action. Tunicamycin (TM, 1 μg/ml, ER stress inducer) and salubrinal (Sal, 50 μM, ER stress inhibitor) were used to produce or block ER stress, respectively. These two agents have been used by others to confirm the occurrence of ER stress [14,15]. Thioridoxin (TR, 1 μM, ASK1 inhibitor) and SP600125 (SP, 25 μM, JNK inhibitor) were added to measured the apoptotic rate induced by CRH.

**Cell viability**

Cell viability was estimated using 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays [16]. After treatment, cells were incubated with MTT (0.5 mg/ml) for 4 h at 37 °C. After medium removal, formed formazan blue was extracted with dimethylsulfoxide (DMSO) and quantified using a spectrophotometer at 490 nm. Experiments were carried out at least three times. The proportion of cell viability was calculated by the following formula: survival% = (test A490/mean control A490) × 100%.

**Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining**

Primary cultured hippocampal neurons were treated as described above. Apoptotic cells were identified using the TUNEL technique per the manufacturer’s instructions (in situ cell death detection kit, Roche Applied Science, Hangzhou, China). Hoechst, the nuclear dye, was used to identify the cells in the field. Labeled neurons were analyzed with a fluorescent microscope.

**Western blot analyses**

Hippocampal neurons were lysed using ice-cold homogenization buffer (150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 50 mM Tris–HCl, pH 7.4) and protein concentration was determined using a standard Coomassie brilliant blue (CBB) total protein assay kit (Jiancheng Bioengineering, Nanjing, China). Equal amounts of protein (50 μg) were subjected to 8%–10% SDS-PAGE and blotted onto polyvinylidene fluoride (PVDF) membranes.
The membranes were blocked for 1 h at 37 °C with 5% skimmed-milk in Tris-buffered saline containing 0.05% Tween-20 (TBST), and then overnight at 4 °C with primary antibodies: anti-GRP78 (1:1000), anti-XBP-1 splicing (1:500), anti-caspase-12 (1:1000), anti-total-IRE1 (1:500), anti-phosphorylated (p)-IRE1 (1:500), anti-total-ASK1 (1:500), anti-phosphorylated (p)-ASK1 (1:500), anti-total-JNK (1:500), anti-phosphorylated (p)-JNK (1:1000), and anti-β-actin (1:1000). After washing with TBST three times, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5000) at room temperature for 2 h, followed by detection using enhanced chemiluminescence (ECL, Pierce, Rockford, IL, USA). Immunoreactive bands were visualized using X-ray films. For quantitative analysis, bands were evaluated with ImageJ software, normalized for β-actin density.

**RNA isolation and real-time RT-PCR**

Total RNA was extracted with TriZol Reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA, USA). Complementary DNA was synthesized from the total RNA (0.5 μg) using the PrimeScript™ RT reagent Kit following the instructions provided by the manufacturer (Takara Biotechnology, Dalian, China). Subsequently, the cDNA was subject to real-time PCR using Power SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK). Each real-time PCR reaction consisted of 2 μl diluted RT product, 10 μl SYBR Green PCR Master Mix (2×) and 250 nM forward and reverse primers in a total volume of 20 μl. Reactions were carried out on 7500 real-time PCR System (Applied Biosystems) for 40 cycles (95 °C for 15 s, 60 °C for 45 s) after initial 10 min incubation at 95 °C. The primers used for real-time PCR were as follows: GRP78 forward: 5′-AAG GTG AAC GAC CCC TAA CAA A-3′; reverse: 5′-GTC ACT CGG AGA ATA CCA TTA ACA TCT-3′; XBP-1 splicing forward: 5′-AGA GTA GCA GCA CAG ACT GCG CG-3′; reverse: 5′-GGA ACT GG-GT-CCT TCT GGG TA-3′; CHOP forward: 5′-GCC TTT CGC CTT TGA GAC AGT-3′; reverse: 5′-TGA CAT ATA GGT GCC CCC AAT T-3′ [17]; GAPDH forward: 5′-GGC ATG GAC TGT GGT CAT GA-3′; reverse: 5′-TTC ACC ACC ATG GAG AAG GC-3′. The fold change in expression of each gene was calculated using the ΔΔCt method, with the housekeeping gene GAPDH mRNA as an internal control.

**Annexin V and propidium iodide staining assay**

Cells were cultured as described above and treated with CRH, CRH + Sal, CRH + TR, CRH + SP, and TM, then rinsed with PBS and harvested in the buffer (PBS-0.05% trypsin). Apoptotic cells in different groups were determined by Annexin V/PI apoptosis detection kit according to manufacturer’s protocol (MultiSciences Biotech, Hangzhou, China). Briefly, the cell pellet was resuspended in 1× binding buffer followed by incubation with 5 μl of Annexin V (conjugated with FITC) and 10 μl of PI in dark for 5 min. The fluorescence of the cells was then analyzed by flow cytometer (Epics-XLII, Becman Coulter). This test discriminates intact cells (Annexin V−/PI−), early apoptotic cells (Annexin V+/PI−) and late apoptotic cells (Annexin V+/PI+).

**Statistical analyses**

Results were presented as mean ± SEM. Statistical analyses were performed by one-way ANOVA, followed by LSD test using SPSS statistical software (v.17.0). The p values less than 0.05 were considered to be significant.

**Results**

**CRH decreased hippocampal neuron cell viability in vitro in a concentration- and time-dependent manner**

In order to determine a concentration of CRH that was cytotoxic to cells in vitro, primary cultured hippocampal neurons were treated with various concentrations (1 × 10⁻⁸ to 1 × 10⁻⁵ M) of CRH for 48 h and cell viability was determined by MTT assays (Fig. 1A). The untreated control group was normalized to 100%. CRH
decreased cell viability in a concentration-dependent manner. From $1 \times 10^{-8}$ M to $1 \times 10^{-5}$ M, CRH gradually decreased cell viability to 78.8%, 75.2%, 68.9% and 42.9% of untreated control, respectively. Therefore, the lower cytotoxic concentration, $1 \times 10^{-8}$ M, was selected to investigate the time-dependent manner of CRH and its relevance to apoptosis.

Then we examined the time-dependent manner of CRH on cell viability in the cultured hippocampal neurons. CRH was applied at $1 \times 10^{-8}$ M for 1, 4, 8, 16, 24, 48 h and analyzed by MTT assays (Fig. 1B). CRH decreased cell viability in a time-dependent manner. For 16, 24, 48 h, CRH produced a significant 15.3%, 17.8%, and 24.6% decrease in neuronal viability as compared with untreated controls. Therefore, the shortest action time of CRH was 16 h, which was selected to examine cell apoptosis.

**CRHR1 participated in CRH-induced neuron apoptosis**

In order to examine the involvement of CRH receptor in CRH-induced apoptosis, CP154526, CRHR1 antagonist, was co-treated with $1 \times 10^{-8}$ M CRH. Apoptosis was determined by TUNEL method. Data demonstrated that the number of TUNEL-positive cells significantly increased in neurons treated with CRH, from 5.2% in untreated control cells to 14.6%, and CP154526 markedly abolished the increase in apoptosis (Fig. 2A, B). These data suggested that CRHR1 participated in the CRH-induced apoptosis.

**CRH induced the protein and mRNA expressions of GRP78, XBP-1 splicing and CHOP**

The effects of CRH on protein levels of ER stress biomarkers, GRP78 and CHOP, were investigated in hippocampal neurons by Western blot. CRH significantly increased the protein levels of GRP78, XBP-1 splicing and CHOP by 139%, 149% and 542%, respectively compared to the untreated control, and the increases of these proteins by CRH were effectively inhibited by salubrinal. As expected, the characterized ER stress inducer, tunicamycin robustly increased the protein levels of GRP78, XBP-1 splicing and CHOP (Fig. 3A, B).

The effects of CRH on the mRNA expressions of GRP78, XBP-1 splicing and CHOP in neurons were also measured by real time RT-PCR. Similarly, CRH increased the mRNA levels of GRP78, XBP-1 splicing and CHOP by 328%, 203% and 552%, respectively compared to the untreated control. Salubrinal inhibited the increases of mRNA levels by CRH. But tunicamycin markedly increased the mRNA levels of GRP78, XBP-1 splicing and CHOP (Fig. 3C).

**CRH induced the cleavage of caspase-12**

Caspase-12, which is identified as the first ER-associated member of the caspase family, is activated by ER stress [18]. This novel caspase is regarded as a representative molecule implicated in the cell death-executing mechanisms relevant to ER stress [19]. In order to examine the involvement of caspase-12 activation in the CRH-induced apoptosis, it was investigated by Western blot. In cultured neurons, the cleavage of caspase-12 occurred after CRH addition, and decreased when co-treated salubrinal. Tunicamycin, also significantly increased the cleavage of caspase-12 (Fig. 4A, B).

![Fig. 2](image-url) – CRHR1 participated in CRH-induced neuron apoptosis. (A) CRH-treated and co-treated with CP154526 (CRHR1 antagonist) neurons were examined with TUNEL method, to identify apoptotic cells, and with the nuclear dye, Hoechst, to identify the cells in the field. Arrows point out some of the TUNEL-positive cells. (B) TUNEL-positive cells were counted in a total of more than 300 cells over 3 random fields and expressed as percentages of the total number of nuclei. Data are expressed as mean ± SEM of three independent experiments. *p < 0.05 compared with untreated control. #p < 0.05 compared with CRH treatment.

**CRH activated IRE1/ASK1/JNK cascade**

In order to determine the signaling pathway of CRH-induced ER stress, the IRE1/ASK1/JNK cascade of ER stress was investigated in hippocampal neurons by Western blot. CRH stimulated phosphorylation, i.e., activation of IRE1, ASK1 and JNK, and the phosphorylation of these proteins by CRH were effectively inhibited by salubrinal. Tunicamycin also enhanced activation of these proteins. However, the expressions of total proteins did not vary (Fig. 5A, B, C, D).

**ER stress contributed to CRH-induced hippocampal neuron apoptosis**

To investigate CRH-induced apoptosis and its relevance to ER stress, Annexin V and PI staining was used to measure the
hippocampal neuron apoptotic rate. This assay was divided into two stages: early apoptotic (Annexin V⁺/PI⁻) and late apoptotic (Annexin V⁺/PI⁺) cells, the two parts of cells represented the total cells of apoptosis. As shown in Fig. 6, the percentage of cells including early and late apoptotic cells was increased significantly when CRH treatment, compared with untreated control. Co-treated with salubrinal, thioredoxin (TR, ASK1 inhibitor), and SP600125 (SP, JNK inhibitor), the apoptotic rates were all decreased, although it was not completely abolished. Tunicamycin also induced observable apoptosis.

Discussion

CRH is a 41-amino acid peptide with multiple biological effects and plays a central regulatory role in the HPA stress response system. Enhanced expression of CRH in both adult [20] and immature [21] rat hippocampal interneurons by stress-related neuronal activation has been demonstrated. Moreover, administration of CRH to the brain of immature rats can induce hippocampal CA3 neuron apoptosis, and importantly, they did not require the presence of stress levels of glucocorticoids [5]. Our present study demonstrates that CRH can independently decrease hippocampal neuron cell viability in a concentration- and time-dependent manner, which confirms above-mentioned results in vitro. Simultaneously, co-treated with CRH and CP154526, CRHR1 antagonist, apoptotic neurons are significantly decreased, suggesting that CRH exerts hippocampus defects by activating CRHR1.

CRH has two distinct G-protein-coupled receptors (GPCRs), CRHR1 and CRHR2. Both receptors have been identified in the hippocampus [22]. Studies in animal models show that the behavioral
and hormonal effects of CRH can be ascribed to CRHR1-mediated actions [23-25]. Clinical studies in humans also support that stress-induced CRH actions are mediated through binding to CRHR1 [26]. While CRHR2, can be found predominantly in subcortical areas and in peripheral tissues, whose specific central and peripheral roles are still under investigation [27,28].

In the present study, we further find evidence for a strong association between apoptotic cell death and ER stress in primary cultured hippocampal neurons exposed to CRH. The characterized ER chaperone, GRP78, either protein or mRNA levels, is up-regulated in CRH treatment group. Moreover, this upregulation is inhibited by salubrinal, the ER stress inhibitor, suggesting that ER stress participates in the CRH-induced hippocampal neuron apoptosis.

So far, the ER stress-mediated apoptotic pathway is only partially characterized, although some ER stress-specific components of the pathway have been identified. CHOP, also known as growth-arrest- and DNA-damage-inducible gene 153 (GADD153), was originally identified in response to DNA damage. However, CHOP induction is probably most sensitive to ER stress conditions [29]. During ER stress, all three arms of the UPR induce transcription of CHOP. CHOP is also regulated post-translationally by phosphorylation on serine residues 78 and 81 by p38 MAPK, which increases its activity. This is interesting, given that p38 is a substrate of ASK1, which is recruited to the IRE1–TRAF2 complex on ER stress. The role of CHOP in ER stress-induced apoptosis has been illustrated in CHOP−/− mice [29]. Our data demonstrate that the expression of CHOP, either proteins or mRNA levels, is up-regulated in CRH-induced neuron defects. Therefore, we conclude that ER stress response is initiated in CRH-induced hippocampal neuron apoptosis, and this response may be significant in the nervous system dysfunction observed during stress.

Another specific component of the pathway is caspase-12, which has been proposed as an initiator caspase and also as the key molecule in the death-driving force in ER stress [30]. However, the precise role of caspase-12 in ER stress-mediated cell death is not clear, because the human gene shows large deletions [31]. Caspase 4 has been proposed to fulfill the function of caspase-12 in humans, but this is under debate. In this study, we show that CRH induces cleavage of caspase-12 in primary cultured hippocampal neurons. Previously, caspase-12 cleavage has been observed in hippocampal neurons lacking the calcium-binding protein hippocalcin [32]. In addition, cells lacking caspase-12 are resistant against degeneration induced by the amyloid β peptide and by tunicamycin causing ER stress [19]. About the mechanism, in part, IRE1, by recruiting TRAF2, mediated induction of caspase-12 oligomerization and cleavage and then activated caspase-12 [33]. However, little consistent data linking caspase-12 to downstream caspase activation is available. Moreover, no definitive

Fig. 5 – Effect of CRH on IRE1/ASK1/JNK cascade. (A) Protein representative photographs of total and phosphorylated IRE1, ASK1, and JNK. (B) Protein levels of total and phosphorylated IRE1. (C) Protein levels of total and phosphorylated ASK1. (D) Protein levels of total and phosphorylated JNK. The level of each protein was quantified by densitometric analysis and normalized to the level of β-actin. Data are expressed as mean±SEM of three independent experiments. *p < 0.05 compared with untreated control. #p < 0.05 compared with CRH treatment.
substrates or human orthologue for caspase-12 have yet been identified, making it difficult to establish a common role for this caspase in ER stress-induced apoptosis.

IRE1 is a protein kinase and an endoribonuclease with a dimerization domain in the ER lumen and the enzymatic domains in the cytosol. The oligomerization and autophosphorylation of IRE1 result in the activation of ribonuclease activity whereby XBP-1 mRNAs are spliced to generate the spliced variant XBP-1s, a more potent transcription factor. A subset of UPR genes participating in ER protein folding and ER-associated degradation (ERAD) are activated by XBP-1s [34].

Though activation of XBP-1 through IRE1 is a protective role, persisting overexpression of IRE1 is reported to result in apoptotic cell death in HEK293T [35], in a mechanism that is independent from its role in XBP-1 splicing. Active IRE1 has been shown to recruit the adaptor molecule TNF-receptor-associated factor 2 (TRAF2). The IRE1–TRAF2 complex formed during ER stress can recruit the apoptosis-signal-regulating kinase (ASK1), which is a mitogen-activated protein kinase kinase kinase (MAP3K) that has been shown to relay various stress signals to the downstream MAPKs JNK and p38 [36]. Overexpression of ASK1 induced apoptosis in several cell types, whereas neurons from ASK1−/− mice exhibited resistance to lethal ER stress, illustrating the importance of ASK1 in ER stress-induced cell death [37]. Activation of JNK has also been reported in response to ER stress and was shown to be IRE1- and TRAF2-dependent [38]. Activation of JNK is a common response to many forms of stress and is known to influence the cell-death machinery through the regulation of BCL2 family proteins [39]. In the current study, we show that CRH activates JNK, and its upstream initiators IRE1 and ASK1, and these changes are inhibited by salubrinal. Moreover, the present data demonstrate that CRH increases apoptotic rate of hippocampal neuron in vitro, which is attenuated by the ASK1 inhibitor thioredoxin, and the JNK inhibitor SP600125. Therefore,
our data indicate that CRH activates the IRE1/ASK1/JNK cascade in primary cultured hippocampal neurons, which may be relevant to stress-induced neuron cell death.

In summary, our studies demonstrate that CRH can decrease hippocampal neuron cell viability in a concentration- and time-dependent manner, and CRHR1 is involved in CRH-induced neuron apoptosis. ER stress, through activating IRE1/ASK1/JNK cascade, plays a key role in the mechanisms underlying CRH-induced neuron apoptosis. However, we acknowledge that the treatment concentration of CRH employed in this study is only experimentally determined in a cell culture model and their link to normal human is not relevant. Therefore, future studies are needed to validate current findings with more complex systems such as organotypic cultures and animal models, even human when stressed. Anyway, our data provide important views in deeply understanding the roles of ER stress and apoptosis in CRH-induced neuron damage. It will offer a preliminary rationale for studies assessing the effects of CRH on stress-mediated physiological dysfunction. It is also worth mentioning that, dissimilar to other stress responses, the mediators of ER stress are specific and well defined; therefore, they could be useful targets for therapy. Inhibitor of the IRE1–TRAF2 interaction by small chemicals or by antagonistic IRE1-interacting adaptor proteins will be a plausible strategy for stress-induced nervous system defects.

Conflict of interest statement

The authors have no financial conflict of interest.

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