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Effects of Corticosterone on Beta-Amyloid-Induced Cell Death in SH-SY5Y Cells

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease characterized by neuronal cell death and memory impairment. Corticosterone (CORT) is a glucocorticoid hormone produced by the hypothalamic-pituitary-adrenal axis in response to a stressful condition. Excessive stress and high CORT levels are known to cause neurotoxicity and aggravate various diseases, whereas mild stress and low CORT levels exert beneficial actions under pathophysiological conditions. However, the effects of mild stress on AD have not been clearly elucidated yet. In this study, the effects of low (3 and 30 nM) CORT concentration on $A\beta_{25-35}$-induced neurotoxicity in SH-SY5Y cells and underlying molecular mechanisms have been investigated. Cytotoxicity caused by $A\beta_{25-35}$ was significantly inhibited by the low concentration of CORT treatment in the cells. Furthermore, CORT pretreatment significantly reduced $A\beta_{25-35}$-mediated pro-apoptotic signals, such as increased Bim/Bcl-2 ratio and caspase-3 cleavage. Moreover, low concentration of CORT treatment inhibited the $A\beta_{25-35}$-induced cyclooxygenase-2 and pro-inflammatory cytokine expressions, including tumor necrosis factor-α and interleukin-1β. $A\beta_{25-35}$ resulted in intracellular accumulation of reactive oxygen species and lipid peroxidation, which were effectively reduced by the low CORT concentration. As a molecular mechanism, low CORT concentration activated the nuclear factor-erythroid 2-related factor 2, a redox-sensitive transcription factor mediating cellular defense and upregulating the expression of antioxidant enzymes, such as NAD(P)H:quinone oxidoreductase, glutamylcysteine synthetase, and manganese superoxide dismutase. These findings suggest that low CORT concentration exerts protective actions against $A\beta_{25-35}$-induced neurotoxicity and might be used to treat and/or prevent AD.

Key Words: Corticosterone, Beta-amyloid, Neurotoxicity, Alzheimer's disease, Oxidative stress, Inflammation

INTRODUCTION

Stress refers to a state of challenged and disturbed homeostasis. In modern society, people are exposed to various physical- and mental-stress situations in daily life. Generally, excessive and continuous stress is known to adversely cause diverse diseases, such as immune, cardiovascular, digestive, and metabolic disorders (Yaribeygi *et al*., 2017). Exposure to high-stress levels in the central nervous system has been reported to cause neurological disorders, including depression, anxiety, post-traumatic syndrome, eating disorder, and addiction (Newell-Price *et al*., 2006; Justice, 2018). Such high-stress levels have long been recognized as a negative factor causing and accelerating a wide range of pathological conditions. Stress exposure activates the hypothalamic-pituitary-adrenal (HPA) axis as a response to regulate and control stress. Activation of the HPA axis stimulates the secretion and production of glucocorticoids (GCs), for instance, cortisol in humans and corticosterone (CORT) in other animals including rodents (Herman *et al*., 2016). GC is a kind of steroid hormone known to have a wide range of effects on almost all physiological systems (De Kloet *et al*., 1998; Abraham *et al*., 2001). Stress is specifically associated with memory functions in the brain (Sandi, 2004; Bermúdez-Rattoni, 2007; Yaribeygi *et al*., 2017). Previous studies have reported that chronic stress and high CORT levels cause functional and structural changes in the hippocampus, an important limbic structure that plays a crucial role in the brain (Woolley *et al*., 1990; McEwen, 1999). Moreover, many experiments have also demonstrated that excessive stress negatively affects cognitive and memory functions (Bodnoff *et al*., 1995; Kim and Diamond, 2002).

Dementia is a representative disease associated with dete-

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riorated memory function and does not mean a single disease. Instead, it is a comprehensive term of conditions in which the normally mature brain is damaged causing memory and cognitive impairment that hinders a person's daily life. Alzheimer's disease (AD) is a type of dementia, accounting for the largest proportion of 60%-80% of all types of dementia (Alzheimer's Association, 2022). When brain tissues in patients with AD were examined under a microscope, two characteristic pathological features are identified, i.e., senile plaques and neurofibrillary tangles, which are caused by the deposition of a peptide known as beta-amyloid (Aβ) and abnormal entanglement of a protein called tau, respectively (Morishima-Kawashima and Ihara, 2002; Tiwari *et al*., 2019). Moreover, these two major pathological features of AD caused overall brain atrophy and neuronal loss (Whitwell, 2010). Several studies have investigated the cause and mechanism of AD, which have not yet been fully elucidated. Aβ is produced by the action of β- and γ-secretases, forming oligomers to aggregates, and is deposited outside the neurons (Sadigh-Eteghad *et al*., 2015). Aβ is known to induce neuron toxicity caused by intracellular calcium level abnormalities, oxidative stress, and inflammation (Demuro *et al*., 2010; Heppner *et al*., 2015; Minter *et al*., 2016; Cheignon *et al*., 2018).

Many risk factors can cause and accelerate AD, including aging, genetic variation, inflammation, and stress (Justice, 2018; Armstrong, 2019). Stress has been considered one of the key risk factors for AD. Studies have reported that chronic stress increased the Aβ production and tau protein phosphorylation in 3xTg-AD mice (Green *et al*., 2006). However, recent studies have interestingly shown the beneficial effects of mild stress on aging and longevity mainly in *Drosophila melanogaster* flies and *Caenorhabditis elegans* (Maglioni *et al*., 2014; Le Bourg, 2016). Furthermore, moderate exposure to GC, a representative stress hormone, had positive effects on N-methyl-D-aspartate excitotoxicity, focal lesions following traumatic brain injury, and hypoxic-ischemic brain damage (Abraham *et al*., 2001). However, the effects of mild stress on neuronal cell death in AD and its underlying mechanisms are not yet fully elucidated. To test this, whether low CORT concentration could protect against $A\beta_{25-35}$-induced cell death was examined in human neuroblastoma SH-SY5Y cells. Furthermore, the relevant molecular targets were investigated focusing on oxidative stress and neuroinflammation.

MATERIALS AND METHODS

Materials

CORT (purity ≥98.5%), $A\beta_{25-35}$, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], anti-actin antibody, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin antibiotic were obtained from Gibco BRL (Grand Island, NY, USA). Dichlorofluorescin diacetate (DCF-DA) was provided by Invitrogen Co. (Carlsbad, CA, USA). Primary antibodies against Bim, poly (ADP-ribose) polymerase (PARP), cyclooxygenase-2 (COX-2), interleukin-1β (IL-1β), nuclear factor erythroid 2-related factor 2 (Nrf2), glutamylcysteine synthetase (GCS), manganese superoxide dismutase (MnSOD), repressor element 1 silencing transcription factor (REST), and brain-derived neurotrophic factor (BDNF) were supplied by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Primary antibodies for 4-hydroxynonenal (4-HNE) and phospho-Nrf2 (p-Nrf2) were obtained from Abcam (Cambridge, UK), and anti-tumor necrosis factor-α (TNF-α) and anti-cleaved caspase-3 antibodies were products from Cell Signaling Technology (Danvers, MA, USA).

Cell culture

Human neuroblastoma SH-SY5Y cell line was obtained from Korean cell line bank (Seoul, Korea). The cells were cultured in DMEM supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 U/mL). Cells were incubated in a humidified 5% CO_2 incubator at 37°C and grown in 6-well plates for 3 days (72 h) with or without CORT (3 nM and 30 nM). Then, the cells were plated at an appropriate density based on each experimental scale. For the treatment of $A\beta_{25-35}$, the cells were switched to the serum-free medium and incubated with or without $A\beta_{25-35}$ for indicated times.

Cell viability assay

Cell viability was analyzed through MTT reduction assays. In the previous study, SH-SY5Y cells had been treated with various concentrations (0-100 μM) to determine the optimal concentrations of CORT which could induce eustress on the cells. (Lee *et al*., 2022). Briefly, it was judged as excessive stress where the cell viability was decreased by more than 20% as in case of 100 μM CORT. When low concentrations of CORT (3- and 30 nM) were treated, the decrease in cell viability was less than 10% from the control group. It was considered as eustress. The cells were treated with a vehicle or CORT (3- and 30 nM) for 72 h and seeded at a density of 5×10^4 cells/300 μL in a 48-well plate. After 24 h, when the cells were stably attached, the existing medium was moved to a serum-free medium with or without $A\beta_{25-35}$ (10 μM). After incubation for 22 h, MTT solution was added and reacted for another 2 h. The formazan crystals formed in the living cells were dissolved with dimethyl sulfoxide (DMSO). The optical density at 540 nm was measured using a microplate reader (Molecular device, LLC., San Jose, CA, USA), and the relative cell viability (%) was calculated as 100% based on the absorbance of the vehicle-treated control group.

DCF-DA staining

To monitor the intracellular accumulation of reactive oxygen species (ROS), the fluorescence-generating probe DCF-DA was used. The cells (1×10^6 cells/ml in a 4-well chamber slide) were rinsed with PBS, and a 10 μM DCF-DA was loaded. After a 15-min incubation at 37°C, the cells were lysed with DMSO. The fluorescence intensities were measured using an Infinite M200 PRO plate reader (Tecan Group Ltd., Mannedorf, Switzerland).

Western blotting

The expression of proteins was measured by western blot analysis. After treatment with $A\beta_{25-35}$ in the presence or absence of CORT, total protein samples were isolated using radioimmunoprecipitation assay buffer (Sigma-Aldrich). Protein samples were separated in 10% or 12% sodium dodecyl sulfate-polyacrylamide gels and transferred to polyvinylidene fluoride membranes (Pall Co., MI, USA). The membranes were blocked with 5% nonfat milk in 0.1% Tween 20 in PBS (PBST) for 30 min at RT and then incubated with primary antibodies

at 4°C overnight. The dilution factors of primary antibodies were as follows: Bim, Bcl-2, PARP, and Nrf2, 1:500; cleaved caspase-3, COX-2, TNF-α, IL-1β, 4-HNE, pNrf2, GCS, NQO-1, and MnSOD, 1:1,000; Actin, 1:4,000. After washing the primary antibodies, the blots were reacted with horseradish peroxidase-conjugated anti-rabbit (1:10,000; Sigma-Aldrich) or anti-mouse secondary antibody (1:10,000; Santa Cruz Biotechnology). The specific bands were visualized by enhanced chemiluminescence western blotting detection reagent (Thermo, Rockford, IL, USA).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, Boston, MA, USA) and IBM SPSS for Windows (SPSS Inc., Chicago, IL, USA). The data were expressed as mean ± standard deviation (SD). Multiple group comparisons were performed by ANOVA followed by the Turkey test as a post-hoc analysis.

RESULTS

Effect of CORT on $A\beta_{25-35}$-Induced Cytotoxicity in SH-SY5Y Cells

To investigate the protective effects of low CORT concentration against cytotoxicity induced by $A\beta_{25-35}$, SH-SY5Y cells were pretreated with 3- and 30 nM CORT for 72 h, and then, 10-μM $A\beta_{25-35}$ was added to the media for 24 h. The cell survival was measured using the MTT reduction assay (Fig. 1). When the cells were treated with $A\beta_{25-35}$, cell viability was significantly lower by 55.27 ± 1.80% than the control group, whereas 3- and 30 nM CORT restored the cell survival to 65.21 ± 3.31% and 67.39 ± 2.27%, respectively. Notably, at each concentration, low CORT concentration alone did not exhibit obvious cytotoxicity.

Effect of CORT on $A\beta_{25-35}$-Induced Apoptotic Signals in SH-SY5Y Cells

To examine the effects of low CORT concentration on $A\beta_{25-35}$-induced apoptotic signals in SH-SY5Y cells, western blot analysis was conducted. As shown in Fig. 2, the cells treated with $A\beta_{25-35}$ (10 μM) and the expression of pro-apoptotic Bim and cleaved caspase-3 were increased, whereas protein levels of anti-apoptotic Bcl-2 and the total form of PARP were lower than that in the control group. However, the Bim expression and cleaved caspase-3 were reduced by the CORT pretreatment. Moreover, the subsequent decrease in Bcl-2 and total PARP levels was effectively restored by CORT. In summary, low CORT concentration significantly attenuated the $A\beta_{25-35}$-induced apoptosis by suppressing pro-apoptotic markers including Bim, and cleavage of caspase-3 and PARP in SH-SY5Y cells.

Effect of CORT on $A\beta_{25-35}$-Induced Inflammatory Responses in SH-SY5Y Cells

The $A\beta_{25-35}$-induced inflammatory responses including the expression of pro-inflammatory enzymes like COX-2 and pro-inflammatory cytokines, such as TNF-α and IL-1β, were examined in SH-SY5Y cells. The $A\beta_{25-35}$ (10 μM) treatment increased the COX-2 protein levels (Fig. 3A) by 1.57 ± 0.11-fold and subsequent TNF-α and IL-1β expressions by 3.24 ± 0.27 and 3.55 ± 0.48 folds, respectively, as compared with the control group. However, pretreatment with 3- and 30-nM CORT effectively attenuated the $A\beta_{25-35}$-induced COX-2 (Fig. 3A), TNF-α, and IL-1β protein expressions (Fig. 3B).

Effect of CORT on $A\beta_{25-35}$-Induced Oxidative Stress in SH-SY5Y Cells

The SH-SY5Y cells were pretreated with CORT (3- and 30 nM) for 72 h before $A\beta_{25-35}$ (10 μM) incubation for 24 h. The

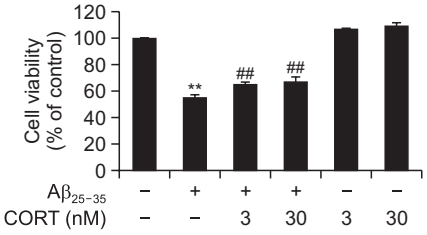

Fig. 1. Protective effect of CORT on $A\beta_{25-35}$-induced cytotoxicity in SH-SY5Y cells. The cells were incubated with media containing 3- and 30 nM CORT for 72 h and treated with a 10 μM $A\beta_{25-35}$ for an additional 24 h. Cell viability was determined using the MTT reduction assay. Data are presented as mean ± SD of three independent experiments, each performed in triplicate. $^{**}p<0.01$ and $^{\#\#}p<0.01$ indicate statistically significant differences from vehicle-treated control group and $A\beta_{25-35}$ alone group, respectively.

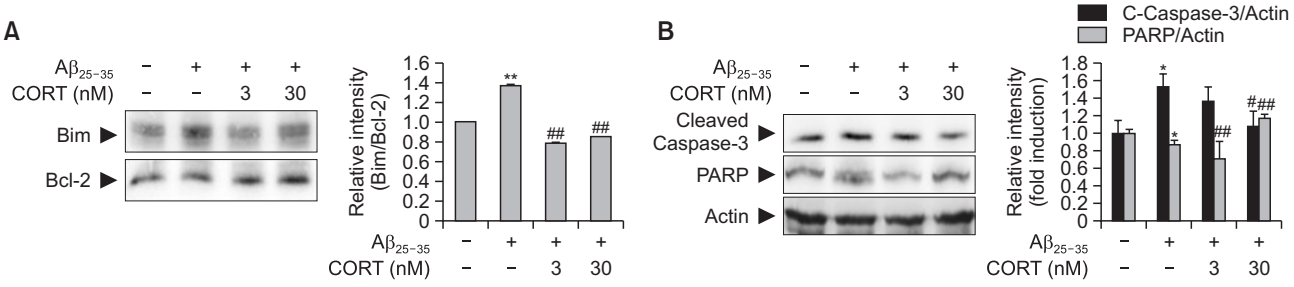

Fig. 2. Effect of CORT on $A\beta_{25-35}$-induced apoptotic signals in SH-SY5Y cells. The cells were pretreated with 3- and 30 nM CORT for 72 h and then incubated with 10 μM $A\beta_{25-35}$ for 24 h. Expression of Bim, Bcl-2 (A), PARP, and cleaved caspase-3 (B) was determined by western blotting. Actin levels were measured as loading controls. Quantitative data were shown as fold induction in the right panel. Data are mean ± SD of three independent experiments. $^{*}p<0.05$ or $^{**}p<0.01$ vs vehicle-treated control group and $^{\#}p<0.05$ or $^{\#\#}p<0.01$ vs $A\beta_{25-35}$ alone group with statistical significance.

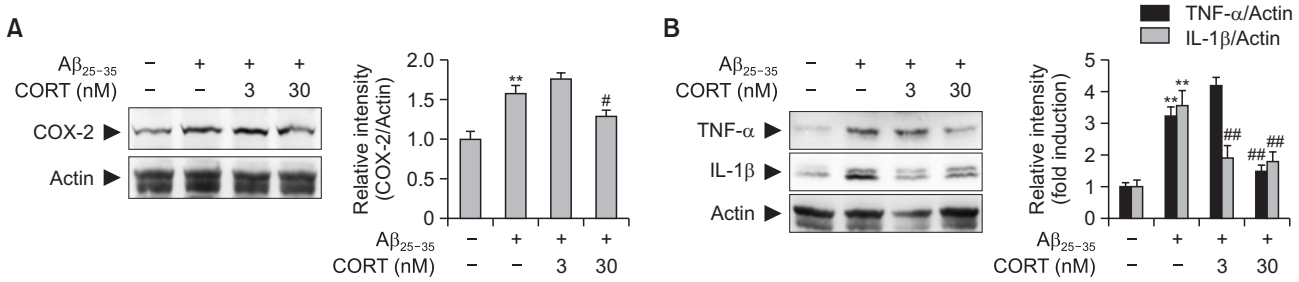

Fig. 3. Effect of CORT on $A\beta_{25-35}$-induced expression of COX-2 and cytokines in SH-SY5Y cells. The cells were pretreated with CORT (3- and 30 nM) for 72 h in the absence or presence of 10 μM $A\beta_{25-35}$ for 24 h. (A) The COX-2 protein expression was determined by western blot analysis. The relative ratio of COX-2 to Actin was represented in the right panel. (B) TNF-α and IL-1β expressions were analyzed by western blot analysis. Quantitative data for TNF-α/Actin and IL-1β/Actin protein levels were presented in the right panel. Data are presented as mean ± SD from three independent experiments. $^{**}p<0.01$ vs vehicle-treated control group and $^{\#}p<0.05$ or $^{\#\#}p<0.01$ vs $A\beta_{25-35}$ alone group with statistical significance.

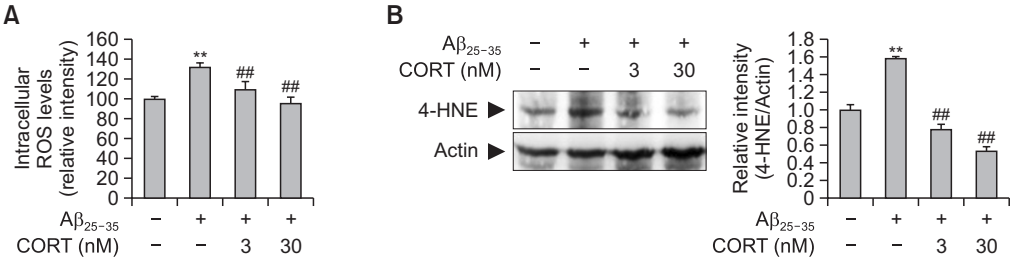

Fig. 4. Effect of CORT on $A\beta_{25-35}$-induced intracellular accumulation of ROS and lipid peroxidation in SH-SY5Y cells. (A) The intracellular ROS levels were determined by DCF-DA fluorescence staining. (B) The 4-HNE was measured by western blotting. Actin levels were measured as loading controls. Quantitative data were shown as fold induction in the right panel. Data are presented as mean ± SD of three independent experiments. $^{**}p<0.01$ vs vehicle-treated control group and $^{\#\#}p<0.01$ vs $A\beta_{25-35}$ alone group with statistical significance.

intracellular ROS levels were increased by the treatment of $A\beta_{25-35}$ as compared with the control group as determined using the DCF-DA fluorescence assay. However, pretreatment with CORT alleviated the $A\beta_{25-35}$-induced ROS accumulation in the cells (Fig. 4A). Furthermore, we have determined one of the representative markers for oxidative stress, 4-HNE, derived from ω-6 polyunsaturated fatty acids (PUFAs), such as linoleic, γ-linolenic, or arachidonic acid by lipid peroxidation. The 4-HNE expression was increased by the $A\beta_{25-35}$ treatment, which was significantly decreased by the pretreatment of 3- and 30 nM CORT (Fig. 4B).

CORT-induced activation of Nrf2 and upregulation of antioxidant enzymes in SH-SY5Y Cells

The Nrf2 is a redox-sensitive transcription factor that plays a cytoprotective role in adaptive cellular defense from oxidative stress and inflammatory response by upregulating phase II detoxifying and antioxidant enzymes (Qu *et al*., 2020). Treating SH-SY5Y cells with $A\beta_{25-35}$ (10 μM) for 24 h did not cause any significant alterations in phosphorylation Nrf2 levels (Fig. 5A) and antioxidant enzyme expression, including GCS, NQO1, and MnSOD (Fig. 5B). However, CORT pretreatment induced Nrf2 activation via phosphorylation (Fig. 5A) and subsequently elevated the GCS, NQO1, and MnSOD protein levels compared with $A\beta_{25-35}$ alone-treated group (Fig. 5B).

CORT-enhanced expression of REST and BDNF in SH-SY5Y Cells

As another molecular mechanism, REST and BDNF expressions, the representative neurohormetic proteins, were examined. Neurohormesis is a phenomenon in which low-dose toxins induce adaptive neuronal stress responses that help cells withstand stress (Mattson and Cheng, 2006). Diverse molecules, such as cell-survival signaling kinases, transcription factors, and histone deacetylases, were known to be involved in neurohormesis. In this sense, whether low CORT concentration could affect $A\beta_{25-35}$-induced REST and BDNF expressions in SH-SY5Y cells was investigated. As shown in Fig. 6A, the REST protein level seemed to be slightly decreased by $A\beta_{25-35}$ treatment for 24 h as compared with the control group, which was enhanced by CORT pretreatment (3- and 30 nM) for 72 h. Moreover, CORT restored $A\beta_{25-35}$-suppressed BDNF expression and even higher levels than that in the control group (Fig. 6B).

DISCUSSION

The pathogenesis of AD is multifactorial and involves genetic, environmental, and lifestyle factors. Over recent years, there has been increasing interest in exploring the potential connection between stress and AD. This relationship is complex and involves intricate molecular, cellular, and behavioral mechanisms. Animal models provide valuable insights into

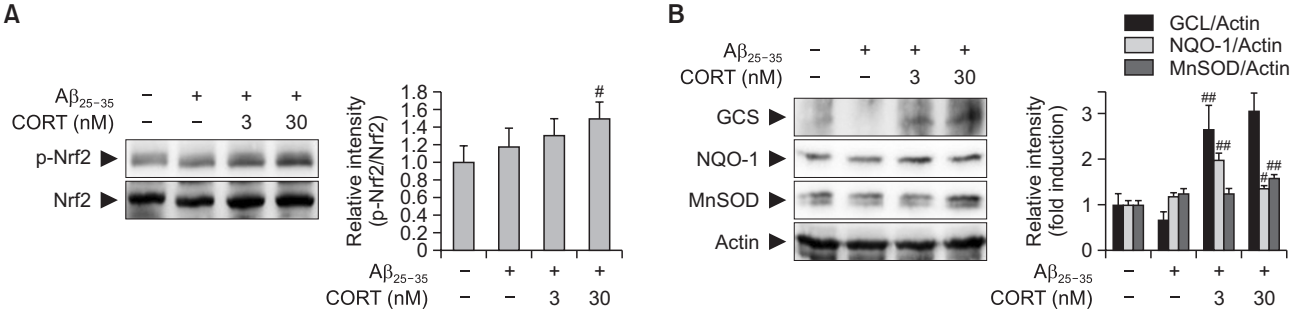

Fig. 5. CORT-induced activation of Nrf2 through phosphorylation and upregulation of antioxidant enzymes. SH-SY5Y cells were pretreated with 3- and 30 nM CORT for 72 h and were incubated for another 24 h in the absence or presence of 10 μM $A\beta_{25-35}$. (A) Nrf2 phosphorylation was evaluated by western blot analysis. Quantitative data for the relative ratio of Nrf2 to actin were indicated in the upper right panel. (B) The protein expression of antioxidant enzymes including GCS, NQO1, and MnSOD was determined by western blot analysis. The relative ratio of aforementioned proteins to Actin was shown in the lower right panel. Data are presented as mean ± SD from three independent experiments. $^{\#}p<0.05$ or $^{\#\#}p<0.01$ vs $A\beta_{25-35}$ alone group with statistical significance.

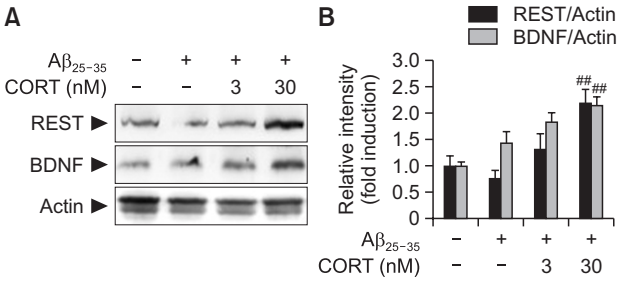

Fig. 6. CORT-enhanced REST and BDNF protein levels in SH-SY5Y cells. The cells were pretreated with CORT (3- and 30 nM) for 72 h and were then incubated with or without 10 μM $A\beta_{25-35}$ for another 24 h. REST and BDNF expressions were analyzed by western blot analysis. Quantitative data and the relative ratio between REST/Actin and BDNF/Actin were represented in the lower panel. Data are presented as mean ± SD from three independent experiments. $^{\#\#}p<0.01$ vs $A\beta_{25-35}$ alone group with statistical significance.

stress-related behaviors and their impact on the development and progression of AD. Animal models have been crucial in elucidating the intricate relationship between stress and AD. Studies using rodent models have demonstrated that chronic stressors, such as social isolation, restraint, or unpredictable stress, can lead to cognitive impairments, memory deficits, and increased susceptibility to AD-like pathology. These stressors often induce anxiety- and depressive-like behaviors, paralleling the mood changes observed in individuals at risk for AD. Additionally, these models have revealed alterations in synaptic plasticity and neurogenesis, further contributing to cognitive decline.

Based on previous studies, stress is a major risk factor for AD and a negative memory modulator; however, a recent study has reported that properly controlled exposure to stress positively affects the human body by enhancing resilience (Osório *et al*., 2017; Faye *et al*., 2018; Babić *et al*., 2020). The relationship between AD and "eustress," with beneficial stress and particularly the effects of moderate stress levels on AD, has not been elucidated. In this study, low CORT concentration, a representative stress hormone, exhibited protective effects on Aβ-induced cell death, inflammation, and oxidative damages by fortifying adaptive survival response in SH-SY5Y human neuroblastoma cells.

Low (3- and 30 nM) CORT concentration demonstrated a protective effect against $A\beta_{25-35}$-induced cytotoxicity in SH-SY5Y cells as measured using MTT reduction assay. Furthermore, $A\beta_{25-35}$ increased the protein levels of pro-apoptotic Bim and cleaved caspase-3 as compared with the control group, which were significantly reduced by CORT pretreatment in the cells. Furthermore, $A\beta_{25-35}$-decreased Bcl-2 and total PARP expression were also restored by CORT pretreatment. These findings suggest that a low CORT concentration has a protective effect against $A\beta_{25-35}$-induced cell death by regulating apoptotic signals.

The Aβ is known to cause neuronal toxicity through various mechanisms. Excessive Aβ accumulations can induce neurotoxicity by enhancing inflammatory responses. In a previous study, representative markers of pro-inflammatory reactions including COX-2, TNF-α, IL-1β, and TNFR1 were elevated by $A\beta_{25-35}$ in SH-SY5Y cells (Xu *et al*., 2018). In the present study, treatment with $A\beta_{25-35}$ in SHSY5Y cells increased the inflammatory mediator expression, such as COX-2, TNF-α, and IL-1β, whereas the expression of aforementioned proteins was reduced by pretreatment with low CORT concentration. This finding suggests that low CORT concentration could reduce $A\beta_{25-35}$-induced neurotoxicity due to its anti-inflammatory actions.

Other mechanisms underlying the Aβ-induced neurotoxicity, oxidative stress and damages were examined. The $A\beta_{25-35}$ treatment increased the ROS intracellular levels, which was effectively reduced by the pretreatment with low CORT concentration in SH-SY5Y cells. In another experiment, the elevated 4-HNE expression by the $A\beta_{25-35}$ treatment alone was decreased when low CORT concentration was pretreated. This finding indicated that low CORT concentration had a protective effect in SH-SY5Y cells against oxidative stress derived from $A\beta_{25-35}$ (Markesbery, 1997; Zhang *et al*., 2010; Ham *et al*., 2017).

Furthermore, neurohormetic regulators have been examined as promising candidates responsible for the protective effects of low CORT concentration. Nrf-2 is an important regulator of cellular defense against oxidative stress by upregulating antioxidant and detoxifying enzymes, such as heme oxygenase-1 (HO-1), GCS, SOD, glutathione S-transferase,

glutathione peroxidase, catalase, sulfiredoxin, and thioredoxin as downstream target genes (Lee *et al*., 2022). SH-SY5Y cells treated with low CORT concentration increased the protein levels of GCS, NQO-1, and MnSOD by Nrf2 activation via phosphorylation. The Nrf-2-ARE pathway activation likely modulates the misfolded protein formation and degradation aggregates in AD (Zhang *et al*., 2010).

REST is another neurohormetic protein and a transcriptional repressor, playing a critical role in stem cells and differentiating neurons and adult neurons. In aging neurons, REST suppresses genes involved in neuronal death, resulting in neuroprotection. Therefore, the loss of REST is implicated with AD pathogenesis (Lu *et al*., 2014). Consistent with the previous findings, pretreatment of low CORT concentration increased the REST and BDNF expressions in SH-SY5Y cells in the present study.

In the present study, one notable weakness of the study lies in the partial inconsistency observed between the effects of 3 nM CORT on cell viability and the associated protein expression pattern such as COX-2, TNF-α, p-Nrf2, MnSOD, REST, and BDNF. While the effect of 30 nM CORT exhibited a satisfactory correlation between cell viability and the protein expression, the unexpected lack of concordance at the lower concentration of 3 nM CORT poses challenges to our current understanding of the cellular response to this hormone. This unexpected finding underscores the complexity of cellular responses to CORT and emphasizes the need for further research to unravel the underlying mechanisms that give rise to such a discrepancy. Addressing these limitations through comprehensive mechanistic studies, alternative experimental approaches, and consideration of dose-dependent effects will enhance the robustness and comprehensiveness of our conclusions.

In conclusion, low CORT concentration could protect against A$\beta_{25\text{-}35}$-induced cell death in SH-SY5Y cells by inflammation suppression and oxidative stress. As a protective molecular mechanism, CORT might fortify the neurohormetic stress responses by Nrf2 activation and REST upregulation, which can augment antioxidant enzyme expression and neurotrophic factors like BDNF. These findings suggest that mild and controllable stress can have positive functions in the brain and shed light on a promising strategy for the prevention and/or treatment of AD.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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