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Role of S-nitrosoglutathione mediated mechanisms in tau hyper-phosphorylation

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ABSTRACT

Hyperphosphorylation and polymerization of microtubule-associated protein tau into paired helical filaments (PHFs) is one of the hallmarks of Alzheimer's disease (AD). Here we report that neuronal tau hyperphosphorylation under AD conditions is regulated by S-nitrosoglutathione (GSNO), an endogenous nitric oxide carrier molecule. In cultured rat cortical primary neurons, we observed that GSNO treatment decreased the β-amyloid ($A\beta_{25-35}$)-induced pathological tau hyperphosphorylation (Ser396, Ser404, and Ser202/Thr205). The decreased tau hyperphosphorylation correlated with decreased activity of calpain and decreased p35 proteolysis into p25 and Cdk5 activation. GSNO treatment also attenuated the $A\beta_{25-35}$-induced activation of GSK-3β which is known to play critical role in tau hyperphosphorylation in addition to Cdk5. Consistent with above studies using cultured neurons, we also observed that systemic GSNO treatment of transgenic mouse model of AD ($APP_{Sw}/PS1_{dE9}$) attenuated calpain-mediated p35 proteolysis and Cdk5/GSK-3β activities as well as tau hyperphosphorylation. In addition, GSNO treatment provided neuro- and cognitive protection in $APP_{Sw}/PS1_{dE9}$ mice. This study describing the GSNO-mediated regulation of tau hyperphosphorylation and cognitive function, for the first time, suggests for therapeutic potential of GSNO as neuro- and cognitive-protective agent for AD.

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1. Introduction

Alzheimer's disease (AD) is the most common form of dementia among older individuals, characterized by extracellular deposits of Aβ aggregates as senile plaques, and abnormal neuronal tau hyperphosphorylation leading to its aggregation to form intracellular paired helical filaments (PHFs) and neurofibrillary tangles (NFTs) that destroy circuitry-linked activities in cortical and hippocampal regions of brain [1]. Unfortunately, no effective therapy is available due to limited understanding of disease pathologies.

Endogenous nitric oxide (NO) synthesized from NO synthases (NOS), plays a key role in numerous physiological and pathological processes [2]. NO is known to exert its biological activities via at least three distinct mechanisms; classical cGMP/PKG mechanism mediating NO-dependent relaxation of vascular smooth muscle [3], peroxynitrite ($ONOO^-$ formed by reaction between NO and superoxide anion) dependent pathological signaling under oxidative stress conditions [4], and S-nitrosoglutathione (GSNO formed by reaction between NO and GSH) dependent redox-based protein modification (S-nitrosylation) [5].

Recently, our laboratory described activities of GSNO in neuro- and cognitive-protection using rats subjected permanent bilateral common carotid artery occlusion (pBCCAO) as a model for chronic cerebral hypoperfusion [6]. GSNO treatment also reduced the Aβ load and ICAM-1/VCAM-1 expression in the brains of pBCCAO rats and increased Aβ uptake by microglia and endothelial cells and decreased neuronal Aβ synthesis by inhibiting activity of BACE1 in *in vitro* cell culture models [6]. Taken together with previously reported role of GSNO in anti-inflammation [6,7], anti-oxidation [8,9], and cerebrovascular and BBB protections [10,11], our study documented the potential neuro-cognitive protective efficacy of GSNO in AD.

Since the finding of abnormally phosphorylated tau protein in PHF which forms the NFT and induces neuronal cell loss in AD brain

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[1], tau hyperphosphorylation-mediated pathology is gaining a more prominent role for the development of AD. Numerous studies have identified a number of protein kinases that cause hyperphosphorylation of tau in AD brain [12,13]. Among these, Cdk5 and GSK-3β are now regarded as the major kinases responsible for pathological tau hyperphosphorylation in AD brain [12,13]. Under AD conditions, Cdk5 is activated aberrantly by intracellular calcium influx and calpain activation [14,15]. Cdk5 is a proline-directed serine/threonine kinase that functions differently from traditional Cdks. Cdk5 does not have a cyclin as its activating partner; instead, it is activated by binding with p35 [16,17]. The p35 localize in membrane through myristoylation and recruit Cdk5 for its activation [16]. Upon the binding with p35, Cdk5 is activated and subsequently undergoes degradation via ubiquitin-mediated proteolysis [17]. Under the pathological conditions, however, p35 is processed to p25 by calpain [16]. Since p25 is resistant to ubiquitin-mediated proteolysis and lacks the myristoylation site, the p25/Cdk5 complex is dissociated from the membrane and gains access to various substrates including tau [16]. It is interest to note that p25 preferentially binds and activates GSK-3β [18]. The p25 is accumulated in the brains of patients with AD with increased tau hyperphosphorylation and neuronal apoptosis [19], thereby suggesting that modulation of calpain activity and thus inhibition of p35 proteolysis to p25 are critical for regulation of aberrant activation of Cdk5 as well as GSK-3β under AD conditions.

In this study, we report that GSNO inhibits pathological *tau* hyperphosphorylation via inhibiting calpain-mediated p35 proteolysis generating p25 and aberrant activation of Cdk5 and/or via inhibiting GSK-3β activity in *in vitro* neuron culture model and $APP_{Sw}/PS1_{dE9}$ AD mouse model.

2. Materials and methods

2.1. Primary neuronal cell culture

Primary cultures of cortical neurons were prepared from the cerebral cortex of embryos of Sprague Dawley rats at embryonic day 17 (E17) as described in our previous report [6]. The cultured neurons were maintained in Neurobasal media (Invitrogen, Carlsbad, CA) supplemented with 2% B27 supplement (Invitrogen), 0.5 mM glutamine, 25 μM glutamate, 50 units/ml penicillin, 50 μg/ml streptomycin under humidified atmosphere of 5% CO_2 and 95% O_2, at 37 °C.

2.2. Western immunoblot analysis

Western immunoblot analysis was performed using antibodies against phospho-tau (p-tau) S^{396} (Cell Signaling Technology, Danvers, MA), p-tau S^{404} (Abcam, Cambridge, MA), p-tau S^{202}/T^{205} (Pierce, Rockford, IL), pan-tau (Cell Signaling Technology), β-actin (Abcam), p35, phospho-GSK-3β (p-GSK-3β) Y^{216}/Y^{279} (Abcam), p-GSK-3β S9 (Cell Signaling Technology), pan-GSK-3β (Cell Signaling Technology)

2.3. Histology and immuno-fluorescent staining

Paraffin-embedded sections from the formalin-fixed brain tissues were stained by with Nissl stain kit (IHCWORLD, Woodstock, MD) to detect Nissl body according to the manufacturer's instruction. The sections were also used for immunofluorescent staining for p-tau S^{396}. BX60 Olympus fluorescent/light microscope equipped with DP-70 camera (Olympus, Tokyo, Japan) was used for imaging. The intensities of fluorescence were quantified by Image-Pro Plus (MediaCybernetics, Bethesda, MD, USA).

2.4. Calpain activity assay

Analysis of calpain activity was performed using the same assay kit (Abcam, Cambridge, MA). Briefly, equal amounts of brain and neuronal lysate or purified active calpain-1 were incubated with substrate (Ac-LLY-AFC) and reaction buffer and calpain-mediated cleavage of substrate was analyzed by fluorometric analysis.

2.5. In vitro kinase assay for Cdk5, and GSK-3β

The brain tissue and neuronal lysates were immunoprecipitated with anti-CDK5 antibody (Abcam) or anti-GSK-3β antibody (BD Transduction Laboratories). The resulted pellets were incubated with biotin-labeled Cdk5 substrate (Biotin-Ahx-PKTPKKAKKL; Enzo life sciences, Farmingdale, NY) or biotin-labeled GSK-3β substrate (*Biotin-RRAAEELDSRAGSPQL*; AnaSpec, San Jose, CA) in kinase assay buffer (5 mM MOPS, pH 7. 2, 2.5 mM β-glycerol-phosphate, 5 mM $MgCl_2$, 1 mM EGTA, 0.4 mM EDTA, 100 μM ATP, and 0.05 mM dithiothreitol). The reactions were then stopped by adding 100 mM EDTA and the levels of phosphorylated substrates in supernatants were analyzed by ELISA using streptavidin coated 96 well plate (Pierce/Thermo Scientific, Rockford, IL) and horse-radish-peroxidase-conjugated anti-phospho-Thr antibody (Cell signaling) for analysis of Cdk5 activity or anti-phospho-Ser antibody for analysis of GSK-3β activity.

2.6. Animals and GSNO treatment

All animal procedures were in accordance with the animal experiment guidelines of the Medical University of South Carolina and National Institute of Health. Wild-type (C57Bl/6J) and $APP_{Sw}/PS1_{dE9}$ mice (The Jackson Laboratories, Bar Harbor, ME) were housed in cages under controlled temperature (21 ± 1 °C) and humidity (55 ± 10%), with a 12-h light/12-h dark cycle. The 5 month old $APP_{Sw}/PS1_{dE9}$ mice were administered with PBS or GSNO (3 mg/kg/day; 50 μL in PBS) on a daily basis for 5 months through intraperitoneally.

2.7. Morris water maze test

Morris water maze was employed to assess spatial learning and memory according to previously published methods with modification [6]. The test was performed in a circular pool (124 cm in diameter/60 cm in depth) filled with water clouded by nontoxic white paint. The circular pool consisted of four equal virtual quadrants. A circular area (radius 20 cm from the center of the platform) was defined as the target zone, equivalent to 4.9% of the total water maze area. All other experimental conditions are identical with our previous report [6].

3. Results and discussion

The excessive phosphorylation of tau in the proline-rich region (residues 172–251) and the C-terminal tail region (residues 368–441) have been implicated in the formation of aberrant tau aggregates known as NFTs in AD brain [20]. To understand the role of GSNO in tau phosphorylation, we studied the primary cortical neuron culture model of Aβ-induced tau phosphorylation. Fig. 1A shows that treatment of cortical neurons with $Aβ_{25–35}$ in culture increased the phosphorylation of tau at Ser^{202}/Thr^{205} in proline-rich region as well as Ser^{396}/Ser^{404} in C-terminal tail region. Treatment of cultured neurons with GSNO (100 μM) decreased the $Aβ_{25–35}$-induced tau phosphorylation in both regions. Since GSNO is a thiol based NO donor, we next investigate the role of S-nitrosothiol group of GSNO in $Aβ_{25–35}$-induced neuronal tau

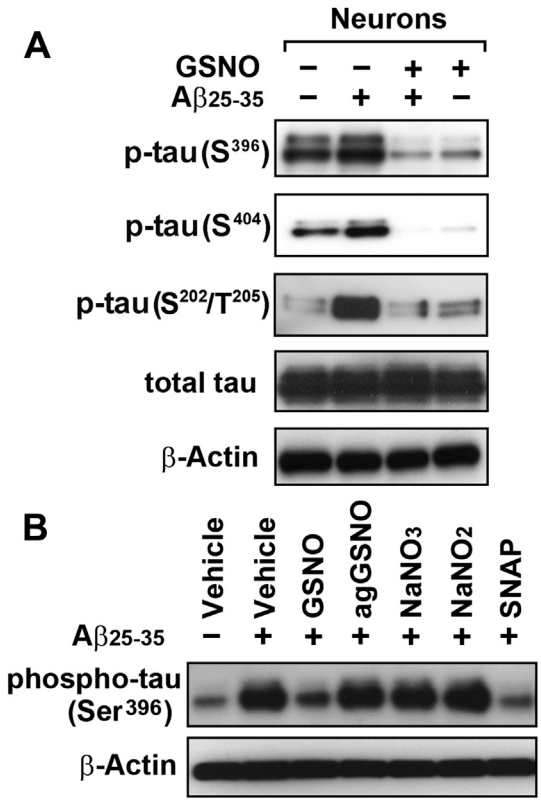

Fig. 1. Effect of GSNO treatment on pathological tau phosphorylation in primary cultured neurons treated with $A\beta_{25-35}$. A. The primary cultured cortical neurons were pretreated with 100 μM of GSNO for 4 h and treated with $A\beta_{25-35}$ (40 μM) for 12 h and neuronal levels of phosphorylated tau (Ser^{396}, Ser^{404}, and Ser^{202}/Thr^{205}), total tau, and β-actin (loading control) were analyzed by Western blot analysis. B. The effects of GSNO metabolites and other S-nitrosothiol donor on $A\beta_{25-35}$-induced tau phosphorylation (Ser^{396}), cultured neurons were pretreated with GSNO, aged or decomposed GSNO (agGSNO), sodium nitrate ($NaNO_3$), sodium nitrite ($NaNO_2$), or S-nitroso-N-acetylpenicillamine (SNAP) for 4 h and treated with $A\beta_{25-35}$ (40 μM).

phosphorylation. Similar to GSNO, treatment of $A\beta_{25-35}$-stimulated neurons with S-nitroso-N-acetylpenicillamine (SNAP), another donor of S-nitrosothiol, also reduced tau phosphorylation. However, aged GSNO (agGSNO), which was decomposed to nitrate and oxidized glutathione under light exposure, or sodium nitrite ($NaNO_2$) and sodium nitrate ($NaNO_3$) had no effect on Aβ-induced tau phosphorylation. These data indicate the specific role of S-nitrosothiol donor in inhibition of $A\beta_{25-35}$-induced tau phosphorylation.

Numerous studies in the last decade have identified a number of protein kinases (e.g. GSK-3β, Cdk5, cAMP-dependent protein kinase/PKA, and stress-activated protein kinases) that cause hyperphosphorylation of tau in AD brain (reviewed in Refs. [12,13]). Among these, Cdk5 and GSK-3β are now regarded as the major kinases responsible for pathological tau hyperphosphorylation in AD brain [12,13]. Under AD conditions, aberrant Cdk5 activation by calpain mediated cleavage of p35 to p25 has been implicated in pathological events leading to neurodegeneration and NFTs [14]. Since NO is reported to reversibly regulate calpain activity via S-nitrosylation of calpain [21,22], we next assessed whether GSNO inhibits calpain-mediated p35 proteolytic process to p25. Fig. 2A–i shows that treatment of purified active calpain with GSNO (10 μM) in cell free system significantly inhibited its enzyme activity. We next examined the effect of GSNO (100 μM) treatment on $A\beta_{25-35}$-induced calpain activation in cultured neuron cells. GSNO treatment significantly attenuated the $A\beta_{25-35}$-induced calpain activation (Fig. 2A–ii). Moreover, GSNO treatment also decreased glutamate (100 μM) or calcium ionophore (5 μM) induced calpain activations (Fig. 2A–ii). These data indicate a direct role of GSNO in inhibition of calpain activity.

Similar to the effect on calpain activity, GSNO treatment also attenuated the proteolysis of p35 detected as p25 levels in cultured neurons treated with $A\beta_{25-35}$ (Fig. 2B). Based on these data, we next examined the effect of GSNO on the regulation of Cdk5 activity. In a time course study, Cdk5 activity in primary cultured cortical neurons was significantly increased at 1 h and reached maximum

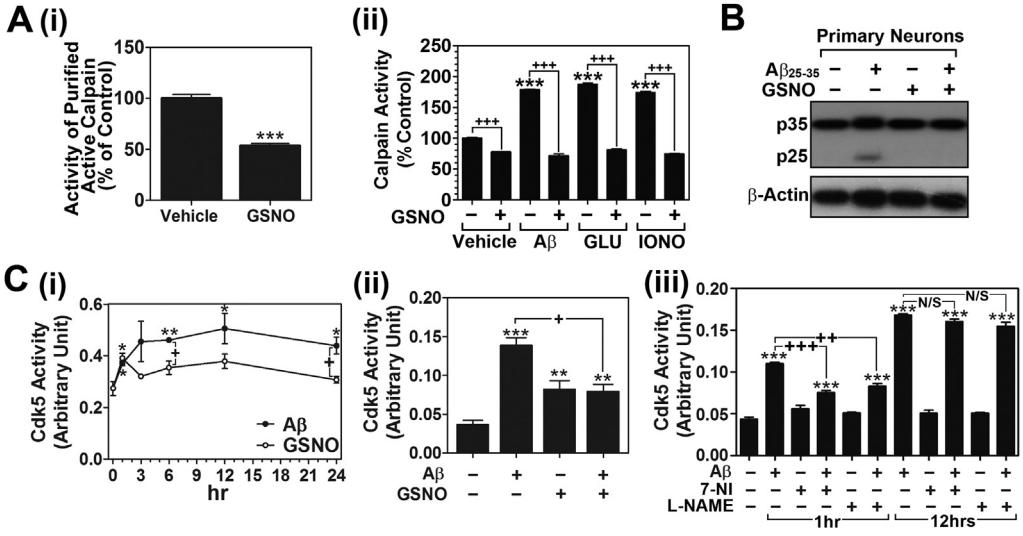

Fig. 2. Effect of GSNO treatment on calpain activation, p35 cleavage to p25, and Cdk5 activation in $A\beta_{25-35}$ treated neurons. A. Effect of GSNO on calpain activities was analyzed in cell free system using purified active calpain-1 (i) and in primary cultured cortical neurons (ii). The cortical neurons were pretreated with GSNO (100 μM) for 4 h and treated with $A\beta_{25-35}$ (Aβ; 40 μM), glutamate (Glu; 50 μM), or ionomycine (IONO; A23187; 10 μM) for 12 h. B. Under the similar experiments, the effect of GSNO on calpain-mediated cleavage of p35 to p25 was examined in cultured neurons treated with $A\beta_{25-35}$. C. The effect of GSNO (100 μM) and $A\beta_{25-35}$ (40 μM) treatment on the activities of Cdk5 was analyzed in a time course manner in primary cultured cortical neurons (i). To examine the effect of GSNO on $A\beta_{25-35}$-induced Cdk5 activities, the cultured neurons were pretreated with GSNO for 4 h and treated with $A\beta_{25-35}$. Following the incubation for 12 h, Cdk5 enzyme activities were analyzed by *in vitro* kinase assay (ii). To examine the role of NOS activation on $A\beta_{25-35}$-induced Cdk5 activities, the cultured neurons were pretreated with L-Nω-nitroarginine methyl ester (L-NAME; 300 μM) or 7-nitroindazole (7-NI; 100 μM) for 4 h and treated with $A\beta_{25-35}$. Following the incubation for 1 h or 12 h, Cdk5 enzyme activities were analyzed (iii). The vertical columns are means of individual data and T-bars are standard error mean. $^{*}p < 0.01$; $^{**}p < 0.005$; and $^{***}p < 0.0001$ as compared to control group. $^{+}p < 0.01$; $^{++}p < 0.005$; $^{+++}p < 0.0001$ as compared to Aβ, Glu, IONO treated groups.

at 12 h following the $A\beta_{25-35}$ treatment (Fig. 2C–i). Interestingly, Cdk5 activity under GSNO (100 μM) treatment was significantly higher than untreated neurons, but was significantly lower than that of activities under $A\beta_{25-35}$ treatment (Fig. 2C–i). In addition, GSNO treatment significantly decreased the Aβ-induced Cdk5 activity (Fig. 2C–ii). Previously, Qu et al. reported that S-nitrosylation of Cdk5 by NO-donors including GSNO increased Cdk5 activity in HEK 293T cells [23]. Therefore, the observed increase in Cdk5 basal activities by GSNO treatment (Fig. 2C–i and ii) may be due to S-nitrosylation of Cdk5. However, under the conditions of $A\beta_{25-35}$-induced calpain-mediated p35 proteolysis to p25, GSNO inhibited aberrant Cdk5 activation via inhibiting calpain-mediated p25 formation, and thus Cdk5-mediated pathological tau phosphorylation. Consistent with previously described $A\beta_{25-35}$-induced Cdk5 activation via activation of neuronal NOS (nNOS) and subsequent S-nitrosylation of Cdk5 [23], we also observed that $A\beta_{25-35}$-induced activation of Cdk5 was dependent on nNOS activity at an early time point (1 h after $A\beta_{25-35}$ treatment) as nNOS inhibitors [L-Nω-nitroarginine methyl ester (L-NAME) and 7-nitroindazole (7-NI)] inhibited the $A\beta_{25-35}$-induced Cdk5 activity (Fig. 2C–iii). However, at a later time point (12 h after $A\beta_{25-35}$ treatment) where Cdk5 activities and p35 proteolysis (Fig. 2B) were aberrantly increased, Cdk5 activities were not affected by L-NAME treatment (Fig. 2C–iii). Along with the data reported by Qu et al. [23], our data suggests that GSNO may have dual roles in regulation of Cdk5 activity; GSNO itself increases the activity of Cdk5 via S-nitrosylation of Cdk5 [23], but under conditions of calpain activation, GSNO inhibits aberrant Cdk5 activation by inhibition of calpain-mediated p35 proteolysis to form p25.

GSK-3β is known to inhibit axonal transport by altering microtubule stability through hyperphosphorylation of tau and PHF/NFT formation [24,25]. GSK-3 is highly active in cells under basal conditions. This is partly due to phosphorylation of a conserved tyrosine residue on the activation loop of the kinase domain (Tyr^{216} in GSK-3β) that is required for kinase activity of GSK-3β [26]. However, phosphorylation of GSK-3β at an N-terminal serine residue inhibits its kinase activity (Ser^{9}). This phosphorylated Ser^{9} acts as a pseudo-substrate and binds to the phosphate-binding pocket on GSK-3β, and thus inhibits its activity by preventing interaction with substrates [27]. To evaluate the effects of $A\beta_{25-35}$ load on neuronal GSK-3β activity, primary cultured neurons were treated with $A\beta_{25-35}$ peptide and its time course effect was analyzed on GSK-3β enzyme activity. As expected, $A\beta_{25-35}$ treatment increased GSK-3β activity over 24 h, but GSNO treatment had no significant effect on basal GSK-3β activity (Fig. 3A). Next, the neuron cells were treated with $A\beta_{25-35}$ peptide in the presence or absence of GSNO to examine the effect of GSNO treatment on $A\beta_{25-35}$-induced activation of GSK-3β. Fig. 3B shows that GSNO treatment had no effect on basal activity but inhibited the $A\beta_{25-35}$-induced GSK-3β activation. The $A\beta_{25-35}$-induced increase in GSK-3β activity and its inhibition by GSNO treatment are in coincidence with phosphorylation status of GSK-3β Tyr^{216} residue (Fig. 3C). However, neither $A\beta_{25-35}$ nor GSNO had effect on phosphorylation of Ser^{9} in GSK-3β (Fig. 3C). These data indicate that GSNO modulates mechanisms regulate GSK-3β activity via dephosphorylaltion of its kinase domain loop (Tyr^{216}) without any effect on N-terminal pseudo-substrate domain (Ser^{9}). Taken together, these studies raise interesting question regarding the potential role of GSNO, a natural biological molecule, in relation to protein secondary modification as a regulator of calpain, Cdk5, and GSK-3β signaling mechanisms in AD processes.

Based on the observed inhibitory role of GSNO in tau hyperphosphorylation in cultured neurons, we next evaluated the therapeutic potential of GSNO in mouse model of AD ($APP_{Sw}/PS1_{dE9}$) which express significant Aβ deposition at 6 months of age and senile plaques at 9 months of age [28]. Starting 5 months of age, $APP_{Sw}/PSEN1_{dE9}$ mice received daily GSNO (3 mg/kg/day/i.p.) for 5 months. At the end of 5 month of GSNO treatment, the animals were subjected to Morris water maze test for evaluation of spatial learning and memory function. Fig. 4A shows that spatial learning and memory functions were significantly compromised in $APP_{Sw}/PS1_{dE9}$ mice. GSNO treatment of $APP_{Sw}/PSEN1_{dE9}$ mice significantly improved the spatial learning performance (escape latency). In addition, GSNO treatment also improved spatial memory performance (percent time in target quadrance), but below the threshold of statistical significance ($p = 0.098$). The improved cognitive functions were well correlated with attenuation of neuronal loss in cortical and hippocampal areas (dentate gyrus/DG and CA1) as observed in Nissl staining (Fig. 4B). Accordingly with the data in *in vitro* neuron culture, we also observed that the brains of $APP_{Sw}/PS1_{dE9}$ mice had an elevated tau phosphorylation at Ser^{202}/Thr^{205} and Ser^{396}/Ser^{404} (Fig. 4C) and increased calpain activity (Fig. 4D–i) and p35 proteolysis to p25 (Fig. 4D–ii) and Cdk5 (Fig. 4D–iii) and GSK-3β (Fig. 4D–iv) activities as compared to wild type control. However, the degree of tau phosphorylation, calpain activity, p35 proteolysis to p25, and Cdk5/GSK-3β activities in $APP_{Sw}/PS1_{dE9}$ mice was markedly reduced by GSNO treatment. Therefore, these data indicate that GSNO-mediated mechanisms also regulate the tau hyperphosphorylation via regulation of calpain/p25/Cdk5 and GSK-3β pathways in brain of AD mice model.

The present study describes the importance of GSNO-mediated mechanisms in neuroprotection and cognitive functions under AD conditions. GSNO is the most abundant S-nitrosothiol in human and animals, formed by redox based reaction between GSH and NO. NO, itself, has been regarded as a cell signaling mediator regulating various physiological processes [2]. However, NO and peroxynitrite are also known to cause nitroso-oxidative damage under inflammatory conditions [29]. The observed opposing roles of NO effectors, such as peroxynitrite in induction of tissue damage of the CNS [29] and AD pathologies (Aβ aggregation and tau modification [30,31]) vs. GSNO in inhibitions of Aβ synthesis,

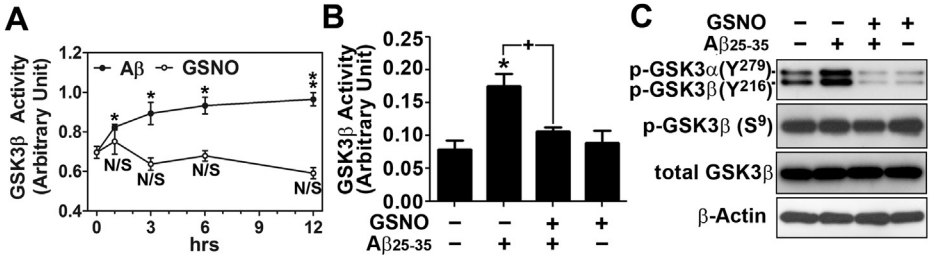

Fig. 3. Effect of GSNO treatment on GSK-3β activation in $A\beta_{25-35}$ treated neurons. A. Effect of GSNO (100 μM) and $A\beta_{25-35}$ (40 μM) treatment on the activities of GSK-3β was analyzed in a time course manner in primary cultured cortical neurons. B and C. To examine the effect of GSNO on $A\beta_{25-35}$-induced GSK-3β activities, the cultured neurons were pretreated with GSNO for 4 h and treated with $A\beta_{25-35}$ for 12 h. GSK-3β enzyme activities were analyzed by *in vitro* kinase assay (B) or Western blot by using antibodies specific to phospho-GSK-3α/β (Tyr279/216; p-GSK-3α-Y279, p-GSK-3β-Y216) and phospho-GSK-3β (Ser9; p-GSK-3β-S9), and total GSK-3β (C). The vertical columns are means of individual data and T-bars are standard error mean. $^{*}p < 0.01$; and $^{**}p < 0.005$ as compared to the control group. $^{+}p < 0.01$ as compared to Aβ treated groups.

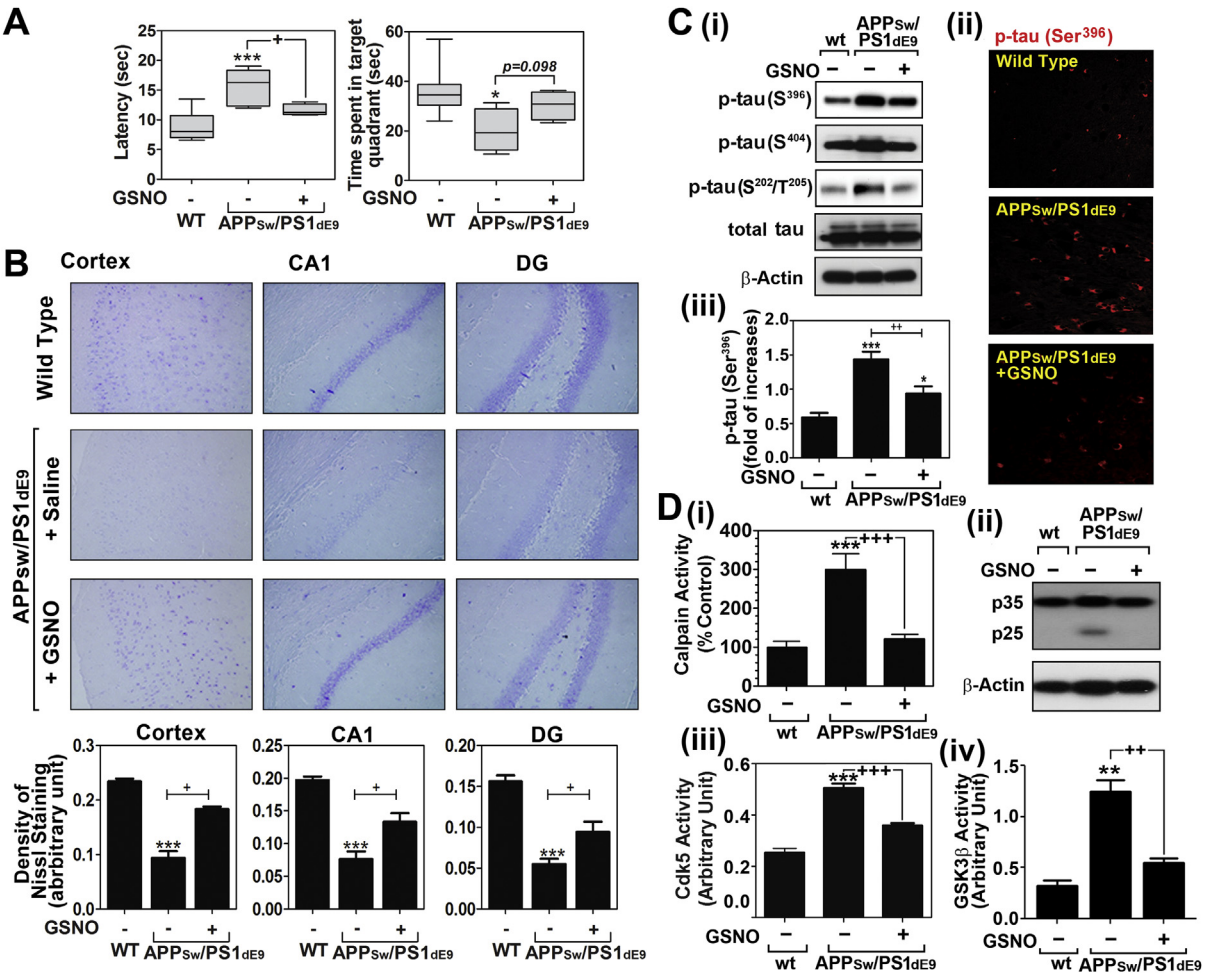

Fig. 4. Effect of GSNO treatment on spatial learning and memory deficits and tau hyperphosphorylation in of $APP_{Sw}/PS1_{dE9}$ mice. A. Analysis of cognitive function (learning and memory function) of wild type (WT/C57BL6) and $APP_{Sw}/PS1_{dE9}$ mice treated with saline or GSNO (3 mg/kg/day). Time (sec) to reach the hidden platform (latency in seconds) for spatial learning performance and time (sec) to spend in target quadrant without platform for spatial memory performance were analyzed by Morris Water maze test (n = 8). The columns show 75% of distribution; horizontal bar in each column is median; and vertical T-bars are minimum and maximum values of the data. For statistical analysis and presentation of the data, one way ANOVA test with Turkey's multiple comparison test was performed. B. Distribution and number of neurons in cortical and hippocampal layers (dentate gyrus; DG and CA1) were analyzed by Nissl staining. C. Brain levels of hyper-phosphorylated tau protein in WT and $APP_{Sw}/PS1_{dE9}$ mice treated with saline or GSNO were analyzed by Western analysis (i) and immunofluorescence staining (ii and iii). D. Calpain activity (i), p35 proteolysis to p25 (ii), Cdk5 activity (iii), and GSK-3β activity (iv) were analyzed in brains of WT and $APP_{Sw}/PS1_{dE9}$ mice treated with saline or GSNO were analyzed as described materials and methods. The vertical columns in panels B ~ D are means of individual data and T-bars are standard error mean. *p < 0.01, **p < 0.005, and ***p < 0.0001 as compared to the wild type (WT) group. $^{+}$p < 0.01, $^{++}$p < 0.005, and $^{+++}$p < 0.0001 as compared to untreated $APP_{Sw}/PS1_{dE9}$ mice group.

inflammation, cognitive deficit [6,32], and tau hyperphosphorylation suggest GSNO as a neuroprotective effector of NO as compared to degenerating role of peroxynitrite. AD involves chronic inflammation and oxidative stress [33]. Since oxidative stress disease conditions reduced the cellular levels of GSNO, caused by reaction between NO and superoxide anion to form peroxynitrite and by decreased levels of GSH, exogenous supplementation is possibly required to the cellular requirement of GSNO/NO for physiological cellular signaling activities. In this study, we also observed that GSNO supplementation inhibited tau hyperphosphorylation under *in vitro* cell culture and *in vivo* AD model. Taken together with the beneficial role of GSNO in anti-inflammation, anti-amyloidogenesis, and cerebrovascular protection [6,32], our data support the possible therapeutic potential of exogenous GSNO supplementation in AD.

Conflict of interest

We declare that we have no conflict of interest related to the publication of this manuscript.

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Transparency document

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