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**NO₂ inhalation causes tauopathy by disturbing
the insulin signaling pathway**

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Abstract

Air pollution has been evidenced as a risk factor for neurodegenerative tauopathies. NO₂, a primary component of air pollution, is negatively linked to neurodegenerative disorders, but its independent and direct association with tau lesion remains to be elucidated. Considering the fact that the insulin signaling pathway can be targeted by air pollutants and regulate tau function, this study focused on the role of insulin signaling in this NO₂-induced tauopathy. Using a dynamic inhalation treatment, we demonstrated that exposure to NO₂ induced a disruption of insulin signaling in skeletal muscle, liver, and brain, with associated p38 MAPK and/or JNK activation. We also found that in parallel with these kinase signaling cascades, the compensatory hyperinsulinemia triggered by whole-body insulin resistance (IR) further attenuated the IRS-1/ AKT/ GSK-3 β signaling pathway in the central nervous system, which consequently increased the phosphorylation of tau and reduced the expression of synaptic proteins that contributed to the development of the tau pathology. These findings provide new insight into the possible mechanisms involved in the etiopathogenesis of NO₂-induced tauopathy, suggesting that the targeting of insulin signaling may be a promising therapeutic strategy to prevent this disease.

Keywords: nitrogen dioxide; tau hyperphosphorylation; synaptic protein loss; insulin resistance; hyperinsulinemia

¹1. Introduction

Air pollution is implicated as a chronic source of the neuroinflammation and reactive oxygen species (ROS) that causes neurodegenerative disease (Block and Calderón-Garcidueñas, 2009). A biological analysis of central nervous system (CNS) tissue exposed to air pollution indicated that multiple cellular targets, including the mitochondria, proteins, lipids, and DNA are sensitive to atmospheric pollutants (Yan et al., 2015; Yan and Yun et al., 2016 and Risom et al., 2005). Additionally, insulin is considered another toxicological target of air contaminants, based on the epidemiological and experimental results showing that long-term exposure to traffic-related air pollution, ozone, and particulate matter 2.5 (PM_{2.5}) impair insulin sensitivity and induce peripheral or whole-body insulin resistance (IR) (Thiering et al., 2013; Hectors et al., 2013; Liu et al., 2014 Zhong et al., 2016 and Haberzettl et al., 2016). Insulin is a well-known hormone that is secreted by pancreatic β -cells and enters the CNS through the blood-brain barrier (BBB) by receptor-mediated transport (Havrankova et al., 1978). Studies on the role of neuronal insulin and its receptor in the brain demonstrated that neurotransmitter release, neuronal outgrowth, tubulin activity, neuronal survival, and synaptic plasticity are all directly modulated by insulin (Cole et al., 2007). Correspondingly, a disrupted insulin-signaling pathway has dire consequences on the structural and functional

¹ Abbreviations: NO₂, nitrogen dioxide; CNS, central nervous system; IR , insulin resistance; BBB, blood brain barrier; IRS-1, insulin receptor substrate-1; PKB /AKT,protein kinase B; GSK-3 β , glycogensynthase kinase-3 β ; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; PSD-95, postsynaptic density-95; Erk1/2, extracellular regulated protein kinases 1/2; ROS, oxygen species; AMPA, alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; NMDA, N-methyl-D-aspartic acid; PI3K, phosphoinositide-3-kinase.

integrity of the CNS. In particular, insulin signaling is proposed to play a causative role in the pathogenesis of neurodegenerative tauopathies which is a group of disorders characterized by an increase in the amount of tau protein or its modification (Lee et al., 2001 and Avila et al., 2004), as evidenced by the observation that impairments of brain insulin/insulin-like growth factor-I (IGF-I) signaling contribute to neurodegeneration due to the increased activation of kinases that aberrantly phosphorylate tau (de la Monte, 2012). Conversely, a series of studies on knockout models lacking insulin, IGF-I, or insulin receptor substrate-2 (IRS-2, an intracellular molecule that mediates insulin signaling) revealed a significant hyperphosphorylated tau (Cheng et al., 2005; Schechter et al., 2005 and Schubert et al., 2003). These findings clearly demonstrate that neuronal insulin signaling plays a direct role in the regulation of tau function and that disruption of intracellular insulin signaling molecules induces a tauopathy-like condition.

Epidemiological and experimental studies show that the phosphorylation of the tau protein was elevated in autopsy samples and in rat brains in response to air pollution (Calderón-Garcidueñas et al., 2012 and Levesque et al., 2011). In addition, the most prevalent tauopathy — Alzheimer's disease (AD), which is potentially provoked by air pollution (Calderón-Garcidueñas et al., 2004 and Block and Calderón-Garcidueñas, 2009) also exhibits increased tau levels and abnormally phosphorylated tau (Tai et al., 2012 and Medina and Avila., 2014). There is an intimate association between atmospheric pollutant exposure and the pathological changes associated with tauopathy, however, the potential molecular mechanism responsible for the air pollution leading to tau dysfunction remains unclear. In light of the fact that insulin signaling can be targeted by atmospheric pollutants and its deregulation

helps augment neurodegenerative tauopathy progression, we hypothesized that brain insulin signaling damage is a candidate etiological factor in the pathogenesis of air pollution-induced tau lesions.

Because air pollution is a complex mixture of gases, particulate matter, and organic compounds, the identification of the specific pollutant that has the greatest effect on health is necessary. Nitrogen dioxide (NO₂) is a crucial component of air pollution. It is one of six criteria air pollutants regulated by the U.S. Environmental Protection Agency (EPA, 2016), and it is included in the computation of the daily air quality index in cities in China (HJ, 2012). Generated primarily from vehicle exhaust, as well as by power plants and gas-burning appliances, this compound has several emission sources. Satellite-derived data estimates of trends in ground-level NO₂ showed that from 1996 to 2012, approximately 3.2 billion people in the world lived in regions where the average NO₂ significantly increased (Geddes et al., 2016). China, predominately characterized as an industrialized and rapidly developing country in East Asia, even exhibited a 2.7-fold increase in NO₂ concentration over the same time span (Seltenrich, 2016). NO₂ in the atmosphere is not just a primary pollutant, but it is also a precursor for several detrimental secondary air pollutants, including nitric acid, the nitrate part of secondary inorganic aerosols, and photo oxidants. Considering its influential role in the environment and climate, most public attention has been focused on the potential harmful effects of NO₂ pollution. During the past decade, considerable evidence has accumulated demonstrating that atmospheric pollutants, including NO₂, are closely related to negative CNS outcomes and the most frequent tauopathy — AD pathology (Calderón-Garcidueñas et al., 2004). However, it is not clear whether NO₂ itself acts as an independent cause of tauopathy

and how this environmental factor contributes to the pathogenesis of this disease. Therefore, we sought to use NO₂ as a predictor of the effects of air pollution and focus on the possible molecular mechanisms underlying the processes of tau pathology regulated by insulin signaling.

2. Materials and methods

2.1 Animals and NO₂ exposure

This research was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the Ministry of Health of the People's Republic of China. The study protocol was approved by the Institutional Animal Care and Use Committee of Shanxi University (Approved Animal Use Protocol Number: HZ20150901). Male C57BL/6J mice, approximately 2 months old, were obtained from Beijing HFK Bioscience Co., Ltd. (Beijing, PRC). The mice were randomly divided into 3 groups, and 10 mice in each group were exposed to 0 (fresh air), 2.5, or 5 mg/m³ of NO₂ for 5 h a day for 4 weeks, using a whole-body inhalation exposure chamber system. To reach the desired NO₂ concentrations, the NO₂ gas was diluted with fresh air at the intake port of the mixing chamber; then, it was delivered to the animals through a tube positioned in the upper left part of the exposure chamber and was distributed homogeneously via a perforated plate. The NO₂ concentration within the chamber was detected by a real-time NO₂ monitor (Wandi, China). Following the last exposure, the mice were fasted for 16 h, and blood samples were then collected for detection of the fasting serum insulin content. The cortex, hippocampus, skeletal muscle, and liver tissues were isolated, quickly frozen in liquid nitrogen, and kept at -80 °C.

2.2 Immunoblot analysis

Total protein was extracted from the treated tissues using RIPA buffer. The protein samples were loaded on a premade 8 – 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel. After electrophoresis, the proteins were transferred to polyvinylidene fluoride membranes (Bio-Rad), and blocked by 3% bovine serum albumin. The membranes were incubated with different primary antibodies at 4 °C overnight. Antibodies including IRS-1 (insulin receptor substrate-1), phosphorylated (P)-IRS-1 (phosphorylation at Tyr612), AKT (protein kinase B), P-AKT (phosphorylation at Ser473), GSK-3 β (glycogen synthase kinase-3 beta), P-GSK-3 β (phosphorylation at Ser9), Erk1/2 (extracellular regulated protein kinases 1/2), P-Erk1/2 (phosphorylation at Thr183/185), JNK (c-Jun N-terminal kinase), P-JNK (phosphorylation at Thr183), tau, P-tau (phosphorylation at Thr231), p38 MAPK (mitogen-activated protein kinase), P-p38 MAPK (phosphorylation at Thr180 + Tyr182), GluA1, GluA2, GluN1, GRIN2A, GRIN2B, and PSD-95 (postsynaptic density-95) were obtained from Bioss Biological Technology Co., Ltd. (1:200, Beijing, PRC). The blots were washed and incubated with an IR Dye 800CW-conjugated secondary antibody (1:5000, LiCor Biosciences, USA) at room temperature for 1 h. The densities of the specific bands were quantified by densitometry using Image-pro Express software (version 6.0) (Media Cybernetics, USA). The band densities were normalized to the amount of β -actin (1:1000, Cell Signaling, USA) protein loaded in each well.

2.3 Immunohistochemistry

The mice were anesthetized and subsequently transcardially perfused with cold 4% paraformaldehyde in phosphate-buffered saline (PBS). The brains were removed, post-fixed in 4% paraformaldehyde overnight, and dehydrated in 15% and 30% sucrose until they sank to

the bottom of the glass jars. The fixed tissues were embedded in Tissue-Tek at the optimal cutting temperature and frozen using crushed dry ice. Coronal sections, 40 μm thick were sliced on a freezing vibratome and were then collected in 0.1 M phosphate buffer (PB). The series sections (10-12 slices) were incubated with rabbit anti-tau and anti-p-tau (Bioss, PRC), and were developed using fluorescent-labeled secondary antibodies (1:5000, LiCor Biosciences, USA). After washing, the sections were incubated with 4'-6-Diamidino-2-phenylindole (DAPI), a fluorescent stain that binds strongly to DNA, to detect the cell nuclei. Representative images were captured using fluorescence microscopy (Olympus ix71, Japan), and were analyzed using the Image-pro Express software, version 6.0 (Media Cybernetics, USA).

2.4 Transmission electron microscope (TEM) observation

Tissue pieces approximately 1 mm^3 in size were rapidly cut from the cortex and hippocampus in the mice brains, and the tissues were fixed in 0.1 M PB containing 4% paraformaldehyde and 1.5% glutaraldehyde for 2 h at room temperature. The pieces were washed with 0.1 M PB, and then post-fixed in 2.5% OsO_4 in 0.1 M PB for 1 h. After washing the samples again in PB, the sections were stained for 1 h with 1% uranyl acetate in 50% ethanol at room temperature in the dark, and they were then dehydrated by graded ethanol and flat-embedded in Araldite. The embedded sections were glued onto a blank Araldite block and trimmed. Ultrathin sections (approximately 70-80 nm thick) were prepared and placed on formvar carbon-coated copper grids. The grids were stained with uranyl acetate for 15-30 min and lead citrate for 3-15 min, examined with an electron microscope (JEOL, JEM 1400, Japan), and photographed.

2.5 Enzyme-linked immunosorbent assay (ELISA)

The collected blood samples were centrifuged for 10 min at 4000 rpm to extract the serum, and were stored at -20 °C until assayed. A mouse insulin ELISA kit, purchased from Westang Biotechnology Inc (Shanghai, China), was used to measure the level of fasting serum insulin according to the manufacturer's instructions.

2.6 Statistical analyses

The data are presented as the means $\pm$ SE unless otherwise indicated. One-way analysis of variance (ANOVA) was used to determine significant differences. A statistically significant difference was defined as *P<0.05, **P<0.01, and ***P<0.001.

3. Results

3.1 NO₂ inhalation causes tauopathy coupled with synaptic loss.

Pathological tau inclusions composed of hyperphosphorylated tau protein are present in several neurodegenerative disorders, called tauopathies (Clavaguera et al., 2014). To determine whether NO₂ inhalation causes tau pathological abnormalities, we first examined the level of total tau and phosphorylated tau (P-tau) protein in the mouse brain following NO₂ dynamic inhalation (2.5 and 5.0 mg/m³). As shown in Fig. 1a and b, there was no significant difference in the expression of total tau protein. However, the levels of tau phosphorylation were elevated in a dose-dependent manner in both the cortex and hippocampus compared with the control group, and a significant difference was observed after the 5.0 mg/m³ exposure. Furthermore, the immunofluorescence staining results confirmed that phosphorylated tau spreads throughout the brain after NO₂ inhalation at 5.0 mg/m³ (see Fig. 1c). These findings clearly demonstrate that NO₂ inhalation induced tauopathy by potentiating the phosphorylation

of tau protein.

Evidence shows that the accumulation of hyperphosphorylated tau which occurs in tau-related brain deficit, tremendously disrupts synaptic function by altering the expression and function of glutamate receptors (Hoover et al., 2010). To further investigate whether NO₂ inhalation-induced tauopathy involves synaptic impairment, we examined the expression of glutamate Alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and N-methyl-D-aspartic acid (NMDA) receptors in the mouse brain, using the proteins GluA1, GluA2, GluN1, GRIN2A, and GRIN2B. As shown in Fig. 2a and b, glutamate receptors expression decreased in cortex and/or hippocampus following NO₂ exposure, but GluN1 expression did not decrease. In particular, the expression of GluA1, GluA2, and GRIN2B in the cortex, GluA1, GluA2, and GRIN2A in the hippocampus was significantly reduced in response to NO₂ inhalation at 5 mg/m³. These results suggest a significant association between NO₂ exposure and synaptic transmission dysfunction.

In addition to regulating the glutamatergic receptor content, tau impacts synaptic activity by interacting directly with post-synaptic signaling complexes (Mondragón-Rodríguez et al., 2012). Postsynaptic densities (PSD) contain glutamate receptors and scaffolding proteins that organize synaptic signal transduction near the postsynaptic membrane. To confirm that NO₂ disrupts the postsynaptic complex, we assessed the thickness of the PSD. TEM revealed a significantly thinner PSD after exposure to 5 mg/m³ NO₂, but a 2.5-mg/m³ NO₂ exposure did not cause obvious morphological changes compared with the control (Fig. 2c). Consistent with this result, the expression of PSD-95, which is a core component of the PSD and is targeted by tau (Mondragón-Rodríguez et al., 2012), significantly decreased following 5-mg/m³ NO₂

inhalation (Fig 2d). Together, our data indicate that hyperphosphorylated tau protein, after NO₂ exposure, triggers the cascade of synaptic protein reduction.

3.2 NO₂ exposure produces central and peripheral IR and induces hyperinsulinemia

The physiological functions of insulin are exerted across a variety of insulin target tissues through several intracellular signaling cascades, one of the most prominent of which is the insulin receptor substrate-1 (IRS-1)/phosphoinositide-3-kinase (PI3K)/ protein kinase B (AKT) pathway (de la Monte, 2012 and Kitagishi et al., 2014). Considering that NO₂ exposure contributed to hyperphosphorylated tau and synaptic dysfunction, we aimed to determine whether these tau-dependant brain deficits were a result of impairments in insulin signaling. To test this hypothesis, we first analyzed the expression of phosphorylated IRS-1 and Akt proteins in the cortical and hippocampal tissues. As shown in Fig. 3a and b, the tyrosine phosphorylation of IRS-1 and Akt were significantly reduced following NO₂ exposure, suggesting the involvement of insulin signaling impairment. Normally, insulin stimulation reduces tau phosphorylation and promotes tau binding to microtubules by activating PI3-K/Akt phosphorylation which therefore, inhibiting the β cytosolic forms of glycogen synthase kinase-3 (GSK-3) in neuron (Hong and Lee, 1997). Based on the deterioration in insulin receptor signaling described above, it is most likely that the aberrant tau phosphorylation, in response to NO₂ inhalation, could be attributed to enhanced GSK-3 β activity through Akt suppression. Accordingly, we assessed the expression of GSK-3 β , and found that phosphorylated GSK-3 β was significantly decreased in this process (Fig. 3c). Thus, we clearly demonstrated that NO₂ inhalation disturbed insulin signaling and contributed to the tauopathy, as well as that the action was mediated by the IRS-1/Akt/GSK-3 β signaling pathway.

The alterations in the IRS-1/PI3K/AKT pathway, particularly the reduced phosphorylation of AKT, are implicated as key markers of IR, a state wherein the normal concentrations of insulin evoke a less-than-normal biological response (Jiang et al., 2013). Considering that insulin exerts its physiological functions in all of the insulin target tissues except the brain, we aimed to determine whether NO₂ derived central IR was accompanied by IR in the peripheral tissues. For this aim, we further measured the activation of IRS-1 and Akt in peripheral insulin target tissues (the skeletal muscle and liver). As indicated in Fig. 4, NO₂ exposure significantly suppressed IRS-1 and Akt activation in muscle (Fig. 4a) and liver (Fig. 4b) tissue. This result, together with the data presented in Fig. 3, indicates that NO₂ exposure triggers systematic IR in both the CNS and peripheral tissue, and these findings imply that deregulation of the insulin signaling cascade occurs in NO₂-induced tau pathology.

In IR states, the cellular response of the insulin signaling pathway is attenuated, and the body's compensatory mechanism typically increases the insulin level in the serum to maintain insulin signaling. Following this logic, if NO₂ exposure contributes to a sustained suppression of brain insulin response (Catalano et al., 2014), it is very likely that hyperinsulinemia would occur in this condition. In the present study, we expectedly observed that the NO₂-treated mice exhibited a significant enhancement in their fasting serum insulin concentration (Fig. 5). Previous studies showed that chronic hyperinsulinemia results in a reduction of insulin transport across the BBB, impairing insulin signaling in the brain (van der Heide et al., 2006 and Neumann et al., 2008). This information confirmed our speculation and provided a further potential reason for the observed brain insulin dysfunction following NO₂ inhalation.

3.3 IR-related tauopathy following NO₂ inhalation is mediated by JNK/p38 MAPK

activation

The activation of multiple serine/threonine kinases (including JNK and p38 MAPK) and their functioning in insulin receptor signaling, as well as the family of IRS proteins, are considered causative factors in the development of IR (Fridlyand and Philipson, 2006 and Kodavanti, 2015). Here, we assessed the activation of JNK and p38 MAPK in insulin target tissues. Compared with the control group, the phosphorylation of p38 MAPK in the skeletal muscle (Fig. 6a) and JNK (Fig. 6b) in the liver was significantly enhanced after NO₂ exposure, whereas the phosphorylation of p38 MAPK and JNK were significantly increased in the cortex and hippocampus (Fig. 6c).

4. Discussion

NO₂ exposure causes negative CNS outcomes and neurodegenerative diseases have been well established in a series of major epidemiological studies (Schikowski et al., 2015 and Yan and Yun et al., 2016). However, the available experimental evidence, especially the evidence based on biomarkers following exposure, is insufficient. A pathological tau lesion characterized by an abnormally phosphorylated tau protein is the predominant feature of a broad range of neurodegenerative diseases grouped under the convenient term 'tauopathies' (Taylor et al., 2002). In this study, we first verified that NO₂ inhalation impaired synaptic structure and function, and caused tauopathy by potentiating tau phosphorylation.

A growing amount of evidence clearly demonstrates that neuronal insulin signaling plays a direct role in the regulation of tau function and that the disruption of intracellular insulin signaling molecules can induce a tauopathy-like condition (Lee et al., 2001; Avila et al., 2004 and de la Monte, 2012). Therefore, we suggested a hypothetical sequence of the pathogenic

steps linking NO₂-induced tau dysfunction to insulin signaling disruption in the CNS. Insulin is synthesized, processed, and secreted by pancreatic β -cells. In each target tissue, insulin triggers its actions by binding to the insulin receptors, which subsequently phosphorylate their substrates, and then activate different signaling pathways. Among these pathways, the IRS-1/PI3K/Akt pathway has been most intensively studied (de la Monte, 2012 and Kitagishi et al., 2014). Normally, in response to insulin, IRS-1 becomes tyrosine phosphorylated (phosphorylation of Tyr608 and Tyr628) and generates the major docking sites for the PI3K, and the IRS-1/PI3K complex is then activate the Akt phosphorylation (Esposito et al., 2001). However, the alterations in the IRS-1/PI3K/AKT pathway, particularly the reduced phosphorylation of AKT, are implicated as key markers of IR (Jiang et al., 2013), a state wherein the normal concentrations of insulin evoke a less-than-normal biological response. The present study showed a significantly reduced phosphorylation of IRS-1 at Tyr628 in skeletal muscle, liver, and brain tissues following NO₂ exposure. Correspondingly, we similarly observed a significant decrease in the phosphorylation of AKT at Ser473 in the NO₂-exposed mouse tissues. These data confirm that NO₂ exposure results in the dysfunction of insulin signaling, and contributes to IR in both peripheral (muscle and liver) and central (hippocampus and cortex) tissues.

In an IR state, target tissues fail to respond properly to normal levels of circulating insulin, and the body responds by increasing serum insulin concentrations designed to maintain insulin signaling (Catalano et al., 2014). Corresponding to this compensatory mechanism, the present study expectedly demonstrated an increased fasting serum insulin level following IR, which directly implicated that physiological hyperinsulinemia occurred in response to NO₂

inhalation. Insulin in the serum is transported by the CSF and can cross the BBB into the brain (Vandal et al., 2015). There is evidence that chronic peripheral hyperinsulinemia (as occurs in IR) leads to a reduction of insulin transport across the BBB and affects all of the actions of insulin in the brain (Neumann et al., 2008). Not surprisingly, in the present study, inhibitory insulin signals such as the diminished phosphorylation of IRS-1 at Tyr628 and decreased phosphorylation of AKT at Ser473 were exhibited in the brain following NO₂ inhalation. These data suggest that NO₂ exposure impairs whole-body insulin signaling, and in a state of systemic IR, compensatory hyperinsulinemia may further worsen the brain insulin signaling response, which contributes to the transition from brain IR to a more severe disease.

The notion that oxidative stress results in the development of IR has been supported by several studies, which showed that a reversal of the imbalance between ROS and antioxidants improves IR in mice and humans (Evans et al., 2003 and Furukawa et al., 2004). Importantly, a possible mechanism of the development of IR through oxidative stress is the activation of multiple serine/threonine kinase signaling cascades, which can act on several potential targets in the insulin signaling pathway, including the insulin receptor and the family of IRS proteins (Evans et al., 2003). Considering that NO₂ inhalation causes systemic oxidative damage in organisms (Ji et al., 2015; Li et al., 2011; Yan et al., 2015; Yan and Yun et al., 2016), we further detected these stress kinases (including p38 MAPK and JNK) in muscles, liver, and brain tissues. The activation of p38 MAPK and/or JNK in insulin target tissues following NO₂ exposure implies that NO₂ exposure-induced IR could be initiated by the regulation of the p38 MAPK and/or JNK pathway.

Generally, insulin and its related hormone IGF-1 reduce tau phosphorylation and promote

tau binding to microtubules by suppressing the activity of GSK-3 β through the IRS-1/PI3-K/Akt signaling pathway (Kitagishi et al., 2014). However, in the present study, NO₂ inhalation significantly suppressed IRS-1/Akt and therefore caused reduced phosphorylation of GSK-3 β , which directly potentiated the phosphorylation of tau protein. In addition to the tau protein, synaptic plasticity is also evidenced can be targeted by insulin signaling through modulation of the activities of excitatory and inhibitory receptors, such as glutamate and GABA receptors (Zhao et al., 2004). In the same study, we also observed a significant decreased in the expression of glutamate receptors and a thinner PSD following NO₂ treatment, indicating a possible synaptic dysfunction coupled with a tau lesion in response to NO₂ inhalation.

5. Conclusions

In the present study, we demonstrated that NO₂ inhalation contributed to synaptic impairment and tauopathy. This action appears to be initiated from a p38 MAPK and/or JNK-mediated abnormality of the IRS-1/PI3K/AKT pathway and systemic IR, and is followed by a GSK-3 β -targeted synaptic loss and tau phosphorylation (see graphical abstract).

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Figure 1

Figure 2

Figure 3

a1

a2

b1

b2

c1

c2

Figure 4

Figure 5

Figure 6