

Scavenging ROS Decreases Amyloid-beta Levels via Activation of PI3K/Akt/GLUT1 pathway in N2a/APP695swe Cells

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Research Article

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Abstract

Dysregulated glucose metabolism in the brain is considered to be the underlying cause of Alzheimer's disease (AD). Abnormal glucose metabolism in AD is associated with decreased glucose transporter 1 (GLUT1) and GLUT3 in the brain, but the underlying mechanisms remains unclear. Here, we reported that GLUT1 expression was decreased in N2a/APP695swe cells and GLUT3 expression was not significantly changed. Flow Cytometry analysis showed a significant increase of intracellular ROS content in N2a/APP695swe cells and GLUT1 expression was upregulated after treatment with the ROS scavenger N-acetyl-L-Cysteine (NAC). Cellular glucose uptake and ATP levels were reduced following decreased GLUT1 expression and increased after upregulating GLUT1. Western blot analyses showed that phosphorylation of PI3K/Akt pathway decreased in N2a/APP695swe cells. A β levels decreased after upregulation of GLUT1 expression and increased after downregulation of GLUT1. After NAC treatment, PI3K/Akt pathway phosphorylation levels and GLUT1 expression were upregulated, glucose uptake and ATP contents were increased, and A β levels were decreased. After adding PI3K/Akt pathway inhibitor LY29004, GLUT1 expression was reduced and A β levels were increased. Besides, the increased glucose uptake and ATP contents by the Akt activator SC79 were hindered with the GLUT1 inhibitor WZB117. A β levels decreased after SC79 treatment and increased after WZB117 treatment. Overall, our data suggest that ROS reduced GLUT1 expression by inhibiting PI3K/Akt pathway activity resulting in impaired glucose metabolism and scavenging ROS prevents A β via activation of PI3K/Akt/GLUT1 pathway in N2a/APP695swe cells.

1 Introduction

Alzheimer's disease (AD) is a progressive degenerative disease of the central nervous system and the main cause of dementia in the elderly ^[1]. The pathological characteristics of AD mainly include β -amyloid (A β) deposition, neurofibrillary tangles (NFTs), of which the neurotoxicity of A β is currently recognized as the central part of AD ^[2]. Recent studies have found that the development of AD is associated with metabolic abnormalities. Particularly, glucose metabolism disorders in the brain are considered to be responsible for the development of AD ^[3]. Impaired brain glucose uptake and utilization are closely associated with AD pathological deterioration and cognitive impairment ^[4,5].

Several studies have shown that the abnormal glucose metabolism in AD is mainly associated with a decrease in glucose transporter 1 (GLUT1) and GLUT3 in the brain, the main glucoses transporter responsible for glucose uptake in the mammalian brain ^[6]. The reduction of GLUT1 and GLUT3 might result in insufficient glucose uptake, compromised glucose metabolism, and ultimately lead to neuronal degeneration ^{[3][7,6]}. The Phosphoinositide 3-Kinase-Akt (PI3K/Akt) pathway has been suggested to play essential roles in the regulation of GLUT1 and GLUT3 ^[8]. Consistently, previous studies have found that abnormal PI3K/Akt signaling is involved in the development of AD ^[9,10]. However, little is currently known about the mechanisms underlying the abnormal PI3K/Akt signaling and reduced expression of GLUT1 and GLUT3 in AD.

Reactive oxygen species (ROS) is an important factor in the early pathological development of AD [11]. ROS triggers the formation and accumulation of A β , meanwhile, A β also promotes ROS production and enhances oxidative stress [12]. In addition, increased level of ROS is associated with decreased expression or activity of key proteins involved in the glucose metabolism pathway, which might result in a low energy state in neurons [13,14]. It has been reported that ROS affects brain energy metabolism by indirectly regulating neuronal cell permeability to glucose [1]. This study investigated the potential roles of ROS in the regulation of GLUT1 and GLUT3 and the underlying mechanisms in N2a/APP695swe cells.

2 Materials And Methods

2.1 Cell culture and treatment

The wild-type mouse neuroblastoma cells (N2a/WT) were purchased from Cell Resource Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). N2a/APP695swe cells were purchased from Obio Technology (Shanghai, China). All cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, Waltham, MA, USA) containing 10% fetal bovine serum (FBS, Gibco), and N2a/APP695swe cells medium were supplemented with an additional 0.04% puromycin (5 mg/ml) to screen cells stably expressing APP695swe. Cells were cultured at 5% CO₂, 37°C. These cells were treated with N-Acetyl-L-cysteine (NAC; 5 mM, Beyotime Institute of Biotechnology, Nantong, Jiangsu, China), LY294002 (1 μ M, Beyotime), SC79 (4 μ g/mL, Beyotime) and WZB117 (10 μ mol/L; Selleck, Houston, Texas, USA), which were dissolved in dimethyl sulfoxide (DMSO; Solarbio, Beijing, China).

2.2 Western blot

Total cellular protein was extracted and quantified according to a standard procedure. Specifically, N2a/WT and N2a/APP695swe cells were homogenized in RIPA lysis buffer and protease inhibitors PMSF at 4°C for 30 min, and centrifuged at 13,000 rpm for 15 min at 4°C to collect the supernatants. The protein concentration was measured with the BCA assay reagent (Beyotime) according to the manufacturer's protocol. Following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, Epizyme, Shanghai, China), the proteins were transferred onto a PVDF membrane (Solarbio). After saturation, the membranes were incubated at room temperature for 10 min in Quick Block™ Western Sealing fluid (Beyotime) and subsequently incubated with primary antibodies against GLUT1 (1:100000, Abcam, Cambridge, MA, USA), GLUT3 (1:1000, Proteintech, Wuhan, China), Akt (1:1000), p-Akt (1:1000), PI3K (1:1000), p-PI3K (1:1000), or β -actin (1:1000), all from SAB (Signalway Antibody, Maryland, USA), at 4°C overnight. The next day, peroxidase-conjugated IgG (Beyotime) was incubated for 40 min and enhanced chemiluminescence (ECL) solution was subsequently applied to the membranes for detection of protein bands.

2.3 Glucose uptake and measurement of ATP level in cells

Glucose uptake by cells and the content of intracellular ATP were measured with Glucose Kit and ATP Assay Kit (Nanjing Jian Cheng, Nanjing, China). Briefly, after cells were treated as indicated for each experiment, cell samples preparation and detection procedures were done following the kit instruction.

2.4 Cell viability CCK-8 assay

Cell viability rates were measured using the CCK-8 Proliferation Assay Kit (Solarbio). Cells were seeded into 96-well plates at a density of 3000/well and grown overnight and then treated with LY294002 (5, 10, 20 or 40 μ M), SC79 (1, 2, 4 or 8 μ g/mL), WZB117 (1, 5, 10 or 15 μ mol/L) or NAC (3, 5, 10 or 15 mM) for up to 72 h. At each time point, each group of cells was treated according to the instruction of the CCK-8 reagent.

2.5 Flow cytometry ROS level assays

According to the ROS Assay Kit (Beyotime) instruction, cells were loaded by an in-situ probe with DCFH-DA, and then cells were collected by centrifugation at 1000 rpm for 5 min. DCF fluorescence was detected by flow cytometry, which was the intracellular ROS level.

2.6 Enzyme-linked immunosorbent assay (ELISA)

The cell culture medium of each group was collected, and the content of $A\beta_{1-40}$ and $A\beta_{1-42}$ were detected according to the instruction of ELISA Kit (SAB, Maryland, USA).

2.7 Statistical analysis

All experiments were performed at least three times and statistical analysis was done using the SPSS13.0 package (SPSS Inc., Chicago, USA). The values for each group are expressed as the means \pm S.E.M. The ANOVA test was used whenever more than two groups were compared, and the significance level was set at $p < 0.05$. A repeated measurement variance test was used to analyze time points comparisons. P values of less than 0.05 ($p < 0.05$) were considered to be statistically significant.

3 Results

3.1 Increased $A\beta$ and ROS levels in N2a/APP695swe cells

Compared with N2a/WT cells, $A\beta_{1-40}$ and $A\beta_{1-42}$ contents were increased in N2a/APP695swe cells (Fig. 1, a + b). Previous studies have reported that hypometabolism of glucose was most likely associated with reduced expression and function of key proteins in the glucose metabolic reaction pathway, which decreased with high levels of ROS [15,14]. Thus, we measured ROS levels by Flow Cytometry techniques, and the results showed that ROS concentration in N2a/APP695swe cells was strongly higher than that in N2a/WT cells (Fig. 1c).

Fig. 1 Increased $A\beta$ and ROS levels in N2a/APP695swe cells

(a + b) N2a/WT and N2a/APP695swe cells culture medium were collected to analyze A β levels. The contents of A β_{1-40} and A β_{1-42} in the APP group were significantly higher than that in the WT group. (c) Intracellular ROS levels were detected by Flow Cytometry. As shown in the column, the ROS contents (P1 value) in the APP group were higher than that in the WT group. * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$, **** $P \leq 0.0001$.

3.2 Decreased GLUT1 expression, the glucose uptake and ATP contents in N2a/APP695swe cells

To detect whether the glucose metabolism of N2a/APP695swe cells was impaired, the glucose uptake and ATP contents of N2a/WT and N2a/APP695swe cells were detected respectively. Our results showed that glucose uptake and ATP contents of N2a/APP695swe cells were significantly lower than that of N2a/WT cells (Fig. 2, a + b). Considering that GLUT1 and GLUT3 are the main transporters responsible for the acquisition of glucose by nerve cells, we tested these two proteins to analyze the possible reasons for the impaired cellular glucose uptake. Western blots results indicated that compared with the WT group, the expression of GLUT1 in the APP group was decreased, and the difference was statistically significant (Fig. 2c), while the expression of GLUT3 was not changed, and the difference was not statistically significant (Fig. 2d).

Fig. 2 Decreased GLUT1 expression, the glucose uptake and ATP contents in N2a/APP695swe cells

The N2a/WT and N2a/APP695swe cells were collected separately from three different culture flasks and the proteins were extracted for the Western blot analysis. (a + b) Measurement of intracellular glucose uptake and ATP contents. (c + d) Western blot analysis of GLUT1 and GLUT3 proteins. GLUT1 expression was decreased in the APP group and GLUT3 was not significantly changed. Numbers 1, 2, and 3 represent proteins extracted at three different dates. * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$.

3.3 GLUT1 enhanced the glucose uptake and ATP contents, reduced A β levels in N2a/APP695swe cells

To verify that the disordered cellular glucose metabolism was caused by low GLUT1 expression, we transfected N2a/WT and N2a/APP695swe cells with GLUT1-overexpressed plasmids, and western blot results showed the protein expression of GLUT1 after transfection (Fig. 3a). As we expected that after upregulated GLUT1, the glucose uptake and ATP contents were increased in both the WT and APP groups, with statistically significant differences (Fig. 3, b + c). Then we proceeded to test the A β levels and the ELISA results showed that both A β_{1-40} and A β_{1-42} contents were reduced (Fig. 3, d + e).

Fig. 3 GLUT1 enhanced the glucose uptake and ATP contents, reduced A β levels in N2a/APP695swe cells

The results from one of three experiments are shown. * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$. (a) GLUT1 overexpression plasmid was transfected into N2a/WT and N2a/APP695swe cells for 24 h and the GLUT1 protein level was detected by Western blot. (b + c) Measurement of intracellular glucose uptake and ATP level. After upregulated GLUT1, the glucose uptake and ATP contents were increased in both two groups.

(d + e) N2a/WT and N2a/APP695swe cells culture medium were collected to analyze A β levels. After overexpression of GLUT1, the contents of A β ₁₋₄₀ and A β ₁₋₄₂ were markedly reduced in the APP group.

3.4 ROS scavenger N-acetyl-L-Cysteine (NAC) increased GLUT1 expression, the glucose uptake and ATP contents, decreased A β levels in N2a/APP695swe cells

Next, we treated the cells with the N-acetyl-L-Cysteine (NAC), which was commonly used as a scavenger of ROS. We explored four concentration gradients (3, 5, 10, 15 mM) according to the drug instruction of NAC and relevant literature^[16]. The CCK-8 results showed that cells incubated with 5 mM were the most active, and almost all cells died after incubation with NAC concentration greater than that (Fig. 4a). After NAC treatment for 24 h, ROS concentrations decreased in N2a/APP695swe cells (Fig. 4b). Western blot results showed that GLUT1 expression was significantly upregulated, compared with the control group (Fig. 4c), the glucose uptake and ATP contents also increased (Fig. 4, d + e). Meanwhile, the contents of A β ₁₋₄₀ and A β ₁₋₄₂ in N2a/APP695swe cells decreased, with statistically significant differences (Fig. 4, f + g).

Fig. 4 ROS scavenger N-acetyl-L-Cysteine (NAC) increased GLUT1 expression, the glucose uptake and ATP contents, decreased A β levels in N2a/APP695swe cells

The results from one of three experiments are shown. * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$. (a) The effect of NAC on viability in N2a/APP695swe cells was analyzed by the CCK-8 assay, 5 mM was chosen as the correct concentration. (b) After NAC treatment for 24 h, ROS concentrations decreased in N2a/APP695swe cells. (c) GLUT1 expression was analyzed by Western blot after being treated with NAC for 24 h. (d + e) Measurement of intracellular glucose uptake and ATP levels. After NAC treatment for 24 h, the glucose uptake and ATP contents were increased. (f + g) After NAC treatment for 24 h, the contents of A β ₁₋₄₀ and A β ₁₋₄₂ decreased in N2a/APP695swe cells.

3.5 Phosphorylation of PI3K/Akt pathway decreased in N2a/APP695swe cells

To clarify the mechanism by which ROS regulated GLUT1, we further tested the PI3K/Akt signal pathway, an important upstream pathway that controlled GLUT1. Our results suggested that the phosphorylation levels of PI3K and Akt in the APP group were lower than that in the WT group, and the difference was statistically significant. The total protein expressions of PI3K and Akt were not a significant variety (Fig. 5, a + b).

Fig. 5 Phosphorylation of PI3K/Akt pathway decreased in N2a/APP695swe cells

The results from one of three experiments are shown. * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$. (a + b) The level of the PI3K/Akt pathway was analyzed by Western blot. Numbers 1, 2, and 3 represent proteins extracted at three different dates.

3.6 NAC upregulated PI3K/Akt/GLUT1 pathway levels, increased the glucose uptake and ATP contents, decreased A β levels

Then we treated N2a/APP695swe cells with NAC and/or PI3K inhibitor LY294002. LY294002 is a potent inhibitor of PI 3-kinase activity based on the quercetin structure, which inhibits PI3K by reversibly binding to the ATP site of PI3K [17]. According to the drug instructions and literature [18], we selected four concentration gradients (5, 10, 20, 40 μ M) to screen the optimal concentration of LY294002 for N2a/APP695swe cells. The CCK-8 results showed that the cell viability was significantly reduced at 20 μ M LY294002 concentration (Fig. 6a), whereas cells became shrunken when the concentration was 40 μ M after 24 h treatment, thus we chose 20 μ M as the drug concentration. After NAC treatment we found that p-PI3K, p-Akt, and GLUT1 expression were increased, while decreased after LY294002 addition (Fig. 6b). Similarly, the glucose uptake and ATP contents were reduced after the cells were co-incubated with LY294002 in the medium containing NAC, with statistically significant differences (Fig. 6, c + d). Interestingly, the contents of A β ₁₋₄₀ and A β ₁₋₄₂ increased after the addition of LY294002 (Fig. 6, e + f). These data indicated that maintaining the activity of the PI3K/Akt/GLUT1 pathway is essential for A β clearance.

Fig. 6 NAC upregulated PI3K/Akt/GLUT1 pathway levels, increased glucose uptake and ATP contents, decreased A β levels

(a) The effect of LY294002 on viability in N2a/APP695swe cells was analyzed by the CCK-8 assay, 20 μ M LY294002 was chosen as the correct concentration. (b) The Western blot results for p-PI3K/Total-PI3K, p-Akt/Total-Akt, and GLUT1 after being treated with NAC and/or LY294002 for 24 h. (c + d) The glucose uptake and ATP levels were increased after NAC treatment for 24 h, while decreased after LY294002 addition. (e + f) N2a/APP695swe cells culture medium was collected to analyze A β levels. The contents of A β ₁₋₄₀ and A β ₁₋₄₂ were reduced after NAC treatment for 24 h, while increased after LY294002 addition.

3.7 Activated PI3K/Akt/GLUT1 increased the glucose uptake and ATP contents, decreased A β levels in N2a/APP695swe cells

To further demonstrate that the PI3K/Akt pathway was dependent on GLUT1 to improve glucose metabolism levels, we co-treated cells with SC79, an activator of Akt, and WZB117, a GLUT1-specific inhibitor. WZB117 binds to amino acid residues in the central channel region of GLUT1 to form three hydrogen bonds thereby inhibiting GLUT1 [19]. Likewise, we selected four concentration gradients (1, 2, 4, 8 μ g/mL) based on drug specification and literature [20] to screen the optimal concentration of SC79 for cells. The CCK-8 results showed that the cell viability was increased at 4 μ g/mL SC79 concentration (Fig. 7a). We also determined the best concentration of WZB117 was 10 μ mol/L based on the instruction and CCK-8 assay results because most of the cells died when the WZB117 concentration was 15 μ mol/L

(Fig. 7b). After SC79 and WZB117 incubated cells together for 24 h, glucose uptake and ATP contents were markedly decreased, and the differences were statistically significant (Fig. 7, c + d). The contents of A β ₁₋₄₀ and A β ₁₋₄₂ were reduced after SC79 treatment for 24 h, while increased after WZB117 addition (Fig. 7, e + f).

Fig. 7 Activated PI3K/Akt/GLUT1 increased glucose uptake and ATP contents, decreased A β levels in N2a/APP695swe cells

The results from one of three experiments are shown. * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$. (a) The effect of SC79 on viability in N2a/APP695swe cells was analyzed by the CCK-8 assay, 4 $\mu\text{g}/\text{mL}$ SC79 was chosen as the correct concentration. (b) The effect of WZB117 on viability in N2a/APP695swe cells was analyzed by the CCK-8 assay, 10 $\mu\text{mol}/\text{L}$ was chosen as the correct concentration. (c + d) The glucose uptake and ATP levels were increased after SC79 treatment for 24 h, while decreased after WZB117 addition. (e + f) N2a/APP695swe cells culture medium was collected to analyze A β levels. The contents of A β ₁₋₄₀ and A β ₁₋₄₂ were reduced after SC79 treatment for 24 h, while increased after WZB117 addition.

Fig. 8 Schematic: ROS reduces PI3K/Akt pathway phosphorylation levels leading to cellular GLUT1 deficiency and impaired glucose uptake, NAC improves this state

4 Discussion

The pathogenesis of AD is complex, and many mechanisms are involved in the pathogenesis of AD in a synergistic manner, such as A β toxicity, oxidative stress, and impaired glucose metabolism [21][22]. During the stage of AD development, the ability of the brain to utilize glucose is progressively impaired, even to the point of decreasing ATP production by 50% [7]. Glucose is the main source of energy for the nervous system and only relies on specific transporter to cross the phospholipid bilayer into cells [23]. Glucose uptake in the brain is mainly reliant on GLUT1 and GLUT3, and is closely related to their levels [24,25][26]. Studies have reported that the accumulation of ROS, the reduction of GLUT1/GLUT3, and the impaired glucose metabolism all occurred in brain regions associated with memory and cognitive function [1][5]. A previous study found that 4-Hydroxynonenal (HNE, an aldehyde product of membrane lipid peroxidation) impaired the glucose transport process, however, GLUT3 protein expression was not altered [27]. In the present study, we found that high levels of ROS and A β , GLUT1 expression was significantly reduced, while GLUT3 showed no significant change. We further showed by NAC treatment and overexpression experiments that in vitro ROS blocked glucose metabolism by decreasing GLUT1 expression. Therefore, it possible that GLUT1 is vulnerable to ROS attack during the shuttling and transport of glucose across the cytoplasm and cytosol, and eventually glucose uptake and glucose metabolism were impaired.

To investigate the mechanism of ROS-induced GLUT1 deficiency, we examined PI3K/Akt pathway levels in N2a/APP695swe cells. Our data showed that ROS reduced GLUT1 expression by suppressing PI3K/Akt pathway activity resulting in impaired glucose metabolism. The latest review summarized the role of PI3K/Akt pathway in AD and supported our results [28]. The article pointed out that abnormalities of the

PI3K/Akt pathway, especially a decrease in Akt phosphorylation, prevented GLUT from transporting glucose into the cell and reduced ATP. Additional studies have also supported our results. Tahir Ali et al. investigated the antioxidant effects of anthocyanins in vivo and in vitro, and found that the reduction in p-PI3K/Akt/GSK3 β pathway levels was caused by ROS induced by amyloid β oligomers (A β O) [10]. ROS could affect energy metabolism via the PI3K/Akt pathway, particularly Akt, which is a metabolically critical sensor [29]. Therefore, we believed that maintaining PI3K/Akt signaling pathway levels was helpful in protecting neuronal cells from damage by AD risk factors.

A β is produced by sequential cleavage of amyloid precursor protein (APP) by β -secretase and γ -secretase [30]. ROS could activate γ -secretase to trigger A β production [31]. Under normal conditions, the production, degradation and clearance of A β are in a dynamic equilibrium. However, various abnormalities lead to impaired A β degradation or clearance, and total A β production increased, while A β generation did not increase [32][33][34]. It is suggested that A β deposition in the brain is mainly due to abnormal clearance. Oxidative stress could produce interference in the clearance of A β by oxidizing the low-density lipoprotein receptor-related protein 1 (LRP1), a key A β clearance transporter in the brain [35]. Interestingly, Winkler, E.A. et al. found that GLUT1 deficiency decreased A β clearance by reducing LRP1 expression in vivo, thereby accelerating A β deposition [36]. Here, we found that A β levels were negatively correlated with PI3K/Akt/GLUT1 pathway levels and ATP. Thus, in the present study, scavenging ROS to reduce A β was likely due to a certain degree of restored cellular energy supply and clearance of A β being enhanced. Meanwhile, clearance of A β required the PI3K/Akt/GLUT1 pathway to maintain ATP levels and that A β accumulation correlated with GLUT1 levels. The specific molecular mechanisms require further investigation. Our data suggested that GLUT1 could work as a therapeutic target for AD, maintaining neuronal glucose metabolism and promoting the clearance of A β .

In conclusion, the present study found that ROS reduced GLUT1 expression by inhibiting PI3K/Akt pathway activity, which results in impaired glucose metabolism in N2a/APP695swe cells. Our results suggest that the PI3K/Akt/GLUT1 pathway might play a bridge role between A β , ROS, and neurometabolic disorders in AD. In future studies, we will continue to explore the function of GLUT1 (including GLUT3) in transporting glucose and the mechanisms regulating its movement between plasma membranes.

Abbreviations

AD, Alzheimer's disease

A β , Amyloid-beta

ATP, Adenosine triphosphate

GLUT, Glucose transporter

NAC, N-acetyl-L-Cysteine

NFTs, Neurofibrillary tangles

N2a, Neuro-2a

WT, Wild type

p-Akt, Phosphorylated Akt

PI3K, Phosphoinositide 3-kinase

p-PI3K, Phosphorylated PI3K

ROS, Reactive oxygen species

Declarations

Statements & Declarations

Ethics Approval and Consent to Participate: Not applicable.

Consent for Publication: All the authors agreed to publish this review.

Conflict of Interest: The authors declare no competing interests.

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Author contributions

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Shijie Li, Jianing Fan, and Xiaoju Li: Conceptualization, revision.

Yu Li: Design, Conceptualization, revision.

Data Availability

All data generated or analysed during the current study are included in this published article.

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Figures

Figure 1

Increased A β and ROS levels in N2a/APP695swe cells

Figure 2

Decreased GLUT1 expression, the glucose uptake and ATP contents in N2a/APP695swe cells The N2a/WT and N2a/APP695swe cells were collected separately from three different culture flasks and the proteins were extracted for the Western blot analysis. (a + b) Measurement of intracellular glucose uptake and ATP contents. (c + d) Western blot analysis of GLUT1 and GLUT3 proteins. GLUT1 expression was decreased in the APP group and GLUT3 was not significantly changed. Numbers 1, 2, and 3 represent proteins extracted at three different dates. * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$.

Figure 3

GLUT1 enhanced the glucose uptake and ATP contents, reduced A β levels in N2a/APP695swe cells

Figure 4

ROS scavenger N-acetyl-L-Cysteine (NAC) increased GLUT1 expression, the glucose uptake and ATP contents, decreased A β levels in N2a/APP695swe cells

Figure 5

Phosphorylation of PI3K/Akt pathway decreased in N2a/APP695swe cells

Figure 6

NAC upregulated PI3K/Akt/GLUT1 pathway levels, increased glucose uptake and ATP contents, decreased A β levels

Figure 7

Activated PI3K/Akt/GLUT1 increased glucose uptake and ATP contents, decreased A β levels in N2a/APP695swe cells

Figure 8

Schematic: ROS reduces PI3K/Akt pathway phosphorylation levels leading to cellular GLUT1 deficiency and impaired glucose uptake, NAC improves this state