J Mol Neurosci (2013) 51:138–147
DOI 10.1007/s12031-013-9966-y

Hypoxia Increases Aβ-Induced Tau Phosphorylation by Calpain and Promotes Behavioral Consequences in AD Transgenic Mice

Lianbo Gao · Shen Tian · Honghua Gao · Yanyuan Xu

Received: 3 December 2012 / Accepted: 14 January 2013 / Published online: 24 January 2013

Abstract Chronic hypoxia has been reported to contribute to the development of Alzheimer's disease (AD). However, the mechanism of hypoxia in the pathogenesis of AD remains unclear. The purpose of this study was to investigate the effects of chronic hypoxia treatment on β-amyloid, tau pathologies, and the behavioral consequences in the double transgenic (APP/PS1) mice. Double transgenic mice (APP/PS1 mice) were treated with hypoxia, and spatial learning and memory abilities of mice were assessed in the Morris water maze. β-amyloid level and plaque level in APP/PS1 double transgenic mice were detected by immunohistochemistry. Protein tau, p35/p25, cyclin-dependent kinase 5 (CDK5), and calpain were detected by western blotting analysis. Chronic hypoxia treatment decreased memory and cognitive function in AD mice. In addition, chronic hypoxia treatment resulted in increased senile plaques, accompanying with increased tau phosphorylation. The hypoxia-induced increase in the tau phosphorylation was associated with a significant increase in the production of p35 and p25 and upregulation of calpain, suggesting that hypoxia induced aberrant CDK5/p25 activation via upregulation of calpain. Our results showed that chronic hypoxia exposure accelerates not only amyloid pathology but also tau pathology via calpain-mediated tau hyperphosphorylation in an AD mouse model. These pathological changes possibly contribute to the hypoxia-induced behavioral change in AD mice.

Keywords Alzheimer's disease · Hypoxia · Calpain · CDK5 · Tau pathology · Behavior

L. Gao (✉) · S. Tian · H. Gao · Y. Xu
Department of Neurology, The 4th Affiliated Hospital of China Medical University, Shenyang 110032, China
e-mail: gaolianbodoctor@126.com

Introduction

Alzheimer's disease (AD), a leading cause of dementia in aged people, is a chronic neurodegenerative disorder characterized by a progressive loss of memory and cognitive function. The pathological features of AD include extracellular neuritic plaques containing β-amyloid (Aβ) peptide and intracellular neurofibrillary tangles (NFTs) which are composed of hyperphosphorylated microtubule-associated protein tau. The major risk factors associated with the pathogenesis of AD, including age, gender, gene polymorphism, high cholesterol, diabetes mellitus, stroke, and brain trauma (Martins et al. 2006; Lahiri et al. 2007). Several studies have also shown that the incidence of AD is greatly increased by hypoxic injury after cerebral ischemia or stroke (Patrick et al. 1999; Bazan et al. 2002; Ballabh et al. 2004; Skoog and Gustafson 2006). Growing evidence has shown that hypoxia could alter the Aβ-induced tau phosphorylation (Cancino et al. 2009; Kitazawa et al. 2009; Manukhina et al. 2010).

Aβ-induced tau phosphorylation is mediated by the activation of various kinases, including glycogen synthase kinase 3 beta (GSK3β), mitogen-activated protein kinase, and cyclin-dependent kinase 5 (CDK5) (Patrick et al. 1999; Mandelkow et al. 1993; Alvarez et al. 2001; Koh et al. 2008). In AD, tau hyperphosphorylation appears to be dependent on the activity of the GSK3β and CDK5 kinases (Lee et al. 2000; Iqbal et al. 2005; Iqbal and Grundke-Iqbal 2006; Mazanetz and Fischer 2007; Engmann and Giese 2009). The activation of CDK5 could mediate the Aβ neurotoxicity. Multiple lines of evidence have also shown that Aβ accelerates and augments NFT formation in tau transgenic mouse models (Cancino et al. 2009; Kitazawa et al. 2009).

CDK5 activity depends on its activator p35, which can be cleaved to be p25 by calpain. Calpain is a Ca^{2+}-dependent cysteine protease that could be activated following Ca^{2+} influx. Aβ has been documented to trigger a cascade of

pathogenic events, such as calcium influx resulting from excitoactivation of glutamate/NMDA receptors and neuronal apoptosis (Bossy-Wetzel et al. 2004). There is considerable evidence that an increased activity of calpain associated with impaired calcium homeostasis may be involved in AD development (Higuchi et al. 2012).

A key feature of cerebral hypoxia caused by ischemia is the delayed neuronal death caused by the initial insult (Lipton 1999). Large increased calcium influx (Picconi et al. 2006; McManus et al. 2004; Monje et al. 2000) has been observed during hypoxia in many systems, which may trigger the calpain activation. Following longer hypoxia events, calpain activation was observed (Roberts-Lewis et al. 1994; Kim et al. 2007). In addition, calpain inhibitors provide neuroprotection for hypoxia both in vitro (Newcomb-Fernandez et al. 2001) and in vivo. Calpain activation is also observed in AD models (Higuchi et al. 2012), suggesting its potential role in hypoxia interference with AD development.

It has been tested that chronic hypoxia increases Aβ deposition in AD transgenic mouse model (Li et al. 2009). The effect of hypoxia on tau-related pathology and behavioral consequences based on them have not been investigated before. This study aimed to investigate the effects of chronic hypoxia exposure to APP/PS1 AD mice. Our results suggest that chronic hypoxia exposure accelerates not only amyloid pathology but also tau pathology in an AD mouse model, and these pathological changes result in decrease in the memory and cognitive function in AD mouse.

Materials and Methods

Transgenic Mice and Hypoxia Treatment

Double transgenic mice (APP/PS1 mice) obtained from the Jackson Laboratory (West Grove, PA, USA, Mo/HuAPP695swe+PS1-dE9) were kept in stainless steel cages in a controlled environment (22–25 °C, 50 % relative humidity, 12-h light–dark cycle), with food and water available. Six-month-old mice were assigned randomly to hypoxia and control groups (n=9 in each group). Each mouse was placed into a 125-ml jar with fresh air. For the hypoxia group, the jar was sealed with a rubber plug. The animal was removed from the jar immediately after the first gasping breath appeared. This hypoxia procedure was performed once a day and repeated for 60 days (Li et al. 2009). All animal experiments were performed in accordance with the care and use of medical laboratory animals and the guidelines of the laboratory animal ethical standards of China Medical University.

Morris Water Maze

Spatial learning and memory abilities of mice were assessed in the Morris water maze as described previously (Wang et al. 2010). Briefly, mice were given behavioral tests with a Morris water maze for eight consecutive days, including visible platform training, a navigation test, and probe trial. Mice were trained individually for 2 days (three trials with an interval of 30 min) to find the visible escape platform. From the third to seventh day, the platform was placed just below the water surface for the place navigation test, and each mouse was subjected to three trials per day at an interval of 1 min between trials. For each trial, the latency and the path length by which the mouse found the hidden platform were recorded. At the eighth day, the platform was removed from the water for the probe trial. The number of times that each mouse crossed the center of the quadrant (where the platform was previously located) at an interval of 1 min was recorded. Activity in the pool was visualized by an overhead camera and tracked using a computer program (ZH0065, Zhenghua Bio-equipments, People's Republic of China). Finally, data on the escape latency, escape latency were analyzed by repeated measures ANOVA with the effect of day and session. And the number of passing times in the normoxia and hypoxia mice were compared and analyzed statistically.

Open-Field Test and Tail Suspension Test

The apparatus consists of a rectangular box (40×50×63 cm) with a floor divided into 20 (10×10 cm) rectangular units (Morais et al. 2012). The animals were gently placed in the right corner of the open field and were allowed to freely explore the area for 5 min. The open field was cleaned with a 5 % water–ethanol solution before behavioral testing to eliminate possible bias due to odors left by previous mouse.

Mice were suspended on the edge of a table 58 cm above the floor by the adhesive tape placed approximately 2–3 cm from the tip of the tail. Immobility time was recorded during a 5-min period. Animals were considered to be immobile when they do not show any body movement and remain hanging passively (Shewale et al. 2012).

Tissue Preparation

After behavioral tests, mice were given an overdose of phenobarbital, and the blood was extracted from the heart for serum insulin measurement. Then, the mice were sacrificed by decapitation, and brains were rapidly removed. Paraffin-embedded sections (5 μm thick) from one brain hemisphere per mouse were prepared for immunohistochemistry. The hippocampus and cerebral cortex were

dissected from the other hemisphere and stored at −80 °C for western blotting analyses.

Immunohistochemistry

Paraffin sections were dewaxed in xylene and rehydrated through a series of decreasing concentrations of ethanol. Routine ABC method was used to detect the distribution of Aβ in the APP/PS1 transgenic mouse (Zheng et al. 2009). The immunostaining procedures were performed in accordance with the standard ABC method. Briefly, sections were rinsed in 0.1 M Tris-buffered saline (TBS, pH7.4), and endogenous peroxidases were quenched with 3 % hydrogen peroxide (H_2O_2) in pure methanol for 10 min. After three washes with TBS, sections were treated with 5 % bovine serum albumin and 3 % goat serum in TBS for 1 h to reduce nonspecific staining. Sections were then rinsed in TBS for 30 min and incubated overnight at 4 °C with mouse antibody Aβ1-42 against Aβ (diluted 1:500; Sigma, St. Louis, MO, USA). Following several rinses, sections were incubated with biotinylated goat anti-mouse IgG at 1:200 for 1 h at room temperature (RT). Sections were rinsed and incubated with ABC solution at RT for 1 h. and Sections were then rinsed in 0.1 M Tris buffer (pH7.6) and treated with 0.025 % 3,3′-diaminobenzidine in the presence of 0.033 % H_2O_2 at RT for 10 min. The sections were dehydrated and cover-slipped for light microscopy. DRG sections incubated without primary antibody were used as negative controls. The images were visualized and digitally photographed by light microscopy (Olympus; AX70U-Photo, Japan).

Plaques are defined as the areas covered by Aβ immunostained regions. To assess the plaque number and Aβ burden (the percentage of the total area of Aβ-positive areas compared with the total area) in the cortex and hippocampus in the APP/PS1 transgenic mouse, five sections from the same reference position were chosen from each mouse (n= 9). Plaque number and Aβ burden of the cortex and hippocampus were quantified. Data were measured by Image-Pro Plus software.

Western Blotting Analysis

Brain tissue fragments were minced into small pieces and homogenized in prechilled lysis buffer (150 mM NaCl, 50 mM Tris–HCl, 1 % Nonidet P-40, 0.25 % sodium deoxycholate, 0.1 % SDS, 1 mM PMSF, 10 mg/ml leupeptin, 1 mM Na_3VO_4, and 1 mM NaF) overnight at 4 °C. The homogenates were collected, centrifuged at 12,000 rpm for 30 min, and quantified for total proteins using the UV-1700 PharmaSpec ultraviolet spectrophotometer (Shimadzu, Kyoto, Japan). The supernatant was removed, aliquoted, and stored at −80 °C. Sixty micrograms total protein of each sample was subjected to SDS-PAGE using 10 % gradient Tris/glycine gels. Next, proteins were transferred to polyvinylidene difluoride membranes (Millipore, Temecula, CA, USA). After blocking in 5 % fat-free milk for 1 h, blots were incubated with the following primary antibodies: mouse anti-tau-pThr231 (1:500, Invitrogen), rabbit anti-tau-pThr205 (1:1,000, Abcam), rabbit anti-tau-pSer396 (1:1,000, Abcam), rabbit anti-tau (1:400, Abcam), rabbit anti-CDK5 (1:5,000, Abcam), rabbit anti-CDK5-pTyr15 (1:1,000, Abcam), rabbit anti-p35/25 (1:200, Cell Signaling Technology), mouse anti-calpain 1 (1:2,000, Abcam), mouse anti-Aβ1-42 (1:1,000, Abcam), and anti-GAPDH (1:10,000, KC-5 G5; Kang Chen, Shanghai, China) at 4 °C overnight. The membranes were washed and incubated with horseradish peroxidase-conjugated second antibody (1:5,000; Santa Cruz, CA, USA) for 2 h at RT. Immunoreactive bands were visualized by the SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Rockford, IL, USA) using ChemiDoc XRS with Quantity One software (Bio-Rad, Hercules, CA, USA).

Statistics

All values are expressed as means±SD. Student's t test was used for evaluation of differences between two groups. Repeated measures ANOVA tests with Tukey's post hoc analysis were used to compare differences in Morris water maze. Differences were considered statistically significant for values of $p<0.05$.

Results

Decreased Learning and Memory Ability of AD Mice Under Chronic Hypoxia Treatment

We first investigated the effects of chronic hypoxia on learning and memory in APP/PS1 mice, using Morris Water Maze tests. In the place navigation (hidden platform) tests (from the third to fifth days), there was no significant difference in the escape latencies between the two groups, though mice in the hypoxia group spent more time searching for the hidden platform than controls (Fig. 1a). In the probe trial on the last day of testing, the mice in the hypoxia group passed over the original platform location significantly fewer times than the controls ($p<0.05$, Fig. 1b), suggesting that the hypoxia mice showed significant cognitive deficits in spatial learning and memory function.

Chronic Hypoxia Does Not Affect Anxiety-Like Behaviors in AD Mice

Chronic hypoxia did not affect anxiety-like behaviors in the open-field test. There was no significant difference in the

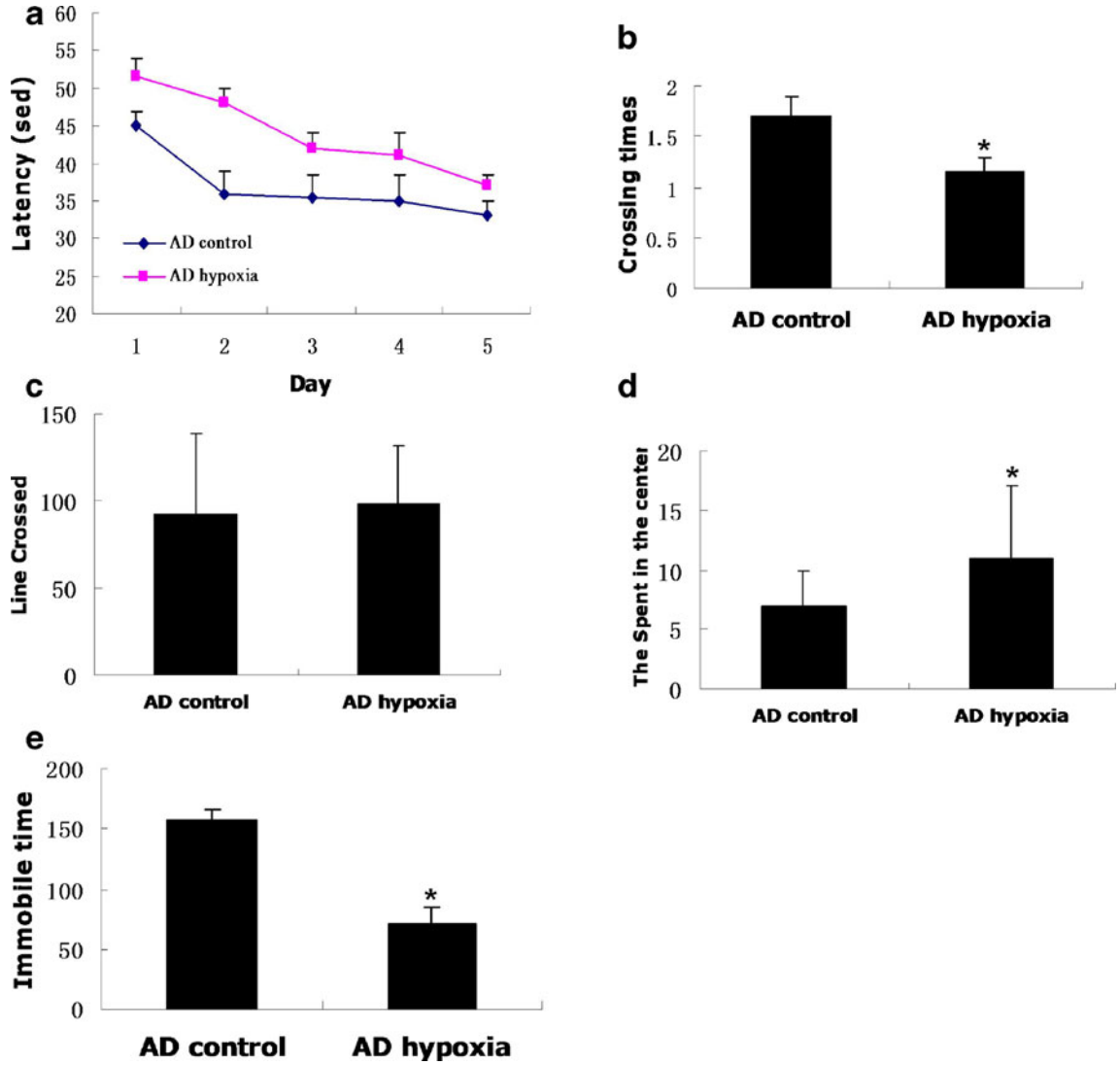

Fig. 1 Two-month hypoxia treatment exacerbates exacerbated cognitive deficits. **a** Morris water maze revealed that in hidden platform tests, the hypoxia group APP/PS1 mice spent more time searching for the hidden platform than the vehicle controls. **b** In the probe trial on the last day of testing, the hypoxia group mice passed the former platform location significantly fewer times than the controls. **c** No significant difference in the number of crossings between the groups. **d** No significant difference in the time spent in the center was found between the groups. **e** Chronic hypoxia treatment significantly increased the immobile time in tail suspension test as compared to the control. *$p<0.05$ by Student's *t* test

number of crossings between the groups ($p>0.05$, Fig. 1c), suggesting that chronic hypoxia did not affect locomotion or activity in AD mice. No significant difference in the time spent in the center was found between the groups ($p>0.05$, Fig. 1d).

Increasing Despair Behaviors of AD Mice Under Chronic Hypoxia Treatment

We also assessed the effects of chronic hypoxia treatment on despair behaviors of AD mice. Chronic hypoxia treatment significantly increased the immobile time in tail suspension test as compared with the control group ($p<0.01$, Fig. 1e).

Chronic Hypoxia Increases Aβ Level and Amyloid Plaque Level in AD Transgenic Mice

We further investigated the effects of hypoxia treatment on plaque level in APP/PS1 double transgenic mice. Neurotic plaque level was significantly increased in the hypoxia AD mice as compared to the controls (Fig. 2). Quantification showed that hypoxia-treated mice had significantly more plaques per field (0.1 mm^2) relative to normoxic controls ($n=9$ each group, Fig. 2). In addition, the plaque area per field in hypoxic mice was also significantly increased ($p<0.01$, $n=9$ each group) as compared to controls.

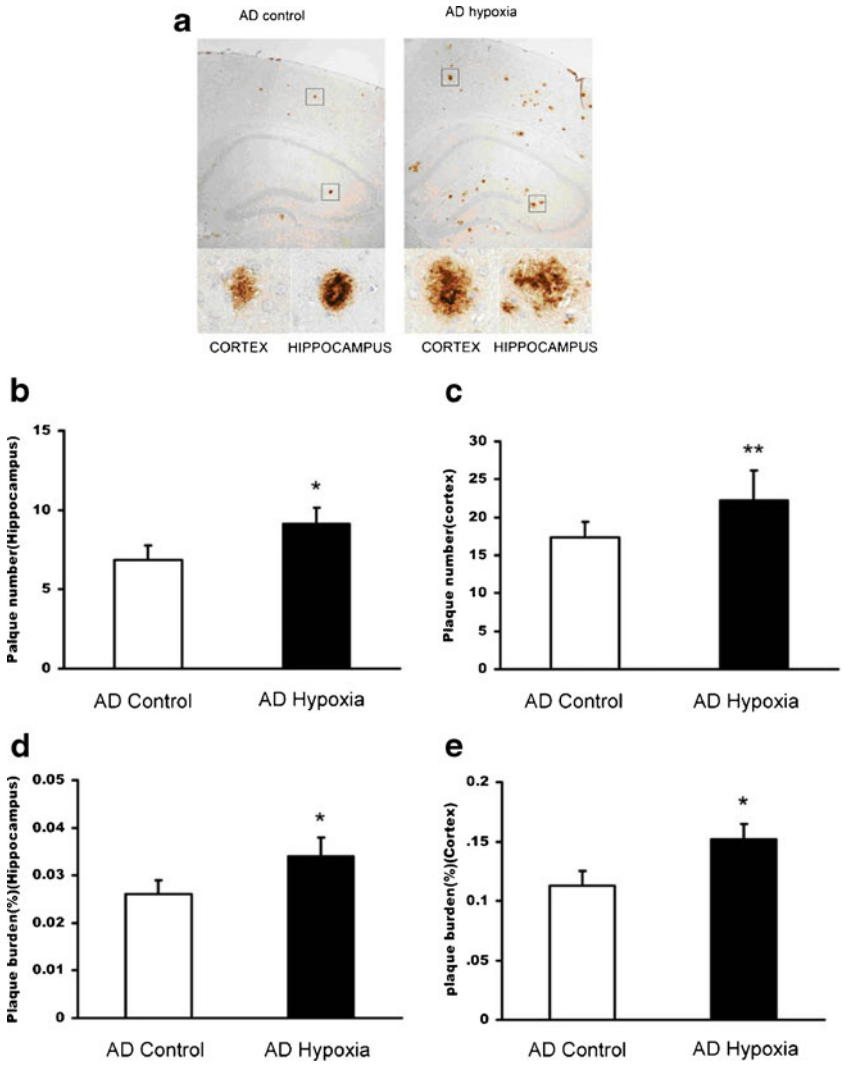

Fig. 2 Two-month hypoxia treatment increased senile plaque level in AD transgenic mice. **a** Immunostaining showed more and larger senile plaques appeared in the hypoxia group mice compared with the control group. *Boxes* indicate the plaques. Quantification revealed that both the number (**b**, **c**) and burden (**d**, **e**) of plaques were increased in the hypoxia group (n=9 in each group). **p<0.01 and *p<0.05 by Student's t test

Furthermore, we also detected the effects of hypoxia treatment on Aβ level in APP/PS1 double transgenic mice. The results showed that the level of Aβ increased significantly both in cortex and hippocampus compared with the AD control (Fig. 3) (p<0.01, n=9 each group). All of these data indicate that repeated hypoxia can increase neuritic plaque level and Aβ level in APP/PS1 double transgenic mice.

Chronic Hypoxia Increases Aβ-Induced Tau Phosphorylation in AD Transgenic Mice

We next examined whether hypoxia-induced Aβ changes resulted in tau hyperphosphorylation using western blotting with the phosphorylated tau antibody (Fig. 4). The phosphorylation of tau at Thr231, Thr205, and Ser396 was significantly increased in the hypoxia mice as compared to the controls (Fig. 4). There was no significant difference in the steady-state levels of total tau between the controls and the hypoxic mice (Fig. 4e). Furthermore, we also examined the Aβ-induced tau phosphorylation in the normal nontransgenic mice (normal hypoxia) treated with hypoxia; compared with the normal hypoxia group, the transgenic mice in the AD hypoxia group (amyloid formation) could upregulate the tau phosphorylation. So we conclude that the tau phosphorylation is amyloid dependent (Fig. 5). These results suggested that chronic hypoxia treatment resulted in tau hyperphosphorylation dependently (Figs. 4 and 5).

Hypoxia Treatment Results in Upregulation of Calpain and Aberrant CDK5/p25 Activation

We then examined whether hypoxia-induced tau hyperphosphorylation is associated with aberrant activations of CDK5, a major kinase associated with abnormal tau phosphorylation in the brain. The activation of CDK5 was detected by measuring the protein levels of phosphorylated CDK5, CDK5 activators p35, and its truncated form p25. As shown in Fig. 6, a significant increase in the phosphorylated CDK5 was found in the hypoxia group as compared with that in the

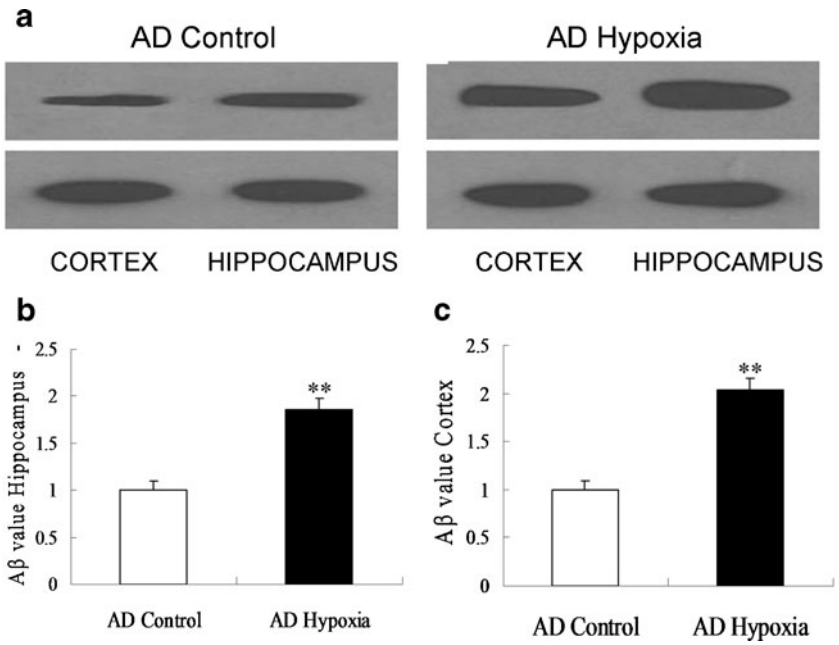

Fig. 3 Aβ level was upregulated in AD transgenic mice after a 2-month hypoxia treatment. **a** Western blotting of Aβ1-42 for both cortex and hippocampus in two groups. **b** Quantification showed that the Aβ level in the AD hypoxia group increased significantly in both cortex and hippocampus compared with the AD control group (n=9 in each group). **p<0.01 by Student's t test

controls (p<0.05, Fig. 6a, c). No significant differences were detected in the total CDK5 protein levels between the two groups (Fig. 6a, b).

The hypoxia-induced increase in the phosphorylated CDK5 was accompanied by a significant increase in the production of p35 and p25 (Fig. 6a, d, e). p25 production though p35 cleavage is shown in Fig. 6f, in which the values represent the percentage of p25 over total (p35+p25). Since calpain mediates the generation of p25 through the proteolytic cleavage of p35, we further determined whether brain calpain protein level was increased in the hypoxia APP/PS1 mice. There was a significant increase in the total calpain 1 protein level in the hypoxia mice compared with the control mice (p<0.01, Fig. 7).

Discussion

Hypoxia, a common consequence resulting from stroke, hypertension, atherosclerosis, and diabetes, is one of the major risk factors for AD (Bazan et al. 2002; Borenstein et al. 2005). Prolonged or severe hypoxia can cause neuronal loss and

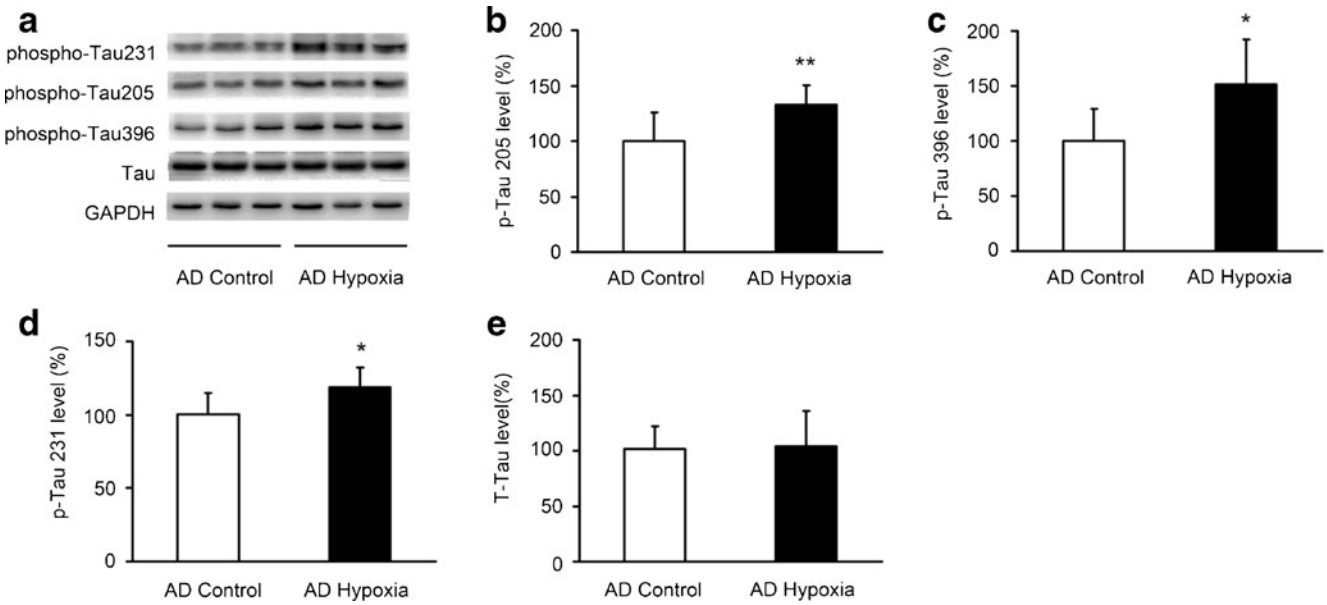

Fig. 4 Two-month hypoxia treatment exacerbates tau pathology induced by Aβ. **a** Western blotting of tau phosphorylation. Quantification revealed that the sites of Ser396, Ser205, and Thr231 (**b**–**e**) of tau phosphorylation were increased in the hypoxia group (n=9 in each group). **p<0.01 and *p<0.05 by Student's t test

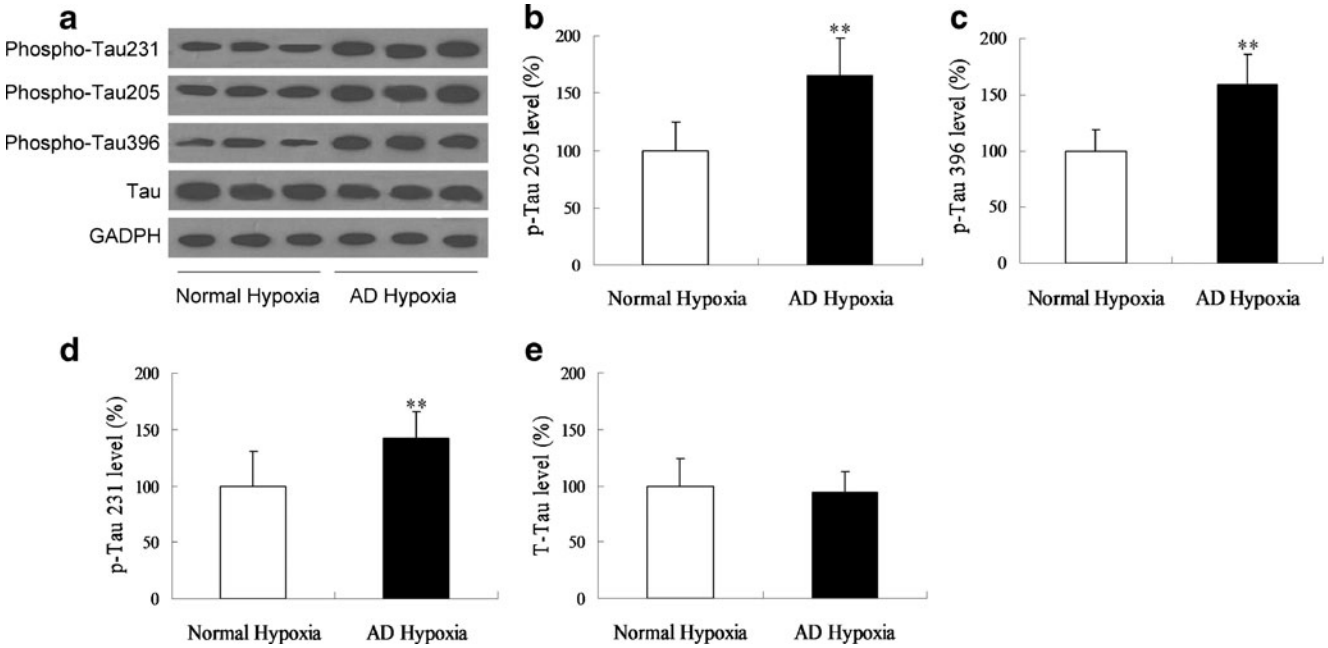

Fig. 5 Tau phosphorylation could not be upregulated in the normal nontransgenic mice (normal hypoxia) treated with hypoxia. **a** Western blotting of tau and tau phosphorylation. Quantification revealed that the sites of Ser396, Ser205 and Thr231 (**b**–**e**) of tau phosphorylation and total tau were increased in the hypoxia group (n=9 in each group). **p<0.01 by Student's t test

memory impairment (Adhami et al. 2006). There is growing evidence suggesting that hypoxia can alter the Aβ-induced tau phosphorylation and promote behavioral consequences in AD transgenic mouse (Cancino et al. 2009; Kitazawa et al. 2009; Manukhina et al. 2010). However, the mechanism of hypoxia remains unknown. The behavioral effect chronic hypoxia brought to the animal is still unclear.

Our present study provides additional evidence showing that chronic hypoxia exposure may be a risk factor for AD. In the APP/PS1 mouse model, chronic hypoxia exposure not

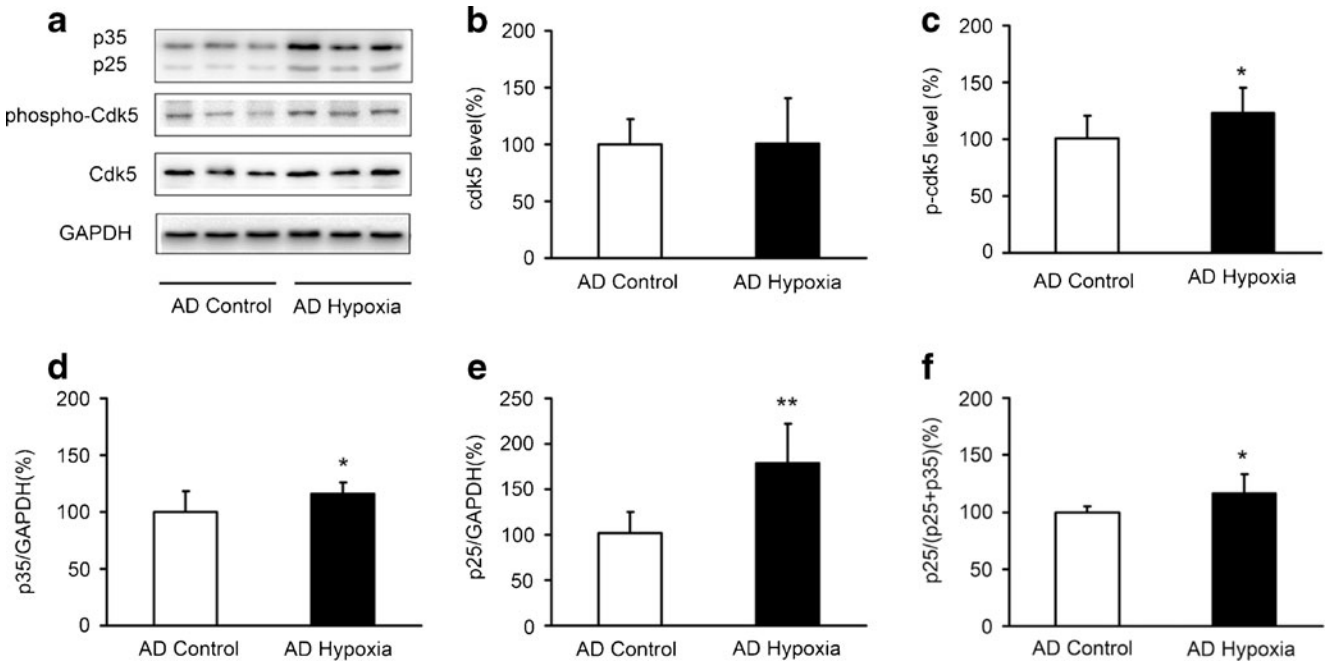

Fig. 6 Two-month hypoxia treatment exacerbates exacerbated Aβ-induced CDK phosphorylation. **a** Western blotting of P-CDK5, p25, p35, and total CDK5. Quantification showed that total CDK5 has not elevated significantly (**b**). P-CDK5 was increased in the hypoxia group (**c**). Both p25 and p35 increased in the hypoxia group (**e**, **f**), and p25 over p25+p35 was also significantly increased (n=9 in each group). **p<0.01 and * p<0.05 by Student's t test

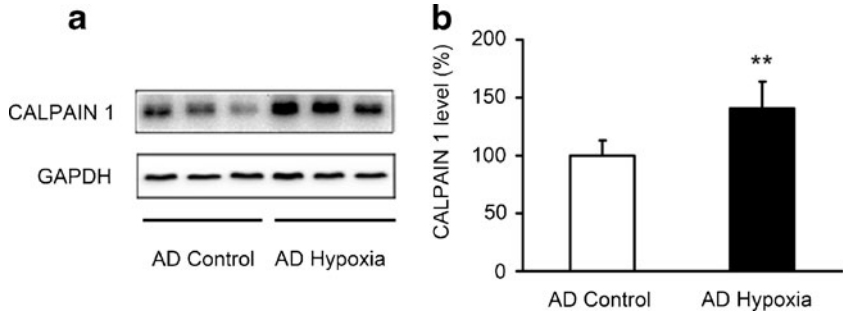

Fig. 7 Two-month hypoxia treatment increases calpain levels. **a** Western blotting of the calpain level. **b** Quantification revealed that calpain level was elevated after hypoxia treatment (n=9 in each group). **p<0.01 by Student's t test

only increases Aβ level but also triggers pathological tau phosphorylation and tangle formation in the brain. We demonstrate that the exacerbation of tau pathology correlates with the increased formation of p25 and subsequent aberrant activation of CDK5/p25. However, it is not well understood how hypoxia triggers these pathological changes in the brain.

NFTs, consist of intracellular hyperphosphorylated tau, are one of the pathological hallmarks of AD (Lee et al. 1991). Phosphorylated tau protein can contribute to the microtubule destabilization, impaired axonal transport, and neuronal death (Busciglio et al. 1995). There is growing evidence that the augmentation of the amount of phosphorylated tau at disease-relevant sites is triggered by Aβ (Cancino et al. 2009; Busciglio et al. 1995; Masliah et al. 2001; Chen et al. 2008). The increased phosphorylation of tau protein induced by Aβ peptide may be the cause of neurotoxicity in cultured neurons. This is supported by the evidence that the inhibition of tau phosphorylation by blocking tau kinases prevents Aβ-induced cell death and that neurons cultured from tau-depleted mice are resistant to Aβ toxicity (Koh et al. 2008; Lopes et al. 2010). These findings indicate that the tau hyperphosphorylation may be the reason for Aβ-induced neurotoxicity. In our study, we observed that chronic hypoxia increased the level of tau phosphorylation at all the detected sites (Ser396, Ser205, and Thr-231), which suggested that chronic hypoxia may aggravate neurotoxicity in AD progress by enhancement of tau pathology.

Calpain is a Ca^{2+}-dependent cysteine protease which is processed into its active configuration following Ca^{2+} influx (Lee et al. 2000). Increasing evidence suggests that Aβ-mediated alternation of Ca^{2+} homeostasis resulted to subsequent calpain activation (Patrick et al. 1999; Grammer et al. 2008; Chaudhary et al. 2012). In vitro study revealed that 1 h after incubation with Aβ, cortical neuron showed a disturbance of Ca^{2+} homeostasis. Aβ peptides promote an imbalance in intracellular calcium levels, both due to Ca^{2+} influx via voltage-sensitive channels and through its release from endoplasmic reticulum. Activated calpains cleave the normal regulatory subunit p35 to p25, thus forming a p25/CDK5 complex with an activity profile substantially higher than when p35 is associated with the kinase.

In our study, there were upregulations of both p25 and p35 in the hypoxia group. Lee et al. (2000) found that adding the calcium in the fresh brain lysates, as well as application of the amyloid β in primary cortical, could stimulate cleavage of p35 to p25. In this study, the increased level of Aβ triggered the conversion of p35 to p25, which is consistent to the former study (Lee et al. 2000). So the increase of the p25 may be caused by the cleavage of the p35. Increasing evidence suggests that p25 was upregulated in AD brains, especially that calpain-mediated protein degradation can be activated in postmortem brains. Parallel to these findings, our data also suggest that there is an upregulation of p25 after chronic hypoxia in an AD mouse model; however, it is not specified to p25 since both p25 and p35 were upregulated with chronic hypoxia treatment. This was also observed in another study (Sato et al. 2008) using AD transgenic mice model. Sato et al. (2008) also found that the subcellular localization of the p25/CDK5 complex is also altered, changing substrate specificity and leading to the hyperphosphorylation of substrates not normally phosphorylated by this kinase, like the cytoskeleton protein tau. Similarly, we observed elevated calpain protein level and an increased cleave from p35 to p25.

We demonstrated that chronic hypoxia in APP/PS1 transgenic mice exacerbated cognitive deficits, characterized by excessive Aβ accumulation and subsequent formation of senile plaques. Increases of Aβ accumulation aggravate neuronal apoptosis which may be the cause for cognitive deficits. And there is also evidence that cognitive deficits are related to imbalance of Ca^{2+} homeostasis which is essential in synaptic plasticity (Toescu and Verkhratsky 2004). In our study, we confirmed that under chronic hypoxia, there were exacerbated cognitive deficits along with Aβ accumulation and tau phosphorylation in AD mice, which suggests the crucial role of hypoxia-induced Aβ changes.

In conclusion, our results showed that chronic hypoxia exposure accelerates not only amyloid pathology but also tau pathology in an AD mouse model, and these pathological changes resulted in decrease in memory and cognitive function. These findings may help better understand the molecular mechanisms underlying the hypoxia-mediated AD pathogenesis. Thus, hypoxia should be considered as a

significant risk factor for AD, and improving cerebral oxygen supply may provide benefits to this devastating disease.

References

Adhami F, Liao G, Morozov YM, Schloemer A, Schmithorst VJ, Lorenz JN, Dunn RS, Vorhees CV, Wills-Karp M, Degen JL, Davis RJ, Mizushima N, Rakic P, Dardzinski BJ, Holland SK, Sharp FR, Kuan CY (2006) Cerebral ischemia-hypoxia induces intravascular coagulation and autophagy. Am J Pathol 169 (2):566–583

Alvarez A, Munoz JP, Maccioni RB (2001) A CDK5-p35 stable complex is involved in the beta-amyloid-induced deregulation of CDK5 activity in hippocampal neurons. Exp Cell Res 264 (2):266–274

Ballabh P, Braun A, Nedergaard M (2004) The blood–brain barrier: an overview: structure, regulation, and clinical implications. Neurobiol Dis 16(1):1–13

Bazan NG, Palacios-Pelaez R, Lukiw WJ (2002) Hypoxia signaling to genes: significance in Alzheimer's disease. Mol Neurobiol 26(2–3):283–298

Borenstein AR, Wu Y, Mortimer JA, Schellenberg GD, McCormick WC, Bowen JD, McCurry S, Larson EB (2005) Developmental and vascular risk factors for Alzheimer's disease. Neurobiol Aging 26(3):325–334

Bossy-Wetzel E, Schwarzenbacher R, Lipton SA (2004) Molecular pathways to neurodegeneration. Nat Med 10(Suppl):S2–S9

Busciglio J, Lorenzo A, Yankner BA (1995) Beta-myloid fibrils induce tau phosphorylation and loss of microtubule binding. Neuron 14 (4):879–888

Cancino GI, Perez de Arce K, Castro PU, Toledo EM, von Bernhardi R, Alvarez AR (2009) c-Abl tyrosine kinase modulates tau pathology and CDK5 phosphorylation in AD transgenic mice. Neurobiol Aging 32(7):1249–1261

Chaudhary P, Suryakumar G, Prasad R, Singh SN, Ali S, Liavazhagan G (2012) Chronic hypobaric hypoxia mediated skeletal muscle atrophy: role of ubiquitin-proteasome pathway and calpains. Mol Cell Biochem 364(1–2):101–113

Chen X, Huang T, Zhang J, Song J, Chen L, Zhu Y (2008) Involvement of calpain and p25 of CDK5 pathway in ginsenoside Rb1's attenuation of beta-amyloid peptide25-35-induced tau hyperphosphorylation in cortical neurons. Brain Res 1200:99–106

Engmann O, Giese KP (2009) Crosstalk between CDK5 and GSK3beta: implications for Alzheimer's disease. Front Mol Neurosci 2:2

Grammer M, Li D, Arunthavasothy N, Lipski J (2008) Contribution of calpain activation to early stages of hippocampal damage during oxygen–glucose deprivation. Brain Res 1196:121–130

Higuchi M, Iwata N, Matsuba Y, Takano J, Suemoto T, Maeda J, Ji B, Ono M, Staufenbiel M, Suhara T, Saido TC (2012) Mechanistic involvement of the calpain-calpastatin system in Alzheimer neuropathology. FASEB J 26(3):1204–1217

Iqbal K, Alonso AC, Chen S, Chohan MO, EI-Akkad E, Gong CX, Khatoon S, Li B, Liu F, Rahman A, Tanimukai H, Grundke-Igbal I (2005) Tau pathology in Alzheimer disease and other tauopathies. Biochim Biophys Acta 1739(2–3):198–210

Iqbal K, Grundke-Iqbal I (2006) Discoveries of tau, abnormally hyperphosphorylated tau and others of neurofibrillary degeneration: a personal historical perspective. J Alzheimers Dis 9(3 Suppl):219–242

Kim MJ, Oh SJ, Park SH, Kang HJ, Won MH, Kang TC, Hwang IK, Park JB, Kim JI, Kim J, Lee JY (2007) Hypoxia-induced cell death of HepG2 cells involves a necrotic cell death mediated by calpain. Apoptosis 12(4):707–718

Kitazawa M, Cheng D, Laferla FM (2009) Chronic copper exposure exacerbates both amyloid and tau pathology and selectively dysregulates CDK5 in a mouse model of AD. J Neurochem 108 (6):1550–1560

Koh SH, Noh MY, Kim SH (2008) Amyloid-beta-induced neurotoxicity is reduced by inhibition of glycogen synthase kinase-3. Brain Res 1188:254–262

Lahiri DK, Maloney B, Basha MR, Ge YW, Zawia NH (2007) How and when environmental agents and dietary factors affect the course of Alzheimer's disease: the "LEARn" model (latent early-life associated regulation) may explain the triggering of AD. Curr Alzheimer Res 4(2):219–228

Lee VM, Balin BJ, Otvos L, Trojanowski JQ (1991) A68: a major subunit of paired helical filaments and derivatized forms of normal tau. Science 251(4994):675–678

Lee MS, Kwon YT, Li M, Peng J, Friedlander RM, Tsai LH (2000) Neurotoxicity induces cleavage of p35 to p25 by calpain. Nature 405(6784):360–364

Li L, Zhang X, Yang D, Luo G, Chen S, Le W (2009) Hypoxia increases Abeta generation by altering beta- and gamma-cleavage of APP. Neurobiol Aging 30(7):1091–1098

Lipton P (1999) Ischemic cell death in brain neurons. Physiol Rev 79 (4):1431–1568

Lopes JP, Oliveira CR, Agostinho P (2010) Neurodegeneration in an Abeta-induced model of Alzheimer's disease: the role of CDK5. Aging Cell 9(1):64–77

Mandelkow EM, Biernat J, Drewes G, Steiner B, Lichtenberg-Kraag B, White H, Gustke N, Mandelkow E (1993) Microtubule-associated protein tau, paired helical filaments, and phosphorylation. Ann N Y Acad Sci 695:209–216

Manukhina EB, Goryacheva AV, Barskov IV, Viktorov IV, Guseva AA, Pshennikova MG, Khomenko IP, Mashina SY, Pokidyshev DA, Malyshev IY (2010) Prevention of neurodegenerative damage to the brain in rats in experimental Alzheimer's disease by adaptation to hypoxia. Neurosci Behav Physiol 40(7):737–743

Martins IJ, Hone E, Foster JK, Gnjec A, Fuller SJ, Nolan D, Gandy SE (2006) Apolipoprotein E, cholesterol metabolism, diabetes, and the convergence of risk factors for Alzheimer's disease and cardiovascular disease. Mol Psychiatry 11(8):721–736

Masliah E, Sisk A, Mallory M, Games D (2001) Neurofibrillary pathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. J Neuropathol Exp Neurol 60(4):357–368

Mazanetz MP, Fischer PM (2007) Untangling tau hyperphosphorylation in drug design for neurodegenerative diseases. Nat Rev Drug Discov 6(6):464–479

McManus T, Sadqrove M, Pringle AK, Chad JE, Sundstrom LE (2004) Intraischaemic hypothermia reduces free radical production and protects against ischaemic insults in cultured hippocampal slices. J Neurochem 91(2):327–336

Monje ML, Chatten-Brown J, Hye SE, Raley-Suman KM (2000) Free radicals are involved in the damage to protein synthesis after anoxia/aglycemia and NMDA exposure. Brain Res 857(1–2):172–182

Morais LH, Lima MMS, Martynhak BJ, Santiago R, Takahashi TT, Ariza D, Barbiero JK, Andreatini R, Vital MABF (2012) Characterization of motor, depressive-like and neurochemical alterations induced by a short-term rotenone administration. Pharmacol Rep 64:1081–1090

Newcomb-Fernandez JK, Zhao X, Pike BR, Wang KK, Kampfl A, Beer R, DeFord SM, Hayes RL (2001) Concurrent assessment of calpain and caspase-3 activation after oxygen-glucose deprivation in primary septo-hippocampal cultures. J Cereb Blood Flow Metab 21(11):1281–1294

Patrick GN, Zukerberg L, Nikolic M, de la Monte S, Dikkes P, Tsai LH (1999) Conversion of p35 to p25 deregulates CDK5 activity and promotes neurodegeneration. Nature 402(6762):615–622

Picconi B, Tortiglione A, Barone I, Centonze D, Gardoni F, Gubellini P, Bonsi P, Pisani A, Bernardi G, Di Luca M, Calabresi P (2006) NR2B subunit exerts a critical role in postischemic synaptic plasticity. Stroke 37(7):1895–1901

Roberts-Lewis JM, Savage MJ, Marcy VR, Pinsker LR, Siman R (1994) Immunolocalization of calpain I-mediated spectrin degradation to vulnerable neurons in the ischemic gerbil brain. J Neurosci 14(6):3934–3944

Sato S, Xu J, Okuyama S, Martinez LB, Walsh SM, Jacobsen MT, Swan RJ, Schlautman JD, Ciborowski P, Ikezu T (2008) Spatial learning impairment, enhanced CDK5/p35 activity, and down-regulation of NMDA receptor expression in transgenic mice expressing tau-tubulin kinase 1. J Neurosci 28(53):14511–14521

Shewale PB, Patil RA, Hiray YA (2012) Antidepressant-like activity of anthocyanidins from *Hibscus rosa-sinensis* flowers in tail suspension test and forced swim test. Indian J Pharmacol 44(4):454–457

Skoog I, Gustafson D (2006) Update on hypertension and Alzheimer's disease. Neurol Res 28(6):605–611

Toescu E, Verkhratsky A (2004) Ca^{2+} and mitochondria as substrates for deficits in synaptic plasticity in normal brain ageing. J Cell Mol Med 8(2):181–190

Wang X, Zheng W, Xei JW, Wang T, Wang SL, Teng WP, Wang ZY (2010) Insulin deficiency exacerbates cerebral amyloidosis and behavioral deficits in an Alzheimer transgenic mouse model. Mol Neurodegener 5:46

Zheng W, Xin N, Chi ZH, Zhao BL, Zhang J, Li JY, Wang ZY (2009) Divalent metal transporter 1 is involved in amyloid precursor protein processing and Abeta generation. FASEB J 23(12):4207–4217