PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2011, 38(10): 908~918
www.pibb.ac.cn

Intrahippocampus Injection of Antibodies to Amyloid β-Protein Precursor Causes Cognitive Deficits and Neuronal Degeneration*

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Abstract In order to evaluate whether APP-Ab injection to hippocampus influence Morris water maze behavior and neuronal degeneration and to further investigate the potential mechanisms, rats were anaesthetized and fixed on a stereotaxic instrument and bilateral injection 1 μl of anti-APP antibody (10 g/L) was made using microsyringe. Meanwhile, NS or IgG-intrahippocampal-injected (1 μl; 10 g/L) rats served as vehicle controls. Antibodies were injected into the hippocampus (AP: -3.0; L and R: 2.0; V: 3.5 mm). The Morris water maze test was performed to test animals' learning and memory ability. After APP-Ab injection, cresyl violet and Fluoro-Jade B staining were used to investigate neuronal degeneration. Immunohistochemistry staining was used to detect MAP-2 and phosphorylated paxillin and tau distribution at hippocampus. APP-Ab injection to hippocampus could prolong the escape latency to find hidden platform and decreased the exploratory time and crossing numbers in the training quadrant. APP-Ab injection was also shown to cause neuronal cell death and degeneration by cresyl violet (CV) staining and Fluoro Jade-B (FJB) staining. Moreover, decreased MAP2 immunoreactivity, increased phosphorylated paxillin and phosphorylated tau immunostaining were observed in the pyramidal cells. It can be concluded that intrahippocampus injection of APP-Ab could induce cognitive deficits and neurodegenerative changes. APP-Ab injection also affected the distribution of MAP2, paxillin and tau protein.

Key words amyloid precursor protein, Morris water maze,neuronal degeneration, hippocampus, paxillin, tau
DOI: 10.3724/SP.J.1206.2011.00024

Alzheimer′s disease (AD) is a progressive neurodegenerative disorder usually characterized by intraneuronal neurofibrillary tangles and extracellular senile plaques. This disease is characterized by irreversible memory impairment, continuous cognitive decline, and behavioral disturbances [1]. β-Amyloid precursor protein (APP) is a transmembrane protein, which is considered to be implicated in certain physiological functions in neurons, such as neurite outgrowth, neuronal survival, and cell-cell adhesion[2-4]. In brain APP are found in neurons where they can undergo fast anterograde axonal transport[5-6] and they are present at synaptic sites and in vesicular structures in the cell body, axon and dendrites[7]. Although these findings suggest that APP functions in the formation and maintenance of synapses, its precise role in longer term memory formation has not been fully elucidated. Despite the physiological role of APP, accumulating lines of evidence suggested an intrinsic central role of APP in the pathogenesis of AD induced by Aβ and have suggested that this disease may be a consequence of a disruption of APP normal function. APP knockout mice develop behavioral and cognitive impairment[8-9]. Moreover, mice expressing mutant APP exhibit age-dependent onset of spatial learning deficits [10]. Perturbation of APP function by intraventricular administration of antibodies targeted against various APP isoforms differentially or antisense oligonucleotides differentially impair behavior and memory in rats[11-13]. These data suggest that APP may be involved in

*This work was supported by grants from The National Natural Science Foundation of China (81071017, 90919004) and the Shanghai Foundation for Development of Science and Technolgy, China (10DZ1976000).

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Received: March 31, 2011 Accepted: June 16, 2011

learning and memory processes. However, the role of this protein *in vivo* in learning and memory has been comparatively little studied.

It has been demonstrated that APP possesses a cytoplasmic Go-stimulating domain that associates with GTP-binding proteins, supporting the idea that APP may act as a cell surface receptor [14–16]. Because the nature of the endogenous ligand that binds APP remains unknown, several groups have studied the effects of an external application of antibodies (APP-Ab, which directed against extracellular domains of APP) on cellular responses[17–20]. They found that the activation of APP by APP-Ab triggers a massive death of cortical neurons in culture, which is preceded by neurite degeneration and nuclear condensation. Also, Zhang *et al.* [21] have found that GM1 could bind the N-terminus of APP and induced a conformational change. These results prompt that conformational change of APP on cell surface may influence its functions. However, to date, the molecular details of these events *in vivo* are not well characterized, and the underlying mechanism remains to be determined. Based on these reports and our previous observations that focal adhesion proteins participated in APP-Ab-induced cell injuries, the aims of the present study are to evaluate whether APP antibody injection to hippocampus influence Morris water maze behavior and to further investigate to what extent MAP2, focal adhesion molecule phosphorylated paxillin and phosphorylated tau changes occur in the rat brain. In addition, we wanted to determine if APP-Ab injection could induce neuronal degeneration.

We demonstrated that APP-Ab injection to hippocampus could prolong the escape latency to find hidden platform and decreased the exploratory time and crossing numbers in the training quadrant where the platform was previously located. APP-Ab injection also caused neuronal cell death and degeneration of pyramidal cells in the CA1 region by cresyl violet (CV) staining and Fluoro Jade-B (FJB) staining. In summary, our studies provide evidence that APP mediated neuronal death and suggest a novel role of APP in learning and memory dysfunction.

1 Materials and methods

1.1 Subjects and antibodies injections

Adult(2～3 months old) male Sprague-Dawley rats ((250 ± 20) g, Shanghai Laboratory Animal Center, China) and APPswe/PS1ΔE9 transgenic mice (were obtained from Key Laboratory of Molecular Medicine, Fudan University, China) were housed in groups of four animals per cage and given food and water ad libitum. The animals were cared complied with the Provisions and General Recommendation of the Chinese Experimental Animals Administration Legislation and were approved by the Science and Technology Department of Shanghai Province. Rats were randomly divided into 3 groups as follows: normal saline injection group (NS; n =10), control rabbit IgG injection group (IgG; n=10) and anti-APP antibody injection group (APP-Ab; n=10). Rats were anaesthetized with 10% chloral hydrate (3.6 ml/kg) and were fixed on a stereotaxic instrument. Bilateral injection 1 μl of anti-APP antibody (10 g/L; affinity purified rabbit polyclonal antibody against the N-terminal region amino acid 46 ～60 of human APP_{695}; Sigma) was made using microsyringe. Meanwhile, NS or IgG-intrahippocampal-injected(1 μl; 10 g/L) rats served as vehicle controls. Antibodies were injected into the hippocampus (AP: -3.0; L and R: 2.0; V: 3.5 mm) or cortex of APP/PS1 transgenic mice, as described previously with some modifications [22]. The needle was kept in the injection site for another 10 min and then slowly withdrawn. Correct placement was visually monitored postmortem. One day after surgery, all rats were initiated Morris water maze test.

1.2 Hidden platform testing

The Morris water maze test was performed as described by Janus [23]. The experimental apparatus consisted of a circular pool (1.6 m in diameter and 0.5 m in height) filled with water (22 ± 1℃) to a depth of 30 cm. A circular escape platform (9.5 cm in diameter) was submerged 1 cm below the surface of the water hidden from the rat′s view. All the rats first underwent visible platform testing for 2 consecutive days to assess swimming ability and to accustom them to the test, followed by hidden platform training for 6 days with 4 trials per day. The position of the hidden platform was constant throughout the 4 trials in an experimental day but released sites changed clockwise among the 4 quadrants on each day. For each trial, the rat was placed in the water facing the pool wall at one of four start quadrant points, and the time required for the rat to find the hidden platform was recorded. Once the platform was found, the rat was allowed to stay on the platform for 30 s and then returned to their cage for 30 s inter-trial interval. If the rat did not find the platform within 90 s, it would be placed on the

platform for 30 s, and the escape latency (finding the submerged escape platform) was recorded as 90 s. The rat-swim-paths were digitally recorded by using microcomputer Running Maze Software (Shanghai Ji-liang Software Technology Co., Ltd., China).

1.3 Probe trial

The day after the completion of hidden platform testing, the platform was removed and the rat was allowed to swim freely for 90 s as the probe test, starting from the same starting location as that used first in hidden platform testing. For the probe trial, two measurements were made: (1) the time spent in the quadrant of the former platform position; (2) the number of crossings of the exact place where the platform had been located.

1.4 Tissue preparation

For other parallel experiments, APP-Ab was injected to hippocampus bilaterally as mentioned above. Rats were deeply anesthetized after injection of antibodies and perfused transcardially with saline for 10 min and then with 4% paraformaldehyde in 0.1 mol/L sodium phosphate buffer (pH 7.4) for 10 min. The brains were removed, post-fixed in the same fixation buffer for 1 day, and then immersed sequentially in 20% and 30% sucrose solution until they sank. Microtome sections (30-μm-thick) were cut coronally. Sections were stored at –20℃ in a cryoprotectant solution for histological analysis. The statistical software Image J was used for the statistical analysis.

1.5 Cresyl violet staining

Some sections were processed for histological assessment of damage by staining with cresyl violet (Sigma). Briefly, the slides were washed and then immersed in 70% and 95% ethanol for 5 min each and in 100% ethanol for 15 min, and then in xylene for 30 min. The slides were then immersed back through the 100%, 95%, 70% ethanol and double distilled water for 5 min in each. The slides were stained for 10 min in filtered cresyl violet solution, and then briefly rinsed in double distilled water. They were then dehydrated again in 70%, 95%, 100% ethanol for 1 min each. The slices were placed in xylene for another 10 min and then coverslipped.

1.6 Fluoro-Jade B staining

To stain for degenerating neurons, a Fluoro-Jade staining procedure was used. As described previously[24], dried sections were dipped in an 80% ethanol solution containing 1% sodium hydroxide for 5 min, 70% ethanol for 2 min and 0.06% potassium permanganate for 10 min. After rinsing with distilled water, the sections were treated in the dark with 0.0001% Fluoro-Jade B staining solution for 20 min followed by three washes of ddH_2O. The sections were then mounted, air-dried and coverslipped with glycerol gelatin. Fluoro-Jade B staining was detected at an excitation of 480 nm and an emission of 525 nm under an epifluorescence microscope and a bright green fluorescence signal indicated degenerative neurons. Images were acquired through a 20 × objective.

1.7 Immunohistochemistry

For immunohistochemistry, endogenous peroxidase activity in the sectioned tissues was blocked with 3% H_2O_2, and nonspecific binding sites were blocked with 10% fetal calf serum. The sections were incubated with rabbit anti-microtubule associated protein-2 (MAP2, 1∶200, Chemicon) or with rabbit anti-Tyr31 phosphorylated paxillin (p-paxillin, 1∶200, Sigma) or anti-Ser202 phosphorylated tau (1∶200, Bioscience) in 0.01 mol/L phosphate-buffered saline (PBS) containing 1% fetal calf serum at 4℃ overnight. Subsequently, biotinylated goat anti-rabbit IgG secondary antibody (1∶200; Santa Cruz) was applied, followed by incubation for 1 h with an avidin-biotin-horseradish peroxidase complex (Elite ABC Kit, Vector Laboratories). The bound antibodies were visualized using with 0.05% diaminobenzidine (DAB; Sigma). Negative controls received the same treatment except that primary antibodies were omitted, and showed no specific staining.

1.8 Statistical analysis

All data were presented as the $\bar{x} \pm s$. The significance of the differences was analyzed *via* one-way ANOVA. Mean values were considered to be statistically significant at $P < 0.05$.

2 Results

2.1 Effects of intra-hippocampus injection of APP-Ab on spatial learning of rats

The Morris water maze (MWM) test was used to examine the spatial cognitive performance of animals. To investigate whether injection of APP antibody (APP-Ab) to hippocampus could damage memory performance, the learning and memory ability of the rats injected with or without APP-Ab was tested by the MWM. The results were shown in Figure 1. In order to determine whether the differences between groups in escape latency were due to the differences in

swimming ability or visual skills, the swimming speed was evaluated (Figure 1a). No significant differences for swimming speed were observed between treatments ($P > 0.05$). While, rats showed significant difference in mean escape latency between the six training days. There was a significant prolongation of latency in rats received intra-hippocampus injection of APP-Ab compared to the controls ($P < 0.05$) as shown in Figure 1b. In the hidden platform trial, the time to find the platform for NS group and IgG-injection group decreased in a day-dependent manner. Although APP-Ab injection group followed this pattern (Figure 1b), group comparisons revealed that APP-Ab injection group presented a longer latency to find the platform than NS and IgG-injection group along time (Figure 1b, on days 1, 2 and 4, $P < 0.05$). These results indicated that APP-Ab injection rats had remarkable cognitive deficit.

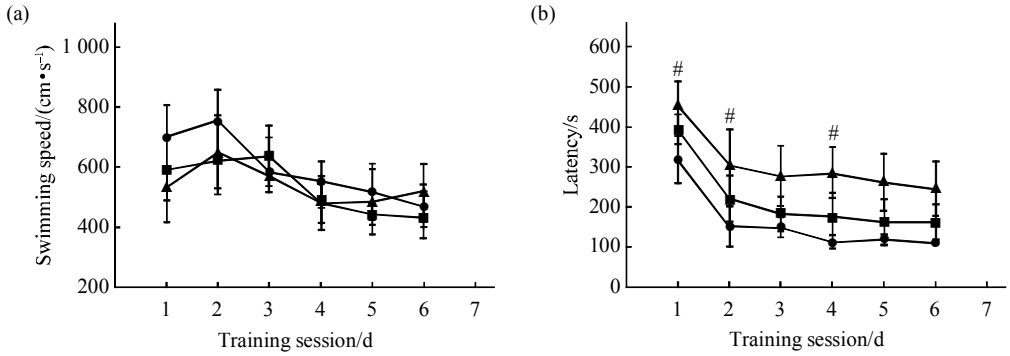

Fig. 1 Performance in Morris water maze test for each group after injection of APP-Ab or IgG

(a) There were no significant changes in the mean swimming speed for each group ($P > 0.05$). (b) The total latency of consecutive six days to find hidden platform was significantly prolonged in APP-Ab injection group compared to the control group ($P < 0.05$). Points in (b) represented average latency to find the platform in every training day. Days 1, 2, 4: showed a significant difference between APP-Ab and IgG injection group; Days 3, 5, 6: showed no significant difference among the groups. $^{\#}P < 0.05$, significantly different from IgG injection group. ●—●: NS; ■—■ : IgG; ▲—▲: APP-Ab.

In the probe trial of the MWM test which investigates memory retrieval ability of the animals, we measured the time that rats spent in the target quadrant. As shown in Figure 2a, NS injection group spent significantly more time ($P < 0.05$) in the training quadrant than APP-Ab injection group, which visited all quadrants equally during 90 s of free swimming. Similar results were obtained from the former platform crossing numbers (Figure 2b). NS and IgG injection groups crossed over the platform location more frequently than APP-Ab injection group ($P < 0.05$). Taken together, these data showed that APP-Ab injection induced spatial learning and memory dysfunction in rats.

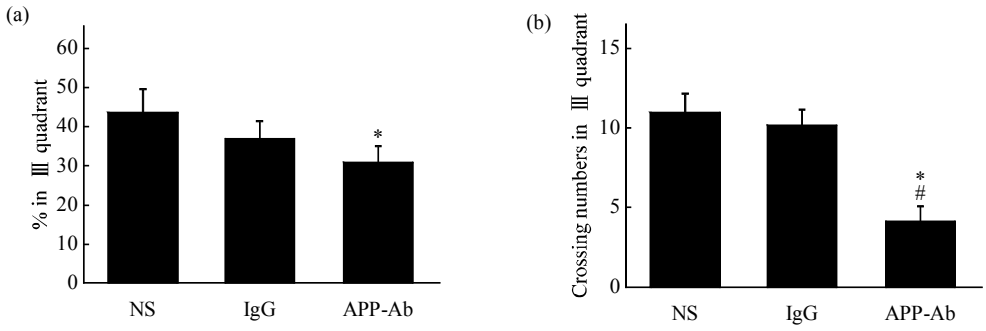

Fig. 2 Probe trial performance for each group after injection

(a) The percent time spent in the quadrant of the former platform position. (b) Crossing times over the former platform location. $^{*}P < 0.05$, significantly different from NS injection group; $^{\#}P < 0.05$, significantly different from IgG injection group.

2.2 Signs of neurodegeneration in APP-Ab injection rats

To assess the extent of neurodegenerative alterations in APP-Ab injection rats, histological analysis was performed using cresyl violet (CV). In control hippocampal tissues (CON), which were not injected with APP-Ab, showed intact neuronal cell layers in pyramidal cell layers and many more cresyl violet-positive neurons were observed as compared with APP-Ab injected tissues. APP-Ab injection rats displayed decreased CV staining in CA1 subfield of hippocampus, which appears at the early stage of 6 h, and becomes evident at 12 h and 24 h (Figure 3a), suggesting a reduced cell density. This decrease seems to correspond to the loss of neurons in this area evidenced by the decreased level of immunoreactivity for the dendritic marker MAP2 (Figure 3b). Previous studies have demonstrated that loss of MAP2 immunoreactivity occurs with CNS injury [25–26]. In J20 APP transgenic mice, analysis of the hippocampus, a region that displays synapse loss in AD, revealed a significant decrease in MAP2 immunoreactivity at 6～8 months, suggesting dendritic loss in hippocampus[27]. Also, in transgenic mice expressing the carboxy terminus of amyloid precursor protein, anti-MAP2 staining showed severe atrophy of large apical dendrites of pyramidal neurons in various cortical regions[28]. Similar to previous studies, we demonstrated a clear decrease in MAP2 staining following APP-Ab injection as compared to the control. As shown in Figure 3b, in control normal hippocampus, somatic compartment of neurons throughout stratum pyramidal layer and granule cell layer contained high level of MAP2, from which MAP2 positive dendritic processes extended in stratum radiatum and distributed in stratum moleculare and molecular layer of dentate gyrus with a high immunoreactive pattern suggesting maintenance of the normal structural integrity of CA1 dendrites [29–30]. While in APP-Ab injection animals, MAP2 staining was dramatically decreased in stratum pyramidal layer and granule cell layer. Only few cells were immunostained in CA1 pyramidal layer throughout the entire 24 h period, although some MAP2 positive cells appeared at late stage.

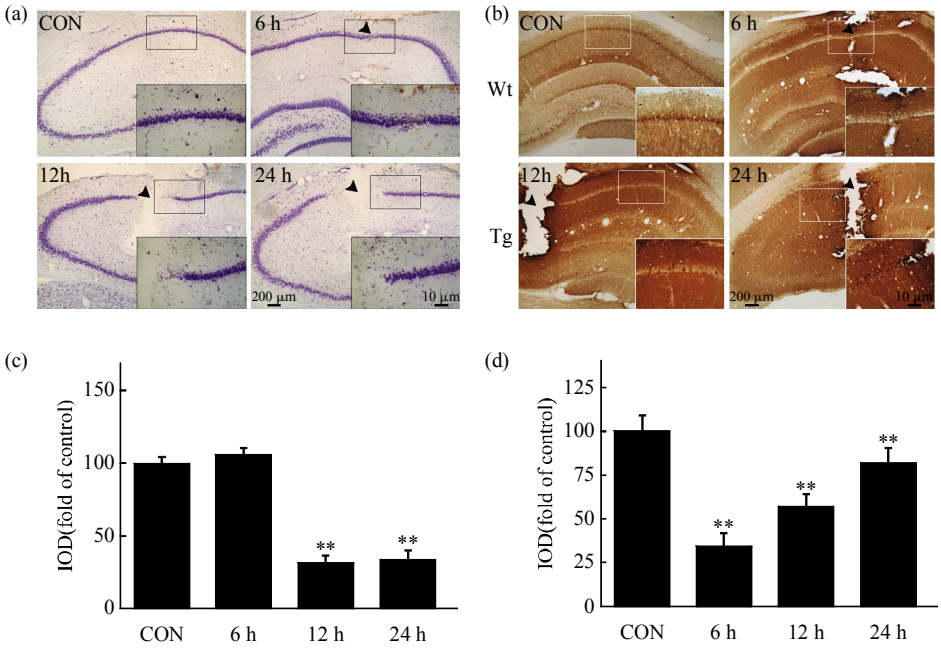

Fig. 3 Histological analysis of the hippocampus following APP-Ab injection for different time

(a) CV staining for dead neurons. The discontinuity and neuronal loss of the pyramidal neuron band in the CA1 subfield was clear following APP-Ab injection. (b) MAP2 immunohistochemistry for structural integrity in the CA1 subfield of hippocampus rat slice with and without injection of APP-Ab. Representative images depicting MAP2 immunostaining in the CA1 of the hippocampus were shown. Control animal presenting MAP2 immunoreactivity in nerve cell bodies and more heavily stained dendrites. Loss of MAP2 immunoreactivity in nerve cell bodies in pyramidal neurons was present at 6 h, 12 h and 24 h after APP-Ab injection. (c) and (d) Quantity analysis showed that with injection time went by, the number of positive cells increased gradually in both groups. Insets in panels (a, b) are high magnification images of CA1 pyramidal cells. CV: Cresyl Violet. Arrowheads show the injection point.

To further confirm neurodegeneration in the CA1 subfield of hippocampus, we then stained for neurodegenerative changes with Fluoro-Jade B (FJB) histofluorescence (a high affinity fluorescent marker for the localization of neuronal degeneration) [24]. Similar to what was observed with CV staining, there was a time-dependent increase in FJB staining in the CA1 region after APP-Ab injection, which plateaued by 24 h of reperfusion (Figure 4a). FJB positive cells were detected in the CA1 area adjacent to the needle track of APP-Ab-injected animals. Few scattered FJB positive cells were found in the hippocampus of the control group. Moreover, we injected APP antibody into APP/PS1 transgenic mice which highly expressed APP protein and to study neuronal degeneration indicated by FJB. Results showed that there were more FJB positive cells in cortex of APP/PS1 transgenic mice than wild type control mice (Figure 4b). These findings, together with the CV and MAP2 staining results indicated a significantly neurodegenerative alterations in APP-Ab injection rats.

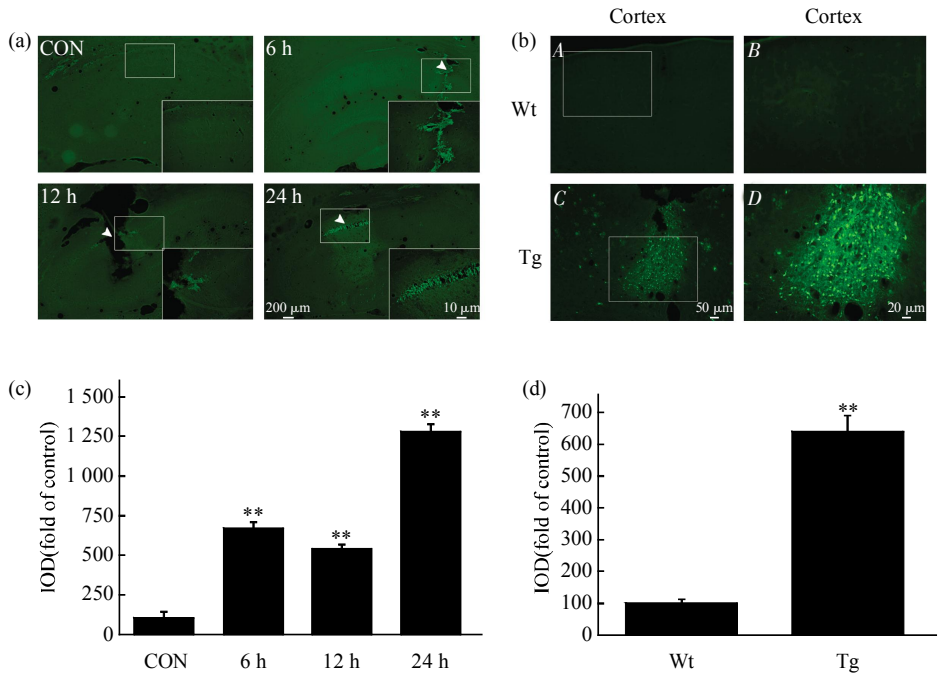

Fig. 4 Fluro-Jade B analysis of the brain following APP-Ab injection

(a) Photomicrographs showed the FJB-positive degenerating cells in the CA1 subfield of hippocampus after injection with APP-Ab for 6 h, 12 h and 24 h. In control IgG-injected group, only few FJB-positive cells were appeared. While, in APP-Ab injected group, FJB staining showed that APP-Ab injection induced increase of the number of FJB-positive cells. Insets in panels A are high magnification images of CA1 pyramidal cells. (b) Photomicrographs showed the FJB-positive degenerating cells in the cortex after injection of APP-Ab into APP/PS1 transgenic mice. *B* and *D* were high magnification of *A* and *C* respectively. FJB: Fluoro-Jade B. Arrowheads show the injection point.

2.3 APP-Ab induces neuronal neurodegeneration associated with increased phosphorylation of paxillin and tau

Neuritic dystrophy in AD is related to abnormal activation of focal adhesion molecules. Paxillin is a scaffolding protein to which other focal adhesions (FA) proteins bind, leading to the formation of the FA contact and initiation of signaling cascades. As reported[31–32], fibrillar Aβ-induced neuronal dystrophy is mediated by the activation of FA proteins and the formation of aberrant FA structures adjacent to Aβ deposits. In AD brain, activated FA proteins including Tyr-31-phosphorylated paxillin are observed associated with the majority of senile plaques where they colocalize with hyperphosphorylated tau. In order to test whether the neuronal injury induced by APP antibody is related with changes in phosphorylation of paxillin and tau, we undertook Tyr31 phosphorylated paxillin and Ser202 phosphorylated tau immunohistochemistry staining. As shown in Figure 5a, faint p-paxillin immunoreactivity was consistently seen in the control brain slides.

While in APP-Ab-injected rats, apparent p-paxillin immunoreactivity occurs in CA1 region of hippocampus. Also, strong immunoreactivity for phosphorylated paxillin at Tyr-31 was observed in senile plaques which were immunostained for Aβ (Figure 5e), while in age-matched control sections, very low levels of diffuse Tyr-phosphorylated paxillin were observed. Tau phosphorylation was also increased after APP-Ab injection in hippocampus, especially in CA3 region (Figure 5b). In hippocampus of normal control rat, phosphorylated tau was weakly stained and was mainly localized to the cell body of neurons (Figure 5b). While in the APP-Ab injection animals, phosphorylated tau positive cells were increased and strong positive staining was observed in both pyramidal cells and their dendrite processes in the CA3 subfield. These results indicated that activation of paxillin and tau is common feature at amyloid deposits in the AD brain.

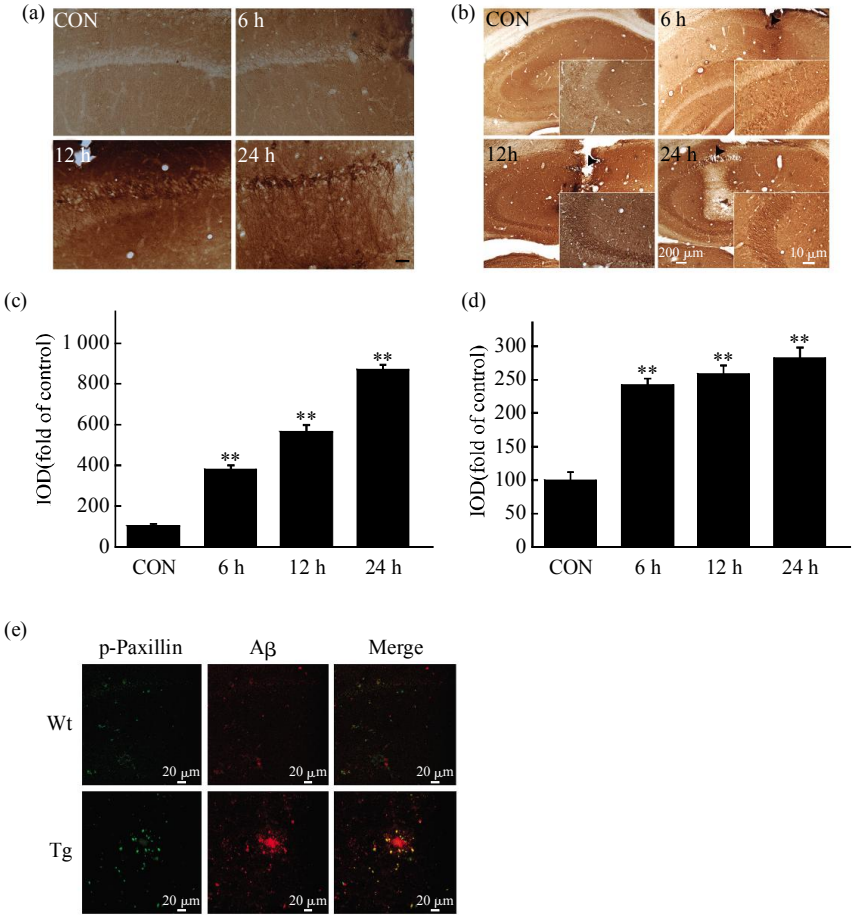

Fig. 5　Immunohistochemistry of phosphorylated paxillin and phosphorylated tau in the hippocampus after APP-Ab injection

The Tyr31 phosphorylated paxillin immunoreactive cells by DAB staining in the CA1 region of the hippocampus were shown in (a, c). In control brain, faint p-paxillin immunoreactivity was consistently seen. Compared to the control rats, there was a significantly increased paxillin staining in the CA1 of hippocampus after APP-Ab injection. Also, strong immunoreactivity for phosphorylated paxillin at Tyr-31 was observed in senile plaques which were immunostained for Aβ (e), while in age-matched control sections, very low levels of diffuse Tyr-phosphorylated paxillin were observed. Tau phosphorylation were also increase after APP-Ab injection in hippocampus, especially in CA3 region (b, d). Arrowheads show the injection point.

These results collectively suggest that phosphorylated paxillin and phosphorylated tau might be closely related with neuronal injuries in APP-Ab-injected rats. Whether the other focal adhesion molecules were associated with APP-Ab-induced injuries or the specifically molecular mechanism remain to be determined.

3 Discussion

APP seems to function as a cell surface receptor involved in regulation of cell function[33]. Because the natural ligand of APP remains unknown, several studies have used antibodies against different extracellular domains of this protein to mimic ligand-receptor interaction [19, 34]. Previous evidence demonstrated that accumulation of wild-type APP in neurons caused a specific type of neuronal degeneration *in vivo* in the absence of extracellular Aβ deposition[35], and antibodies against APP N-terminus killed primary cultured neurons and immortalized neuronal cells over expressing APP[20, 36], implicating functional deregulation of surface APP in pathological mechanism in AD.

Molecular crosslinking by antibody has been best investigated for the cell surface Fc receptor (FcR). It has been demonstrated that IgG binding to FcR induces little crosslinking of FcR and that the crosslinking of FcR can be best induced with IgM antibody or IgG antibody plus a second antibody. To discuss whether activation of this APP-mediated cell damage is an artifact induced only by antibody crosslinking, we therefore examined whether injection of anti-rabbit IgG was also induced memory damage. We also used normal saline (NS) as control. In this study, using antibody ligand mimicking, we showed that an APP N-terminal antibody induced neuronal injury and memory dysfunction *in vivo*, confirming that deregulation of APP′s functions alters memory formation and consolidation.

Previous investigation found that the intracerebroventricular injection of antibodies that do not distinguish between different APP isoforms were reported to result in impairment of acquisition, but not of consolidation and memory retrieval[12]. While in our research that the memory dysfunction were analysed with Morris water maze, we found that hippocampus injection of antibody raised against APP significantly increased escape latency, at both 1, 2 and 4 days compared with NS injection group. Escape latency in IgG injection group also increased compared with NS injection group, although the difference was not significant. In APP-Ab injection group, there were decreased searching time and crossing numbers in the target quadrant where the platform was previously located. The retention deficit observed in hidden platform in the early days after anti-APP injection, when a large proportion of the antibody is still present, might reflect the blockade of a normal function of APP in memory processes. In contrast, the recall analysis 5 or 6 days later indicated that there was no difference in three groups for the escape latency, probably because the antibody treatments did not induce permanent effects on APPs and the antibody molecules were no longer present in the brain after 5 days. Therefore, it appears that the anti-APP effect on memory function observed in the hidden platform testing is transient, as is the presence of the anti-APP antibody in the brain. All these results presented suggested APP to be directly involved in the former of memory formation and specifically in the early phases of acquisition. The discrepancy between other reports in probe trial might result from the different injection pattern which induced more severe memory injury for hippocampus injection, because of the importance of hippocampus for memory formation and consolidation.

In addition, disruption of MAP2 distribution further confirmed this presumption. MAP2 is an early marker for damage of neuronal cytoskeleton following experimental TBI[37], its disruption could contribute to the development of cognitive dysfunction after brain injury[38]. Depletion of MAP2 immunoreactivity is an early response to neuronal damage[39], so the reduction of MAP2 immunoreactivity in CA1 region is an indication of cell damage. In this study, we investigated that MAP2 immunostaining in CA1 subfield of hippocampus appeared an acute decrease then partly restore, suggesting that short-term effects on these major neuronal marker proteins resulted from the presence and transient blockade of APP function by the antibodies. There is also an induction of accumulation of MAP2 immunoreactivity in neuronal somata, which further supports the dystructive role of APP-Ab because in the normal rat hippocampus well-defined MAP2 immunoreactivity is virtually restricted to dendrites. However, we could neither confirm the recovery of MAP2 disruption nor correlate it with the transient cognitive deficits, return of MAP2 immunoreactivity in the hippocampus occurred 24 h after APP-Ab injection in the rats. Therefore, future study should be conducted in a chronic time period following APP-Ab in various severities to examine the potential recovery of MAP2.

These results were further supported by the findings that the anti-APP antibody also induces

aberrant distribution of phosphorylated paxillin. Dystrophy in AD is mediated by aberrant activation of focal adhesion proteins, including paxillin, FAK and LIM kinase, among others[31]. Paxillin is a multidomain adaptor protein localized to focal adhesions, which function is regulated by tyrosine phosphorylation. The role of APP in memory formation has been attributed to its involvement in cell-to-substrate adhesion processes. We found that APP-Ab injection could induce hyperphosphorylated paxillin and tau surrounding injection site (Figure 5), suggesting that aberrant cross-linking of APP on cell surface induced by APP antibody may lead to deregulation of kinase and phosphatase activities responsible for paxillin and tau hyperphosphorylation. The data suggests that the APP involvement in memory formation most probably through mediating FA signaling. These data are in agreement with our previous studies that APP-Ab treatment to primary cultured cells could induce the increased expression of phosphoratlated paxillin and FAK. However, further investigation is required to determine the mechanism by which APP induces phosphorylation of paxillin and through which mechanism affect memory.

Our findings suggest that intrahippocampus injection of APP-Ab could induce cognitive deficits and neurodegenerative changes. APP-Ab injection also affected the distribution of MAP2 and paxillin and tau protein. These results show that APP may play an important role in the process of memory formation and specifically in the early phases of acquisition. The present findings support the relevance of APP-dependent mechanisms in the pathogenesis of Alzheimer′s disease.

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海马注射 β-淀粉样蛋白前体蛋白抗体诱导神经元的退行性变及学习记忆障碍 *

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摘要　探讨海马内注射淀粉样蛋白前体蛋白(APP)抗体诱导细胞表面 APP 的铰链是否影响大鼠的水迷宫行为学以及是否诱导神经元的退行性改变，并进一步探讨其可能的机制．成年雄性 SD 大鼠海马内分别注射生理盐水、对照 IgG 和 APP 抗体．水迷宫行为学检测测试动物的学习和记忆能力．Cresyl Violet(CV) 和 Fluoro-Jade B 染色观察神经元的退行性变．免疫组织化学方法检测 MAP-2 和 磷酸化 paxillin 及磷酸化 tau 蛋白在海马的异常表达和分布．海马内注射 APP 抗体可延长动物的寻台潜伏期，减少大鼠在平台所在象限的探索时间和穿梭次数．CV 和 Fluoro Jade-B 染色结果显示，海马注射 APP 抗体可导致海马锥体细胞的死亡和退变．同时伴 MAP2 免疫染色的减少和磷酸化 paxillin 及磷酸化 tau 的免疫染色的增加．上述结果表明，海马内注射 APP 抗体可诱导学习和记忆功能障碍及神经元的退行性改变，其机制可能与 MAP-2 和磷酸化 paxillin 及磷酸化 tau 的异常表达分布有关．

关键词　淀粉样蛋白前体蛋白，水迷宫，神经元退行性变，海马，paxillin，tau
学科分类号　Q42　　**DOI**: 10.3724/SP.J.1206.2011.00024

* 国家自然科学基金(81071017, 90919004)和上海市科学技术发展基金(10DZ1976000)资助项目．
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收稿日期：2011-03-31，接受日期：2011-06-16