μ -Opioid Receptor Attenuates A β Oligomers-Induced Neurotoxicity Through mTOR Signaling

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SUMMARY

Aims: μ -opioid receptor (OPRM1) exerts many functions such as antinociception, neuroprotection, and hippocampal plasticity. A body of evidence has shown that OPRM1 activation could stimulate downstream effectors of mechanistic/mammalian target of rapamycin (mTOR). However, it is not clear whether OPRM1 protects neurons against β -amyloid peptide (A β) neurotoxicity through mTOR signaling. **Methods:** The effects of OPRM1 activation on A β oligomers-induced neurotoxicity were assessed by cell viability and neurite outgrowth assay in primary cultured cortical neurons. The activities of mTOR, protein kinase B (Akt) and p70 ribosomal S6 kinase (p70 S6k) upon OPRM1 activation by morphine were measured by immunoblotting their phosphorylation status. **Results:** Morphine dose-dependently attenuated A β oligomers-induced neurotoxicity. A β oligomers downregulated mTOR signaling. Morphine significantly rescued mTOR signaling by reversal of A β oligomers' effect on mTOR and its upstream signaling molecule Akt, as well as its downstream molecule p70 S6k. Moreover, the neuroprotective effect of morphine could be reversed by OPRM1 selective antagonist and phosphatidylinositol 3-kinases (PI3K), Akt and mTOR inhibitors. Furthermore, endogenous opioids–enkephalins also attenuated A β oligomers-induced neurotoxicity. **Conclusions:** Our findings demonstrated OPRM1 activation attenuated A β oligomers-induced neurotoxicity through mTOR signaling. It may provide new insight into the pathological process and useful strategy for therapeutic interventions against A β neurotoxicity.

Introduction

μ -opioid receptor (OPRM1), one of the rhodopsin class of G protein-coupled receptors (GPCRs), is widely expressed in sensory neurons and has a broad range of biological effects [1]. Besides its critical role in antinociception, many other functions have been described such as neuroprotection, hippocampal plasticity, gastrointestinal motility, and modulation of the immune response [2–4]. It has been demonstrated that OPRM1 activation by morphine promotes cell proliferation and also affects neuronal differentiation both *in vitro* and *in vivo* [5–7]. Several studies also demonstrate that morphine protects neurons against microglia-mediated neuroinflammation and oxidative stress [8,9]. Preconditioning with morphine induces neuroprotective effects against hypoxia/hypoglycemia [10,11]. Such neuroprotection is shown to be OPRM1 dependent [9,11].

Mechanistic/mammalian target of rapamycin (mTOR), which is widely distributed throughout all eukaryotic cells, belongs to the phosphatidylinositol kinase-related kinase (PIKK) family. PIKK family members contain serine/threonine kinase domain in carboxyl terminus that resemble the catalytic domain of

phosphatidylinositol 3-kinases (PI3Ks) and PI4Ks [12]. PI3K activation concomitantly leads to the protein kinase B (Akt) activation, which further activates mTOR by phosphorylating and inactivating the tuberous sclerosis complex. mTOR primarily controls cell growth in response to favorable nutrients and other growth stimuli, whereas in the adult with relatively limited growth, mTOR influences aging and other aspects of nutrient-related physiology such as protein synthesis, ribosome biogenesis and cell proliferation [13]. In central nervous system (CNS), mTOR plays a critical role in neuronal plasticity and learning and memory and has been shown to associate with many neurodevelopmental and neuropsychiatric disorders [14]. Brain-derived neurotrophic factor (BDNF)-dependent survival of primary hippocampal neurons requires mTOR activation [15]. Conditional knockout of intrinsic negative regulators of mTOR such as phosphatase and tensin homolog (PTEN) or tuberous sclerosis complex 1 promotes axon regeneration after CNS injury [16]. Moreover, mTOR activation promotes changes of dendritic morphology and formation of synaptic contacts [17]. mTOR-mediated translational control is required for late-long-term potentiation (L-LTP) and memory reconsolidation [18].

Alzheimer's disease (AD) is a neurodegenerative disease leading to a progressive decline of the cognitive functions. Extracellular accumulation of β -amyloid peptide (A β) has been reported to be a major etiological cause of AD [19,20]. Intensive research has been carried out to clarify the molecular mechanisms of A β in mediating the neuronal death. Several studies demonstrate that A β exposure induces a rapid but sustained reduction of the phosphorylation of mTOR and its downstream effector p70 ribosomal S6 kinase (p70 S6k) in murine neuroblastoma cells and hippocampal slices [21–23]. Furthermore, reduced mTOR phosphorylation is manifested in the cortex or hippocampus of transgenic AD mouse models and in lymphocytes of AD patients [22,24–26]. Glycogen synthase kinase 3 (GSK3) inhibitor ameliorates A β pathology and reactivates mTOR [26]. Moreover, mTOR activation has been shown to play a key role in cytoprotective effect of erythropoietin in AD [27]. Thus, mTOR activation could slow or reverse the pathological process of AD.

OPRM1 activation has been shown to stimulate p70 S6k and other downstream effectors of mTOR, eukaryotic initiation factor 4E-binding (eIF4E-binding) proteins (4E-BP1 and 4E-BP2) [28]. Furthermore, the activation of these downstream effectors of mTOR could be blocked by rapamycin [28]. Therefore, whether such effect is involved in neuroprotection mediated by OPRM1 needs to be illuminated. In current study, we demonstrated that OPRM1 activation could activate mTOR and protect neurons against A β -induced toxicity. Besides activating PI3K/Akt, MAP kinase kinase (MEK1/2)-extracellular signal regulated kinases (ERK1/2) signaling cooperates with the PI3K/Akt axis to modulate mTOR activity [29]. Moreover, protein kinase C (PKC) which is similar to Akt can be modulated by mTOR. Expression of PKC has been suggested to play a role in the preservation of cell survival and the formation and consolidation of memory [30–32]. Increased activity of PKC in neurons is required in neuroprotection against several exogenous insults [33]. The phosphorylation of Akt has been reported with OPRM1 activation by [D-Ala², N-Me-Phe⁴, Gly⁵-ol]-enkephalin (DAMGO) [28,34]. Moreover, PKC and ERK1/2 activation is observed after OPRM1 activation [35]. Thus, the signaling pathway leading to mTOR activation by OPRM1 was further investigated.

Materials and Methods

Materials

Morphine and naloxone were supplied by the National Institute on Drug Abuse. Rapamycin, H-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂ (CTAP), mouse monoclonal anti- β -actin antibody and hexafluoroisopropanol were purchased from Sigma Chemical Co (St. Louis, MO, USA). All the other antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). A β -(1–40) was purchased from Biosource International (Camarillo, CA, USA). Cell counting kit-8 (CCK-8) kits were purchased from Dojindo laboratories (Kumamoto, Japan). Cellomics neurite outgrowth assay kit was purchased from Thermo Scientific (Pittsburgh, PA, USA). All the cell culture reagents were purchased from GIBCO (Grand Island, NY, USA).

Primary Rat Cerebral Cortical Neuron Culture

Primary rat cerebral cortical neurons were prepared as previously described [36]. Neurons were prepared from embryonic day 17 (E17) rat embryos (Sprague-Dawley, Slac Laboratories, Chinese Academy of Sciences, Shanghai, China). The embryos were collected, and the cortices were dissected out in ice-cold Hank's-buffered salt solution without Ca²⁺ and Mg²⁺. Dissected pieces of cortical tissue were pooled together and transferred to an enzymatic dissociation medium containing 0.05% trypsin-EDTA. Then, enzymatic dissociation medium was aspirated, and the tissue was triturated with a sterile Pasteur pipette in MEM with 10% fetal bovine serum (v/v), 5% horse serum (v/v), 2 mM L-glutamine, 100 units/mL penicillin, and 100 μ g/mL streptomycin plus 2000 IU/mL DNase. After centrifuging at 800 $\times$ g for 8 min, the pellets were resuspended in MEM medium and plated on poly-L-lysine precoated 96-well or 24-well plates. After culturing in a humidified atmosphere of 95% air and 5% CO₂ at 37°C for 2 h, the medium was replaced with neurobasal medium plus 2% B27 and 2 mM GlutaMAX^{TM}-I supplements.

Cell Viability

Cell viability was measured as previously described [36]. Briefly, cells were plated in 96-well plate at the density of 1–2 $\times$ 10⁴ cells per well. On day 7 after culturing, half of the medium was aspirated, and 1 μ M morphine was added for 2 h before the addition of 1 μ M A β -(1–40) oligomers prepared as previously described [36]. 10 μ M naloxone, 5 μ M CTAP or 1 μ M rapamycin were added 10 min before morphine. Then, the cells were incubated for 24 h, and cell viability was measured with CCK-8 kit according to manufacturer's instructions. The absorbance of the samples was read by Varioskan Flash plate reader (Thermo Scientific) at 450 nm and 650 nm. Nuclear size and cell permeability were assessed using Cellomics HCS multiparameter cytotoxicity 2 kits according to the manufacturers' instructions, and cells were then scanned and analyzed using the ArrayScan HCS Reader with appropriate image-processing BioApplication software module.

Neurite Outgrowth

The neurite outgrowth assay was performed using Cellomics neurite outgrowth assay kit according to the manufacturer's instructions. In brief, primary cultured cerebral cortical neurons were treated with A β -(1–40) oligomers and morphine and then stained with neurite outgrowth primary antibody plus Dylight^{TM} 488-conjugated secondary antibody and Hoechst33342. The stained cells were scanned by Cellomics KineticScan reader (Thermo Scientific).

Western Blot

After treatment, cortical neurons were extracted with lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na₃VO₄, 1 μ g/mL leupeptin, and 1 $\times$ protease inhibitor cocktail (Sigma, Shanghai, China). After centrifuging at 12 000 $\times$ g for 5 min, sample loading buffer was

added to the supernatants and boiled for 5 min. Approximately 30–40 μ g of protein was subjected to SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% milk and then incubated with primary antibodies against mTOR phosphorylated at Ser2448 (1:1000), mTOR (1:1000), Akt phosphorylated at Thr308 (1:1000), Akt (1:1000), p70 S6k phosphorylated at Thr389 (1:1000), p70 S6k (1:1000), and β -actin (1:5000) overnight at 4°C. Membranes were then incubated with goat anti-rabbit/mouse HRP-conjugated secondary antibody (1:10,000) for 1 h at room temperature, visualized with SuperSignal West Pico Chemiluminescent Substrate (Pierce Chemical, Rockford, IL, USA) and analyzed by Image J (National Institutes of Health) software.

Statistical Analysis

Data were presented as mean $\pm$ standard error of the mean (SEM). All experiments were carried out at least three times independently with triplicate repeats in cell viability assay. The results were analyzed by analysis of variances (ANOVA) followed by Dunnett's test using GraphPad Prism 5.0 statistical analysis software (GraphPad Software, La Jolla, CA, USA), with $P < 0.05$ taken to indicate significant difference.

Results

OPRM1 Activation by Morphine Protected A β Oligomers-Induced Neurotoxicity

To evaluate whether OPRM1 activation could protect the neurons against the injury induced by exposure to A β , 1 μ M A β -(1-40) oligomers and OPRM1 agonist morphine were administered to primary cultured cerebral cortical neurons. As shown in Figure 1A, 24 h exposure of cortical neurons to A β -(1-40) oligomers decreased the cell viability to $46.5 \pm 4.1\%$ of control, while co-incubation of A β -(1-40) oligomers with 0.01, 0.1, 1 μ M morphine increased the cell viability to $48.4 \pm 3.9\%$, $67.9 \pm 2.9\%$ ($P < 0.01$ vs. A β -(1-40) oligomers treatment), $77.9 \pm 2.6\%$ ($P < 0.001$ vs. A β -(1-40) oligomers treatment).

Synaptic changes reflected by monitoring neurite outgrowth provides subtler indication of neuronal function. As shown in Figure 1B left panel, normal neuronal cells had distinguishable cell bodies and neurites with corresponding nucleus. The dendrites and axon were clearly defined and neurons communicated with each other as indicated by numerous branches and cross-points. The neurons treated with A β oligomers demonstrated ambiguous outlines of cell bodies and retraction of preformed neurites (Figure 1B, middle panel). The volumes of neurons decreased, and staining of cytoplasm and neurites was dimmer than that of normal neurons. Furthermore, karyopyknosis and nuclear accumulation were observed. 1 μ M morphine administration significantly rescued the morphology damage as indicated by the recovery of normal-sized nucleus and integral neurites (Figure 1B, right panel).

Toxic insults, such as A β oligomers, often cause cells to undergo necrosis or apoptosis, which can be manifested by changes in nuclear size and/or morphology [37,38]. Meanwhile, cell permeability can be increased by the impaired cell membrane integrity

Figure 1 Morphine attenuated A β oligomers-induced neurotoxicity in primary cultured cortical neurons. **(A)** Primary cultured rat cerebral cortical neurons were incubated with 0, 0.01, 0.1, and 1 μ M morphine for 2 h before 1 μ M A β -(1-40) oligomers was added. Cells were then cultured for 24 h, and cell viability was measured by CCK-8 assay. **(B)** Primary cultured rat cerebral cortical neurons were incubated with 1 μ M morphine for 2 h before 1 μ M A β -(1-40) oligomers was added for another 24 h. Then, the neurons were stained with Hoechst 33342 for nuclei and neurite primary antibody for cell bodies and neurites. Representative images were taken by Cellomics KineticScan HCS Reader. Magnification was 512×512 pixels ($10 \times$ for parameter settings, one pixel represents 0.625μ M). **(C–D)** Primary cultured rat cerebral cortical neurons were incubated with 1 μ M morphine for 2 h before 1 μ M A β -(1-40) oligomers was added for another 24 h. Nuclear size **(C)** and cell permeability **(D)** were measured and calculated using multiparameter HCS cytotoxicity kit and Cellomics BioApplication Modules. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[39]. To further evaluate the neurotoxicity of A β oligomers and the protective role of morphine on *in vitro* cultured neurons more vividly, we applied multiparameter HCS neurotoxicity kits to assess the change of nuclear size and cell permeability. As shown in Figure 1C, 24 h exposure of cortical neurons to A β -(1-40) oligomers decreased the nuclear size to $74.4 \pm 1.5\%$ of control ($P < 0.001$ vs. control), while co-incubation of A β -(1-40) oligomers with 1 μ M morphine significantly increased the nuclear size to $81.8 \pm 2.3\%$ of control ($P < 0.05$ vs. A β -(1-40) oligomers treatment). In line with nuclear abnormal morphology, cell membrane permeability increased to $248.4 \pm 12.8\%$ of control ($P < 0.001$ vs. control) after incubation with A β -(1-40) oligomers, while co-incubation of A β -(1-40) oligomers with 1 μ M morphine significantly decreased the cell permeability to $203.5 \pm 11.4\%$ of control ($P < 0.05$ vs. A β -(1-40) oligomers treatment). These results

collectively demonstrated the apparent neuroprotective properties of morphine.

To further confirm that the neuroprotective effect of morphine is through OPRM1 activation, opioid receptor antagonist naloxone or OPRM1 selective antagonist CTAP was applied 10 min prior to morphine and A β oligomers administration. 10 μ M naloxone reduced the cell viability to $46.5 \pm 4.9\%$ of control, and 10 μ M CTAP reduced the cell viability to $56.9 \pm 1.5\%$ of control (Figure 2A). These data indicated that the neuroprotective effect of morphine was dependent on OPRM1 activation.

The Attenuation of A β Oligomers-Induced Neurotoxicity by OPRM1 Activation was through mTOR Activation

To investigate whether mTOR activation is involved in protective effect of morphine, mTOR inhibitor rapamycin was applied. As shown in Figure 2A, 1 μ M rapamycin reduced the cell viability to $56.4 \pm 3.6\%$ of control. To further evaluate the role of mTOR signaling pathway in these processes, the activation of mTOR and p70 S6k was examined. A β -(1-40) oligomers induced a progressive reduction of phosphorylated mTOR (0.44 ± 0.09 -fold of control) and p70 S6k (0.50 ± 0.12 -fold of control), meanwhile, the total forms of mTOR and p70 S6k slightly decreased but without statistically significance (Figure 3A and B). This inhibition of mTOR and p70 S6k was rescued by 1 μ M morphine pretreatment (Figure 3A and B, 1.02 ± 0.17 -fold of control for phosphorylated mTOR; 0.90 ± 0.11 -fold of control for phosphorylated p70 S6k). Moreover, the effects of morphine were prohibited by OPRM1 selective antagonist CTAP (Figure 3A and B), indicating the neuroprotective effect of morphine through modulating mTOR signaling pathway was mediated by receptor activation. Taken together, these results revealed that pharmacological activation of mTOR by

OPRM1 activation provided definite protective effects against A β oligomers-induced cytotoxicity in primary cultured neurons.

PI3K/Akt Mediated the mTOR Activation by OPRM1 Activation

Next, the signaling pathway leading to mTOR activation was investigated by employing specific inhibitors. As shown in Figure 4, blockade of PI3K or Akt signaling pathway abolished morphine-mediated cell toxicity improvement against A β oligomers treatment ($43.4 \pm 2.0\%$ and $37.2 \pm 2.9\%$ of control, respectively, $P < 0.001$ vs. A β oligomers treatment), while blockade of MEK1/2 and PKC did not affect the action of morphine in primary cortical neurons.

Moreover, the activation of Akt was assessed. As shown in Figure 3A and B, A β -(1-40) oligomers induced a progressive reduction of phosphorylated Akt (0.53 ± 0.03 -fold of control), and 1 μ M morphine pretreatment could block such effect (0.77 ± 0.04 -fold of control). Further, OPRM1 selective antagonist CTAP could reverse the effect of morphine. All these results demonstrated that PI3K/Akt signaling but not MEK1/2 and PKC played a role in morphine's attenuation of A β oligomers-induced neurotoxicity.

Endogenous Opioid Peptides Attenuated A β Oligomers-Induced Neurotoxicity

Similar to morphine, endogenous opioids including endorphins, enkephalins (ENKs), and endomorphins can activate OPRM1. We further investigated whether two forms of ENKs—Met-ENK and Leu-ENK could attenuate A β oligomers-induced toxicity. As expected and shown in Figure 5, co-incubation of A β -(1-40) oligomers with 10 μ M Met-ENK and 0.1 μ M Leu-ENK increased the cell viability to $73.7 \pm 1.8\%$ and $72.7 \pm 4.6\%$ of control, respectively ($P < 0.001$ vs. A β oligomers treatment), and the protection could be eliminated by blockade of opioid receptor antagonist naloxone, $50.6 \pm 5.5\%$ of control ($P < 0.001$ vs. Met-ENK treatment) and $53.4 \pm 3.5\%$ of control ($P < 0.05$ vs. Leu-ENK treatment), respectively. The protection could also be eliminated using mTOR inhibitor rapamycin which decreased cell viability to $58.0 \pm 3.0\%$ of control ($P < 0.05$ vs. Met-ENK treatment) and $50.2 \pm 2.5\%$ of control ($P < 0.001$ vs. Leu-ENK treatment), respectively. These data suggested that both Met-ENK and Leu-ENK possessed beneficial effect against toxic A β and the protective function was dependent on opioid receptor activation and cascade mTOR activation.

Discussion

In the present study, we demonstrated that morphine dose-dependently attenuated A β oligomers-induced neurotoxicity. Morphine's effect was shown to be dependent on activation of OPRM1 and was mediated by reversal of A β oligomers' downregulation of mTOR signaling. Moreover, such effect is also crucial in the attenuation of A β oligomers-induced neurotoxicity by endogenous opioids ENKs.

As one of the most indispensable translational conductors in the eukaryotic system, mTOR and its regulation have been intensely

Figure 2 Morphine attenuated A β oligomers-induced neurotoxicity in primary cultured cortical neurons through OPRM1 and mTOR activation. Primary cultured rat cerebral cortical neurons were incubated with 1 μ M morphine 2 h before 1 μ M A β -(1-40) oligomers was added for another 24 h. 10 μ M naloxone, 5 μ M CTAP, or 1 μ M rapamycin was added 10 min before the administration of morphine, respectively. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure 3 Morphine activated mTOR signaling through OPRM1 activation. Primary cultured rat cerebral cortical neurons were incubated with 1 μ M morphine 2 h before 1 μ M A β -(1-40) oligomers was added for another 24 h. 5 μ M CTAP was added 10 min before the administration of morphine. Then cell extracts were analyzed by immunoblotting with antibodies against mTOR phosphorylated at Ser2448 (p-mTOR), total mTOR, Akt phosphorylated at Thr308, total Akt, p70 S6K phosphorylated at Thr389 (p-p70 S6K), total p70 S6K (A), and the relative densities were quantified (B). * P < 0.05; ** P < 0.01; *** P < 0.001. ms, morphine.

Figure 4 PI3K-Akt signaling pathway was involved in the morphine-induced attenuation of A β oligomers-induced neurotoxicity in rat cerebral cortical neurons. Primary cultured rat cerebral cortical neurons were pretreated with morphine (1 μ M) alone or with PI3K inhibitor LY294002 (10 μ M), Akt inhibitor IV (0.2 μ M), MEK1/2 inhibitor U0126 (1 μ M), MEK1 inhibitor PD98059 (10 μ M), or PKC inhibitor Calphostin C (1 μ M) as indicated for 2 h. Cultures were then exposed to 1 μ M A β -(1-40) oligomers for 24 h, then cell viability was measured by CCK-8 assay. *** P < 0.001.

Figure 5 Neuroprotective effects of endogenous opioid peptides enkephalins in A β oligomers-induced neurotoxicity involved opioid receptor and mTOR activation. Primary cultured rat cerebral cortical neurons were pretreated with 10 μ M Met-ENK or 0.1 μ M Leu-ENK, opioid receptor antagonist naloxone (10 μ M), mTOR inhibitor rapamycin (1 μ M) as indicated for 2 h, cultures were then exposed to A β -(1-40) oligomers for 24 h. Then, cell viability was measured by CCK-8 assay. * P < 0.05, ** P < 0.01, *** P < 0.001.

investigated in the pathogenesis of AD. It is widely acknowledged that decreased synaptic plasticity caused by soluble A β oligomers emerges well before the senile plaque formation in the brain and correlates much better with the presence and degree of subtle, intermittent impairment of hippocampal neuronal function as well as episodic cognitive deficits [40]. mTOR and its downstream signaling pathways are significantly involved in modulating long-lasting synaptic plasticity [18,41,42] and consolidation of long-term learning and memory [18,43,44]. Both of these processes are dramatically impaired in the progress of AD. A line of evidence has demonstrated that mTOR signaling is impaired in AD mouse models or in cells exposed to A β [21–26]. Moreover, decreased mTOR signaling is observed in the lymphocytes of Alzheimer's

patients, and it is statistically correlated with Mini Mental Status Examination (MMSE) scores [22]. However, mTOR signaling is reported to be upregulated in postmortem human AD brains, especially in tangle-bearing neurons [45] as well as in a mouse model of AD containing a tau mutation [46]. Significant increase of the mTOR signaling positively correlates with total tau and phospho-tau in the medial temporal cortex in AD patients [47,48]. Specifically, there is a report that A β -(1-42) oligomers activates Akt and mTOR in primary cortical neurons and inhibition of mTOR and PI3K/Akt prevents its toxic effects [49]. In current study, we observed apparent downregulation of mTOR signaling following 24 h of A β exposure in primary cultured neurons. We also confirmed our findings *in vivo* by assessing mTOR expression in hippocampus of SAMP8 mice (data not shown), a senescence accelerated mouse model of AD which

featured with early deposition of A β [50]. The disparity between our study and the increased Akt, mTOR levels in A β -(1-42) oligomers-treated neurons [49] could be attributed to the different A β peptides (A β -(1-40) in our study). Besides, the different preparation methods for oligomers could also contribute to the disparity. A β -(1-40) was dissolved in water and aged for 48 h in our study, whereas A β -(1-42) was dissolved in sodium phosphate buffer, and the formation of oligomers was monitored in their study. Nevertheless, our data strongly support the view that A β impairs mTOR signaling and are consistent with a previous report that rapamycin exacerbates neurotoxicity of A β [21].

mTOR is mainly linked to the PI3K/Akt signaling pathway, but its activity can be modulated by other signaling molecules such as ERK1/2, and it can regulate the activities of other signaling molecules such as PKC. Our results demonstrated that PI3K/Akt was exclusively involved in the activation of mTOR by morphine. One of the main translation effectors of mTOR is the p70 S6k. Activation of p70 S6k mediated by mTOR results in the translation of mRNAs that encodes both ribosomal proteins and translational elongation factors [51], which may directly contributed to the neuroprotective effects of morphine.

Besides binding to and activating OPRM1, morphine is also a κ -opioid and δ -opioid receptor agonist. Specific antagonist of OPRM1, CTAP, blocked morphine's effect to attenuate A β oligomers-induced neurotoxicity and activation of mTOR and p70 S6k. Further, mTOR activation could not be elicited in cells lacking endogenous OPRM1 (data not shown). These data indicate that morphine activated mTOR signaling mainly through OPRM1.

Met-ENK, an endogenous opioid peptide, has been demonstrated to be elevated in dentate gyrus of human AD brains and contributes to the cognitive impairments [52]. Our study demonstrated that Met-ENK reduced A β oligomers-induced neurocytotoxicity, suggesting that the elevated Met-ENK in AD brain could be a compensatory process and the peptide's effect on cognitive impairments could reflect other opioid receptor function such as inhibiting adult neurogenesis [53].

In summary, our current study demonstrated that OPRM1 activation attenuated A β oligomers-induced neurotoxicity in primary cultured cortical neurons through mTOR signaling. Our results may provide better understanding of the pathological process of AD and could provide a possible target for modulating neuroprotection.

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Conflict of Interest

The authors declare no conflict of interest.

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