Physiology and Pharmacology 27 (2023) 192-201
Original Article

Estradiol preserves synapse-related proteins against oligomeric amyloid beta in the hippocampal cultured cells: possible involvement of protein kinase M zeta

Sogol Atabaki-Mehr[1, 2], Saeed Airian[2], Hamid Gholami Pourbadie[1*]

1. Department of Physiology and Pharmacology, Pasteur Institute of Iran, Tehran, Iran
2. Department of Cell and Molecular Sciences, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran

ABSTRACT

Introduction: Estradiol has been shown to facilitate synaptic long-term potentiation (LTP) mainly through translocation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor from intracellular pool to the post synaptic membrane. On the other hand, oligomeric amyloid beta (oAβ) decreases number of AMPA receptors in the synapses. It is well known that trafficking of AMPA receptors is governed by an atypical and autonomously active isoform of PKC called protein kinase M zeta (PKMζ). In spite of these evidence, the effect of estradiol on PKMζ expression is not yet studied. We aim to examine the possible protective effect of estradiol on PKMζ and AMPA receptor subunits against oAβ in hippocampal primary cell culture.

Methods: Primary cell culture was prepared from postnatal (P0 to P3) rat pups. They were decapitated and the brains were removed. Hippocampi were isolated and collected in cold phosphate buffer saline. Then, they were trypsinized at 37°C for 15min. The cells were treated with 1μM OAβ or vehicle for 24h and then with 100nM estradiol for another 24h. Using the western blot analysis, the expression level of AMPAR subunit glutamate receptor 1 (GluA1), GluA2 and PKMζ were determined.

Results: OAβ decreased the level of GluA1, GluA2 and PKMζ. Estradiol did not change the molecule levels in healthy cells; however, it preserved their expression levels in OAβ treated cells.

Conclusion: These findings suggest that estradiol may restore expression level of synapse related molecules in an Alzheimer' disease cell model, in part, through acting on PKMζ signaling pathway.

Keywords:
Alzheimer's disease (AD)
Estradiol
Protein kinase M zeta (PKMζ)
Amyloid beta
α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)

Introduction

Alzheimer's disease (AD), which is the most common form of dementia, is a neurodegenerative disease characterized by progressive synaptic dysfunction and amnesia.

AD affects women and men unequally (Carter et al.,

* Corresponding author: Hamid Gholami Pourbadie, h_gholamipour@pasteur.ac.ir
Received 2 March 2022; Revised from 29 June 2022; Accepted 12 July 2022

Citation: Atabaki-Mehr S, Airian S, Gholami Pourbadie H. Estradiol preserves synapse-related proteins against oligomeric amyloid beta in the hippocampal cultured cells: possible involvement of protein kinase M zeta. Physiology and Pharmacology 2023; 27: 192-201. http://dx.doi.org/10.61186/phypha.27.2.192

2012). It is reported that approximately 66% of AD are women and after the age of 65 the incidence of AD in men is 9.1% (i.e. 1 in 11) while this rate is 16.7% for women (i.e. 1 in 6) (Brookmeyer et al., 2011; Uddin et al., 2020). Even under the same conditions, women develop more severe memory deficit than men (Irvine et al., 2012), which may reflect a role of sex hormones, including estrogens on brain function.

Principal pathological hallmarks of AD are extracellular accumulation of amyloid beta (Aβ) plaques and intracellular neurofibrillary tangles. Multiple evidence suggests the superior importance of amyloidogenic pathway in AD pathogenesis. For instance, all four genes, amyloid beta precursor protein (APP), presenilin 1, presenilin 2 and ε4 allele of apolipoprotein E involved in familial or sporadic forms of AD, increase production and deposition of Aβ in the cerebral parenchyma (Selkoe and Podlisny, 2002). Human Aβ42 oligomers has been shown to induce tau hyperphosphorylation and cause neuritic dystrophy in cultured rat neurons while co-administration of Aβ antibodies fully prevents accompanied taupathy (Selkoe and Hardy, 2016). Furthermore, Aβ oligomers obtained from AD patients' brains was reported to impair learning and memory, inhibit long-term potentiation (LTP), enhance long-term depression (LTD) and decrease dendritic spines when directly administered to mouse's hippocampus (Shankar et al., 2008).

APP is a transmembrane protein that cleaved by two proteolysis enzymes: α-secretase, producing non-amyloidogenic secreted APP and β-secretase, producing amyloidogenic fragments and Aβ peptides (Evin and Weidemann, 2002). Soluble forms of Aβ have been shown to be even more toxic than fibrillar form for synaptic function (Shankar et al., 2008; Sengupta et al., 2016). In this regard, it has been shown that Aβ oligomer internalizes the glutamate receptors on the postsynaptic membranes and thus suppresses synaptic transmission (Guntupalli et al., 2016). α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is a tetramer composed of two dimers of four subunits, GluA1-GluA4. Pyramidal neurons of hippocampus and cortex express GluA1 or GluA2-containig AMPA subunits (Lu et al., 2009). Reports have shown that AMPA receptor levels are declined in different types of AD model (Rui et al., 2010), but the underlying mechanism still remains to be understood. AMPA channels with GluA1 subunits have been shown to play an important role in inducing long-term changes in synapses, LTP, known as cellular basis of learning and memory, while GluA2 contributes in LTP maintenance (Aliakbari et al., 2021; Plant et al., 2006). Interestingly, studies to date have shown that Aβ causes synaptic dysfunction by affecting both types of the channel (Aliakbari et al., 2021; Amini et al., 2021; Miñano-Molina et al., 2011).

Protein kinase M zeta (PKMζ), an atypical and autonomously active isoform of protein kinase C PKC (Ling et al., 2002), is believed to maintain LTP and establish remote memories (Shema et al., 2007). On the other hand, PKMζ inhibition has been shown to suppress maintenance of late-LTP (Aliakbari et al., 2021; Schuette et al., 2016). PKMζ acts mainly through facilitation of AMPA receptor trafficking and increasing the number of GluA2 containing AMPA receptor at synapses. Additionally, interactions between PKMζ, GluA2 and post synaptic density protein-95 (PSD-95) caused to prevent internalization of AMPA receptor from the membrane (Shao et al., 2012). Although there is strong evidence that the number of GluA2 containing AMPA receptors on the membrane surface is decreased in amyloidopathy (Miñano-Molina et al., 2011; Aliakbari et al., 2021; Amini et al., 2021), it is not yet clear how the PKMζ is changed in amyloidopathy.

Many reports have elucidated that estradiol induced improvements in memory through synaptic efficacy alteration and have protective effects against AD (Kramár et al., 2013). Estradiol has positive impact on the LTP enhancement by action on NMDA or AMPA receptor function in the hippocampus of male adult rats (Foy et al., 1999). In this regards, hippocampal slices *in vitro* that were subjected to estradiol have shown increases in N-methyl-D-aspartate receptor NMDAR and AMPAR transmission and LTP enhancement within minutes (Smith and McMahon, 2005). Study have indicated that, estradiol induced rapidly increase of hippocampal dendritic spine density and trafficking of GluA2 and PSD-95 in specific spines (Avila et al., 2017).

Although there is strong evidence on modulating effect of estradiol on synaptic efficacy through rapid increase of GluA2 containing AMPA receptor on the post-synaptic membrane, the effect of estradiol on PKMζ as an up-stream enzyme for the new AMPA receptor insertion in health and AD condition is still unclear. Therefore, in this study, we investigated the possible role of estradiol

in preserving synapse related proteins against oligomeric form of Aβ in cultured hippocampal cells.

Materials and methods

Preparation of AB oligomers

Oligomeric Aβ was produced using synthetic Aβ-42 (Abcam, USA) as previously described (Pourbadie et al., 2018). Briefly, Aβ 1–42 was dissolved in hexafluor-2-propanol to yield a 1mM. It is dried under hood in the room temperature, and then kept at -80℃. Aβ oligomers (oAβs) were prepared freshly by dissolving the peptide film with dimethyl sulfoxide (DMSO, Merck) and cold serum free F-12 medium without phenol red to obtain a 100μM stock. The stocks were then incubated at 4℃ for 24h.

Primary culture of hippocampal neurons

All experiments were carried out according to the Review Board and Ethics Committee of Pasteur Institute (Authorization code IR.PII.REC.1398.013 June 2019) and conforming to the European ommunities Council Directive 2010-63-EU. Primary hippocampal cell cultures were prepared from newborn (P0 to P3 days) Wistar rats. They were decapitated and the brains were removed. Hippocampi were isolated and collected in cold phosphate buffer saline (PBS) containing glucose and antibiotics (penicillin and streptomycin 1%) solution. They were trypsinized with trypsin (1 x, Gibco; 50μl per hippocampus) in PBS at 37℃ for 15min. Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS) was used to inhibit trypsin. Cells were mechanically dissociated and pelleted at 1000g. They were re-suspended in Neurobasal medium with 2% B27 (Gibco), 1% L-glutamine and 1% penicillin and streptomycin antibiotics (Gibco). This medium is a specific culture for neurons that provides almost pure neuronal population by suppressing glial cells (Valian et al., 2021). Primary cells were plated at a density of 100,000 cells/cm^2 onto poly-l-lysine (Sigma)-coated 12-well plates. Half of the growth medium was replaced once per week.

After two weeks, cells were treated with oAβ (1μM) (Heshmati-Fakhr et al., 2018; Yousefi et al., 2019). Twenty-four hours later, cells were treated with estradiol (Sigma; 100nM) (Kajta et al., 2001). After 24h, the supernatant was removed and cells were harvested in RIPA lysis buffer containing protease/phosphatase inhibitor cocktail (Roche, Germany) with a cell scraper and kept at -80℃ until use.

Western blot analysis

Twenty-four hours after the last treatment, cells were harvested, homogenized and lysed using lysis buffer. The lysates mixed with 2x laemmli sample buffer and then were heated in order to linearize the proteins. Twelve percent gel and sodium dodecyl sulfate-polyacrylamide gel electrophoresis were used to separate proteins of the cell lysates. The protein bands were transferred to polyvinylidene fluoride membranes that were then incubated in blocking buffer containing skim milk 2% and Tween-20 1% in Tris-buffered saline (TBS-T) for 2h. The membrane was incubated with the primary antibodies overnight at 4℃ and washed three times each 10min. The following primary antibodies were used: anti-glutamate receptor A1 (anti- GluA1, 1:1000, Merck Millipore) and anti-glutamate receptor A2 (GluA2, 1:1000, Merck Millipore), anti-PKMζ antibody (1:500, Millipore) and rabbit polyclonal anti-β-actin antibody (1:1000, Invitrogen). The membranes were incubated in horseradish peroxidase-conjugated secondary antibody (1:3000, Invitrogen) for 2h at room temperature and washed with TBS-T three times each 10min. The protein bands were illuminated using chemiluminescence detection kit (ECL, Amersham Bioscience) and were then exposed to an autoradiography film (Kodak). The Image J 1.50i software (National Institutes of Health) was used to quantify and normalize the protein bands to corresponding β-Actin band.

Statistical analysis

Shapiro-Wilk test was used to check the normal distribution of the data. After passing the normality test, one-way ANOVA followed by Tukey's posttest was used to determine the significant difference between groups. Data were presented as mean±SEM and *P*-values <0.05 was considered as significance. Analyses were performed using GraphPad Prism 6 software.

Results

Primary cells were treated after 14 days of culture. Representative images of cell morphology on day 14 are shown in Figure 1. No significant changes of cell morphology or neurite length was found following treatment with oAβ or estradiol (data not shown).

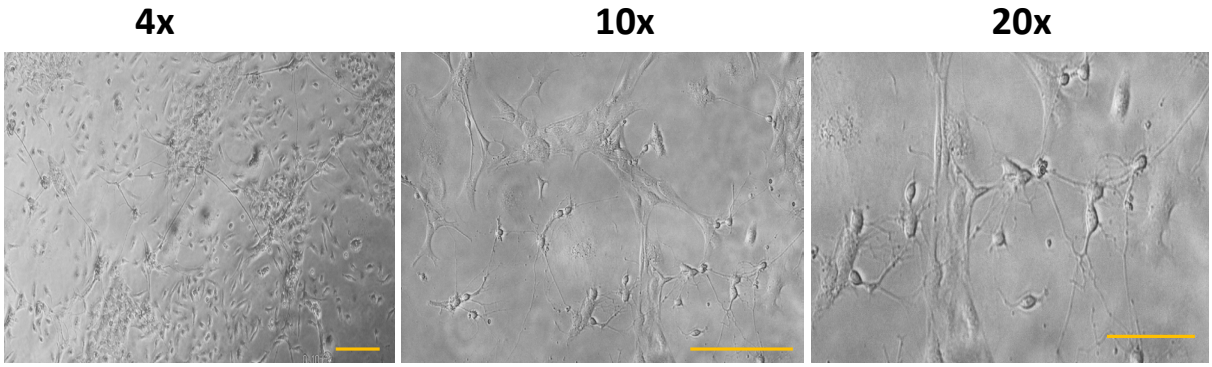

FIGURE 1. Representative images of hippocampal primary cell at day 14 of culture. Scale bar 4x and 10x: 100µm, 20x: 50 µm.

Estradiol reversed down expression of PKMζ induced by oligomeric Aβ in primary hippocampal cells

As depicted in Figure 2, incubation of primary hippocampal cell with 1µM oligomeric Aβ resulted in declined PKMζ level. Figure 2A depicts representative band densities of PKMζ in different groups. One-way ANOVA showed a significant difference among groups [F(3, 12)= 37.55, P<0.0001]. Further analysis with Tukey's Posttest revealed a significant difference between control and OAβ groups (P<0.01). However, treatment of the cells with estradiol reversed the Aβ effect and enhances PKMζ in Aβ treated cells toward control level. No significant difference was found between control cells and those treated with estradiol alone.

Estradiol preserved GluA1 and GluA2 AMPA receptor subtypes against OAβ in primary hippocampal cells

Figure 3 demonstrates that GluA1 and GluA2, the key subunits of AMPA receptors, expression level in primary hippocampal cells in different groups. Figure 3A and C depict representative band densities of GluA1 and GluA2, respectively. One-way ANOVA revealed a significant difference of GluA1 level among groups [F(3, 8)= 34.47, P<0.0001]. Tukey's posttest showed a significant decrease of GluA1 in cells treated with oAβ compared with control group (P<0. 001). Treatment of the cells with estradiol inhibited the declining effect of oAβ on GluA1 level. Estradiol alone significantly increased GluA1 level compared to the control group (P<0.05).

As shown in Figure 3D, GluA2 level was significantly decreased in oAβ treated cells compared to the control group (P<0.01). However, estradiol reversed the effect of oAβ and increased the level of GluA2 toward the control level. No significant difference of GluA2 level was found between control and estradiol alone (E2).

Analysis of GluA2/GluA1 ratio revealed a significant difference among experimental groups. The ratio was significantly decreased in oAβ treated cells compared to the control and E2 groups (P<0.01). Treatment of cells with estradiol restore the GluA2/GluA1 ratio in oAβ treated cells (Fig. 4).

Discussion

In the present study, we found that oligomeric Aβ decreased the expression level of PKMζ in primary hippocampal cells. Also, AMPA receptor subtypes, GluA1 and GluA2, were down-regulated in these cells following treatment with Aβ oligomers. While estradiol did not change PKMζ level in intact cells, it restored PKMζ and GluA1 and GluA2 level in Aβ treated cells.

One of the main hallmarks of Alzheimer's disease is the overproduction and accumulation of various forms of Aβ, which in advanced stages manifests as amyloid plaques deposition in brain tissue. Various studies have shown that Aβ oligomers are the most toxic form of amyloid to neurons (Shankar et al., 2008). They cause progressive synaptic dysfunction, induce neuronal apoptosis in brain regions involved in memory formation and eventually lead to memory loss. It has been shown that Aβ oligomers suppress synapses (Pourbadie et al., 2015; Pourbadie et al., 2016) by provoking the internalization of AMPA channels through protein kinase C dependent phosphorylation of serine 880 in GluA2 subunit (Liu et al., 2010). Two main forms of AMPA receptors are of particular importance in synaptic transmission, GluA1 and GluA2 containing AMPA channels. GluA1-containing AMPA channels are involved in induction of LTP, while those containing GluA2 are mainly involved in

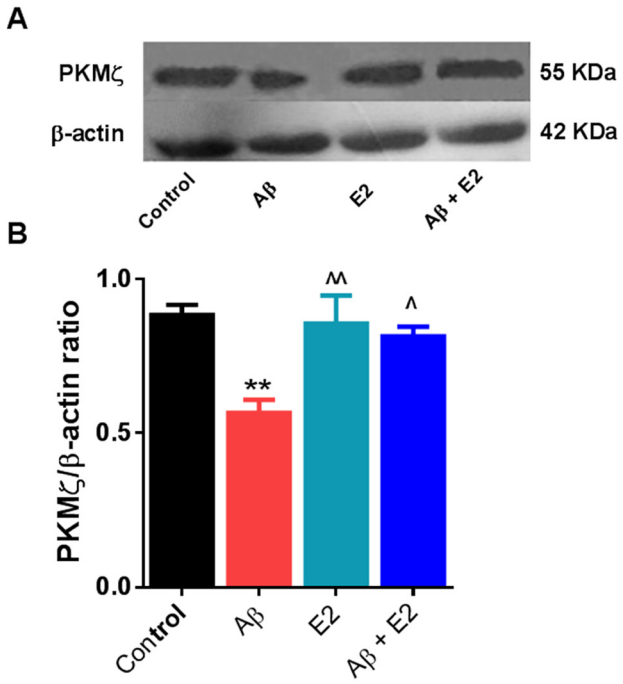

FIGURE 2. Effect of estradiol on PKMζ against Aβ in primary hippocampal cells. A) representative Western blot and band densities of PKMζ and corresponding β-actin in different groups. B) oAβ treated cells express lower PKMζ compared to the control group. Incubation of oAβ treated cells with estradiol for 24h increased the level of PKMζ toward the control level. No significant difference was found between control and estradiol alone groups. Data expressed and mean±SEM (n=4-5). $^{**}P<0.01$ compared to control group, $^{\wedge}P<0.05$ $^{\wedge\wedge}P<0.01$ compared to Aβ group.

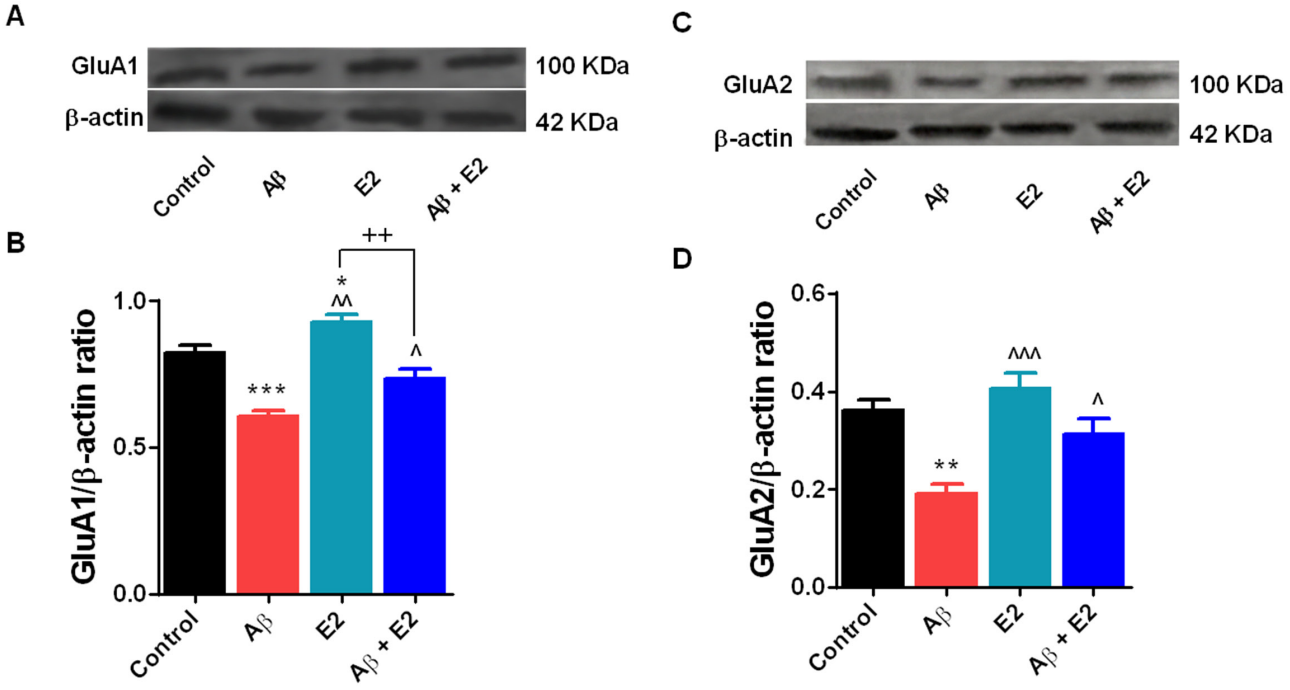

FIGURE 3. Effect of oligomeric Aβ and estradiol on AMPA receptor subtypes in primary hippocampal cells. A) Representative western blot and band densities of GluA1 and the corresponding β-actin in different groups. B) oAβ treated cells express lower GluA1 compared to the control group. Estradiol restored GluA1 level in oAβ treated cells. Estradiol alone also enhanced GluA1 expression level in healthy cells as compared with control group. C) Representative western blot and band densities of GluA2 and the corresponding β-actin in different groups. D) oAβ treated cells expressed lower GluA2 compared to the control group. However, treatment with estradiol increased GluA2 expression level in oAβ treated cells toward the control group. There was no significant difference of GluA2 between estradiol (E2) and control group. Data expressed and mean±SEM (n=4-5). *P<0.05, **P<0.01 and ***P<0.01 compared to control group. ^P<0.05, ^^P<0.01 and ^^^P<0.01 compared to Aβ group.

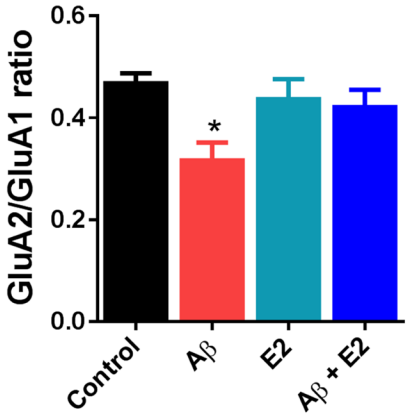

FIGURE 4. GluA2/ GluA1 ratio. OAβ decreased GluA2/GluA1 ratio in hippocampal pyramidal cells. Estradiol (E2) enhance the ratio but did not reach to the significant level compared to the Aβ group. $^{*}P<0.05$ compared to the control group.

maintenance of LTP. In other words, GluA1-channels play a role in short-term memory while GluA2 mediates long-term memory. The expression of both types has been shown to decrease with age (Pagliusi et al., 1994) or in AD (Zhang et al., 2018; Zhao et al., 2010).

In this study, we found that Aβ oligomer reduced GluA1 and GluA2 expression. The GluA2/ GluA1 ratio was also decreased, indicating a more prominent decline of GluA2 due to Aβ. In agreement, GluA2 down-regulation due to Aβ has been shown in multiple studies (Liu et al., 2010; Zhao et al., 2010). GluA2 decreases the channel permeability to Ca^{2+}, but GluA1 subunit allows high permeability the channel to Ca^{2+} (Cantanelli et al., 2014). Decreased GluA2/ GluA1 ratio induced by oAβ may render the neuronal cells vulnerable to excitotoxic synaptic dysfunction following disproportionate Ca^{2+} influx. Estradiol may protect neuronal cells by preserving GluA2 containing AMPA channels. In this regard, GluA2 knockdown has been reported to result in dendritic spine shrinkage, hippocampal cell death, learning and memory deficits and seizure vulnerability (Konen et al., 2020). Estradiol may combat Aβ, in part, through preserving or enhancing PKMζ/GluA2-AMPA receptors pathway in synapses. Consistently, PKMζ is shown to preserve long-term memories by increasing GluA2-containing AMPA receptors at synapses (Aliakbari et al., 2021; Amini et al., 2021; Yao et al., 2008).

In 2002, an isoform of protein kinase C, resembling catalytic domain of protein kinase C with irreversible activity, was identified which is expressed exclusively in brain (Ling et al., 2002). This enzyme, PKMζ, increases the trafficking of GluA2-AMPA channels in favor of increasing the channel quantity on the synaptic membrane through N-ethylmaleimide-sensitive factor dependent pathway (Lüthi et al., 1999). It also blocks removal of AMPA receptors from synaptic sites. Additionally, PKMζ has been reported to maintain long-term memories by increasing the number of GluA2-containing AMPA receptors at postsynaptic sites (Yao et al., 2008). Interestingly, in this study we found that treatment of hippocampal primary cells with Aβ oligomer reduced the expression level of PKMζ. This finding was in contrast to our previous finding that we did not observe a significant decline of PKMζ level due to Aβ (Amini et al., 2021). This discrepancy may be due to different methodological approach. Indeed, in the previous study, Aβ oligomer was injected locally into the dorsal part of the hippocampus, while PKMζ expression levels were assessed in the whole hippocampus. Therefore, the ventral part of the hippocampus that was not exposed to Aβ may mask the PKMζ change in the dorsal part. However, in this study cultured hippocampal cells were uniformly exposed to the Aβ oligomer.

AD is the five and eight leading cause of death, respectively for women and men in the United States (Alzheimer's and Dementia 2017). Treatment of postmenopausal women with estrogen-only or estrogen-progestogen hormonal replacement therapy slowdowns cognitive decline and decrease risk of AD (Zandi et al., 2002). Therefore, the lack of sex hormones such as estrogens can play a key role in cognitive function. In this regard, it has been shown that estradiol can improve memory impairment in AD models (Koss and Frick, 2019). Estradiol has also been shown to rapidly localize AMPA

channels at the synaptic membrane surface and enhance synaptic function and subsequent cognitive function (Avila et al., 2017). It is not yet known that how estradiol increases the number of AMPA channels, especially GluA2 containing AMPAs in the synaptic membrane.

In this study, we showed that estradiol prevented the decline of GluA1 and GluA2 level due to Aβ in hippocampal primary cells. We found that estradiol alone enhanced GluA1, but not GluA2, in healthy hippocampal primary cells. Consistently, Zadran et al. (2009) reported that membrane levels of GluR1 (GluA1) but not GluR2/3 (GluA2/3) subunits of AMPA receptors are increased following administration of estradiol on acute hippocampal slices. Estradiol may have genomic and non-genomic effect on synaptic function. We did not investigate AMPA channel trafficking on synaptic membrane. In the first phase, estradiol may cause the AMPA channel to move from intracellular pool to the synaptic membrane, which occurs in a fraction of a second, thereby increasing synaptic function as previously reported (Hasegawa et al., 2015).

It should be also acknowledged that high level of GluA2 in intact cells may reflect a ceiling effect, occluding estradiol relevant synaptic GluA2-AMPA enhancement. There are multiple studies showing that estradiol may enhance activation of NMDA receptors/channels or an increase in AMPA receptor function (Zhang et al., 2018; Zhao et al., 2010). However, the underlying mechanism is not yet elucidated. Interestingly, we found that estradiol maintained PKMζ expression in hippocampal primary cells against Aβ. It has been reported that hippocampal primary neurons are more sensitive to Aβ neurotoxicity compared with cortical primary cells. Furthermore, primary hippocampal cells treated with Aβ peptide (Aβ 25-35) showed a decrease in GluA2 level (Resende et al., 2007). In parallel with our finding, it is reported that GluA2/GluA1 ratio is decreased due to Aβ that contributed in LTD induction (Miñano-Molina et al., 2011). On the other hand, estradiol has been shown to facilitate the expression of LTP at CA3-CA1 synapses (Foy et al., 1999). It was also reported that only E2 application or E2 plus progesterone *in vivo* resulted in increased expression of AMPA receptors and AMPA receptor binding levels in the hypothalamus (Brann and Mahesh, 1994; Fugger et al., 2001; Ferri et al., 2014).

Conclusion

In conclusion, the findings of our study indicate that estradiol restores GluA1 and GluA2 expression level in Aβ treated primary hippocampal cells. It may act partly through preserving PKMζ molecule in these cells. However, further studies are needed to elucidate how estradiol modulate PKMζ expression.

Acknowledgement

This research was financially supported by a grant no, 972808 from National Institute for Medical Research Development (NIMAD).

Conflict of interest

We have no conflicts of interest to disclose.

References

Aliakbari S, Sayyah M, Mirzapourdelavar H, Amini N, Naghdi N, Pourbadie HG. Overexpression of protein kinase Mζ in the hippocampal dentate gyrus rescues amyloid-β-induced synaptic dysfunction within entorhinal-hippocampal circuit. Neurobiol Aging 2021; 101: 160-71. https://doi.org/10.1016/j.neurobiolaging.2021.01.017

Alzheimer's Association. 2017 Alzheimer's disease facts and figures. Alzheimer's & Dementia 2017; 13: 325-373.

Amini N, Azad RR, Motamedi F, Mirzapour-Delavar H, Ghasemi S, Aliakbari S, et al. Overexpression of protein kinase Mζ in the hippocampus mitigates Alzheimer's disease-related cognitive deficit in rats. Brain Res Bull 2021; 166: 64-72. https://doi.org/10.1016/j.brainresbull.2020.11.001

Avila JA, Alliger AA, Carvajal B, Zanca RM, Serrano PA, Luine VN. Estradiol rapidly increases GluA2-mushroom spines and decreases GluA2-filopodia spines in hippocampus CA1. Hippocampus 2017; 27: 1224-9. https://doi.org/10.1002/hipo.22768

Brann DW, Mahesh VB. Excitatory amino acids: function and significance in reproduction and neuroendocrine regulation. Front Neuroendocrinol 1994; 15: 3-49. https://doi.org/10.1006/frne.1994.1002

Brookmeyer R, Evans DA, Hebert L, Langa KM, Heeringa S G, Plassman BL, et al. National estimates of the prevalence of Alzheimer's disease in the United States. Alzheimers Dement 2011; 7: 61-73. https://doi.org/10.1016/j.jalz.2010.11.007

Cantanelli P, Sperduti S, Ciavardelli D, Stuppia L, Gatta V, Sensi SL. Age-dependent modifications of AMPA receptor

subunit expression levels and related cognitive effects in 3xTg-AD mice. Front Aging Neurosci 2014; 6: 200. https://doi.org/10.3389/fnagi.2014.00200

Carter CL, Resnick EM, Mallampalli M, Kalbarczyk A. Sex and gender differences in Alzheimer's disease: recommendations for future research. J Womens Health (Larchmt) 2012; 21: 1018-23. https://doi.org/10.1089/jwh.2012.3789

Evin G, Weidemann A. Biogenesis and metabolism of Alzheimer's disease Aβ amyloid peptides. Peptides 2002; 23: 1285-97. https://doi.org/10.1016/S0196-9781(02)00063-3

Ferri S L, Hildebrand P F, Way S E, Flanagan-Cato L M. Estradiol regulates markers of synaptic plasticity in the hypothalamic ventromedial nucleus and amygdala of female rats. Horm Behav . 2014; 66: 409-420. https://doi.org/10.1016/j.yhbeh.2014.06.016

Foy MR, Xu J, Xie X, Brinton RD, Thompson RF, Berger TW. 17beta-estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. J Neurophysiol 1999; 81: 925-9. https://doi.org/10.1152/jn.1999.81.2.925

Fugger HN, Kumar A, Lubahn DB, Korach KS, Foster TC. Examination of estradiol effects on the rapid estradiol mediated increase in hippocampal synaptic transmission in estrogen receptor α knockout mice. Neurosci Lett 2001; 309: 207-9. https://doi.org/10.1016/S0304-3940(01)02083-3

Guntupalli S, Widagdo J, Anggono V. Amyloid-β-induced dysregulation of AMPA receptor trafficking. Neural Plast 2016; 2016: 3204519. https://doi.org/10.1155/2016/3204519

Hasegawa Y, Hojo Y, Kojima H, Ikeda M, Hotta K, Sato R, et al. Estradiol rapidly modulates synaptic plasticity of hippocampal neurons: involvement of kinase networks. Brain Res 2015; 1621: 147-61. https://doi.org/10.1016/j.brainres.2014.12.056

Heshmati-Fakhr N, Sotoodehnejadnematalahi F, Yousefi N, Sayyah M, Hosseini SM, Pourbadie HG. Triggering microglia through toll-like receptor 2 pathway induced interferon β expression in cell and animal model of Alzheimer's disease. Neuroreport 2018; 29: 1456-62. https://doi.org/10.1097/WNR.0000000000001132

Irvine K, Laws KR, Gale TM, Kondel TK. Greater cognitive deterioration in women than men with Alzheimer's disease: a meta analysis. J Clin Exp Neuropsychol 2012; 34: 989-98. https://doi.org/10.1080/13803395.2012.712676

Kajta M, Budziszewska B, Marszal M, Lason W. Effects of 17-beta estradiol and estriol on NMDA-induced toxicity and apoptosis in primary cultures of rat cortical neurons. J Physiol Pharmacol. 2001; 52: 437-46

Konen LM, Wright AL, Royle GA, Morris GP, Lau BK, Seow PW, et al. A new mouse line with reduced GluA2 Q/R site RNA editing exhibits loss of dendritic spines, hippocampal CA1-neuron loss, learning and memory impairments and NMDA receptor-independent seizure vulnerability. Mol Brain 2020; 13: 1-19. https://doi.org/10.1186/s13041-020-0545-1

Koss W, Frick KM. Activation of androgen receptors protects intact male mice from memory impairments caused by aromatase inhibition. Horm Behav 2019; 111:96-104. https://doi.org/10.1016/j.yhbeh.2019.01.002

Kramár EA, Babayan AH, Gall CM, Lynch G. Estrogen promotes learning-related plasticity by modifying the synaptic cytoskeleton. Neuroscience 2013; 239: 3-16. https://doi.org/10.1016/j.neuroscience.2012.10.038

Ling DSF, Benardo LS, Serrano PA, Blace N, Kelly MT, Crary JF, et al. Protein kinase Mζ is necessary and sufficient for LTP maintenance. Nat Neurosci 2002; 5: 295-6. https://doi.org/10.1038/nn829

Liu SJ, Gasperini R, Foa L, Small DH. Amyloid-beta decreases cell-surface AMPA receptors by increasing intracellular calcium and phosphorylation of GluR2. J Alzheimers Dis 2010; 21: 655-66. https://doi.org/10.3233/JAD-2010-091654

Lu W, Shi Y, Jackson A C, Bjorgan K, During MJ, Sprengel R, et al. Subunit composition of synaptic AMPA receptors revealed by a single-cell genetic approach. Neuron 2009; 62: 254-68. https://doi.org/10.1016/j.neuron.2009.02.027

Lüthi A, Chittajallu R, Duprat F, Palmer MJ, Benke TA, Kidd FL, et al. Hippocampal LTD expression involves a pool of AMPARs regulated by the NSF-GluR2 interaction. Neuron 1999; 24: 389-99. https://doi.org/10.1016/S0896-6273(00)80852-1

Miñano-Molina AJ, España J, Martín E, Barneda-Zahonero B, Fadó R, Solé M, et al. Soluble oligomers of amyloid-β peptide disrupt membrane trafficking of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor contributing to early synapse dysfunction. J Biol Chem 2011; 286: 27311-21. https://doi.org/10.1074/jbc.M111.227504

Pagliusi SR, Gerrard P, Abdallah M, Talabot D, Catsicas S. Age-related changes in expression of AMPA-selective glutamate receptor subunits: is calcium-permeability altered in hippocampal neurons? Neuroscience 1994; 61: 429-33. https://doi.org/10.1016/0306-4522(94)90422-7

Plant K, Pelkey KA, Bortolotto Z A, Morita D, Terashima A, McBain CJ, et al. Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. Nat Neurosci 2006; 9: 602-4. https://doi.

org/10.1038/nn1678

Pourbadie HG, Naderi N, Janahmadi M, Mehranfard N, Motamedi F. Calcium channel blockade attenuates abnormal synaptic transmission in the dentate gyrus elicited by entorhinal amyloidopathy. Synapse 2016; 70: 408-17. https://doi.org/10.1002/syn.21915

Pourbadie HG, Naderi N, Mehranfard N, Janahmadi M, Khodagholi F, Motamedi F. Preventing effect of L-type calcium channel blockade on electrophysiological alterations in dentate gyrus granule cells induced by entorhinal amyloid pathology. Plos one 2015; 10: e0117555. https://doi.org/10.1371/journal.pone.0117555

Pourbadie HG, Sayyah M, Khoshkholgh-Sima B, Choopani S, Nategh M, Motamedi F, et al. Early minor stimulation of microglial TLR2 and TLR4 receptors attenuates Alzheimer's disease-related cognitive deficit in rats: behavioral, molecular, and electrophysiological evidence. Neurobiol Aging 2018; 70: 203-16. https://doi.org/10.1016/j.neurobiolaging.2018.06.020

Resende R, Pereira C, Agostinho P, Vieira A, Malva JO, Oliveira CR. Susceptibility of hippocampal neurons to Aβ peptide toxicity is associated with perturbation of Ca2+ homeostasis. Brain Res 2007; 1143: 11-21. https://doi.org/10.1016/j.brainres.2007.01.071

Rui Y, Gu J, Yu K, Hartzell HC, Zheng JQ. Inhibition of AMPA receptor trafficking at hippocampal synapses by β-amyloid oligomers: the mitochondrial contribution. Mol Brain 2010; 3: 1-13. https://doi.org/10.1186/1756-6606-3-10

Schuette SR, Fernández-Fernández D, Lamla T, Rosenbrock H, Hobson S. Overexpression of protein kinase Mζ in the hippocampus enhances long-term potentiation and long-term contextual but not cued fear memory in rats. J Neurosci 2016; 36: 4313-24. https://doi.org/10.1523/JNEUROSCI.3600-15.2016

Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med 2016; 8: 595-608. https://doi.org/10.15252/emmm.201606210

Selkoe DJ, Podlisny MB. Deciphering the genetic basis of Alzheimer's disease. Annu Rev Genomics Hum Genet 2002; 3: 67-99. https://doi.org/10.1146/annurev.genom.3.022502.103022

Sengupta U, Nilson AN, Kayed R. The role of amyloid-β oligomers in toxicity, propagation, and immunotherapy. EBioMedicine 2016; 6: 42-9. https://doi.org/10.1016/j.ebiom.2016.03.035

Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, et al. Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 2008; 14: 837-42. https://doi.org/10.1038/nm1782

Shao CY, Sondhi R, van de Nes PS, Sacktor TC. PKMζ is necessary and sufficient for synaptic clustering of PSD-95. Hippocampus 2012; 22: 1501-7. https://doi.org/10.1002/hipo.20996

Shema R, Sacktor TC, Dudai Y. Rapid erasure of long-term memory associations in the cortex by an inhibitor of PKM zeta. Science 2007; 317: 951-3. https://doi.org/10.1126/science.1144334

Smith CC, McMahon L. Estrogen-induced increase in the magnitude of long-term potentiation occurs only when the ratio of NMDA transmission to AMPA transmission is increased. J Neurosci 2005; 25: 7780-91. https://doi.org/10.1523/JNEUROSCI.0762-05.2005

Uddin MS, Rahman MM, Jakaria M, Rahman MS, Hossain MS, Islam A, et al. Estrogen signaling in Alzheimer's disease: molecular insights and therapeutic targets for Alzheimer's dementia. Mol Neurobiol 2020; 57: 2654-70. https://doi.org/10.1007/s12035-020-01911-8

Valian N, Heravi M, Ahmadiani A, Dargahi L. Comparison of Rat Primary Midbrain Neurons Cultured in DMEM/F12 and Neurobasal Mediums. asic Clin Neurosci. 2021 Mar-Apr; 12: 205–212. https://doi.org/ 10.32598/bcn.12.2.1568.1

Yao Y, Kelly MT, Sajikumar S, Serrano P, Tian D, Bergold PJ, et al. PKM zeta maintains late long-term potentiation by N-ethylmaleimide-sensitive factor/GluR2-dependent trafficking of postsynaptic AMPA receptors. J Neurosci 2008; 28: 7820-7. https://doi.org/10.1523/JNEUROSCI.0223-08.2008

Yousefi N, Sotoodehnejadnematalahi F, Heshmati-Fakhr N, Sayyah M, Hoseini M, Ghassemi S, et al. Prestimulation of microglia through TLR4 pathway promotes interferon beta expression in a rat model of Alzheimer's disease. J Mol Neurosci 2019; 67: 495-503. https://doi.org/10.1007/s12031-018-1249-1

Zadran S, Qin Q, Bi X, Zadran H, Kim Y, Foy MR, et al. 17-Beta- estradiol increases neuronal excitability through MAP kinase-induced calpain activation. PNAS 2009; 106: 21936-41. https://doi.org/10.1073/pnas.0912558106

Zandi P P, Carlson M C, Plassman B L, Welsh-Bohmer K A, Mayer L S, Steffens D C, et al. Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. JAMA 2002; 288: 2123-2129. https://doi.org/10.1001/jama.288.17.2123

Zhang Y, Guo O, Huo Y, Wang G, Man HY. Amyloid-β induc-

es AMPA receptor ubiquitination and degradation in primary neurons and human brains of Alzheimer's disease. J Alzheimers Dis 2018; 62: 1789-801. https://doi.org/10.3233/JAD-170879

Zhao WQ, Santini F, Breese R, Ross D, Zhang XD, Stone DJ, et al. Inhibition of calcineurin-mediated endocytosis and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors prevents amyloid β oligomer-induced synaptic disruption. J Biol Chem 2010; 285: 7619-32. https://doi.org/10.1074/jbc.M109.057182