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Repression of the eIF2 α kinase PERK alleviates mGluR-LTD impairments in a mouse model of Alzheimer's disease

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Abstract

Mounting evidence indicates that impairments of synaptic efficacy/plasticity may be a key step in the development of Alzheimer's disease (AD) pathophysiology. Among the two major forms of synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD), much less is known about how LTD is regulated in AD and its molecular mechanisms. Recent studies indicate that metabotropic glutamate receptor 5 (mGluR5) may function as a receptor/co-receptor for Amyloid beta (A β). Herein we examined mGluR-LTD in hippocampal slices from aged APP/PS1 mutant mice that model AD. Our findings demonstrate that mGluR-LTD is blocked in APP/PS1 mice, and that the mGluR-LTD failure is reversed by either genetically or pharmacologically suppressing the activity of PERK, a kinase for the mRNA translation factor eIF2 α . These data are congruent with recent evidence that inhibition of eIF2 α phosphorylation via PERK suppression and reversal of *de novo* protein synthesis deficits can mitigate cognitive deficits in neurodegenerative diseases. Together with reports indicating that mGluR5 may mediate A β synaptotoxicity, our findings offer insights into novel therapeutic targets for AD and other cognitive syndromes.

Keywords

Alzheimer's disease; mGluR-LTD; PERK; eIF2 α ; protein synthesis; synaptic plasticity

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1. Introduction

Abundant evidence indicates that synaptic dysfunction that results in compromised synaptic efficacy/plasticity (the ability of synapses to strengthen or weaken over time) may be a key step during the development of Alzheimer's disease (AD) pathophysiology (Selkoe, 2002, Oddo et al., 2003, Jacobsen et al., 2006, Ma et al., 2010, Tomiyama et al., 2010, Ma and Klann, 2012). Thus, understanding the molecular signaling mechanisms underlying these synaptic impairments could yield insights into therapeutic targets for AD. Long-term potentiation (LTP) and long-term depression (LTD) are the intensely studied forms of synaptic plasticity, that work in concert to mediate learning and memory as well as several other forms of experience-dependent changes in brain function (Malenka and Bear, 2004). LTP has been extensively studied in AD, either in the context of exogenous application of soluble beta-amyloid (A β), abnormal accumulation of which represents a brain pathology hallmark for AD, or in various transgenic mouse models of AD (Walsh et al., 2002, Rowan et al., 2005, Li et al., 2011, Ma and Klann, 2012). In contrast, very few studies have examined how the molecular mechanisms of LTD are altered in AD models. Moreover, to our knowledge, most of the previous LTD studies in AD have been conducted either in the context of acute exogenous A β application or relatively young (less than one year old), but not aged, transgenic mouse models of AD (Kim et al., 2001, Wang et al., 2002, Raymond et al., 2003, Li et al., 2009, D'Amelio et al., 2011, Ma et al., 2012, Megill et al., 2015).

There are two well studied, mechanistically distinct forms of LTD: N-methyl-D-aspartate receptor (NMDAR)-dependent LTD and metabotropic glutamate receptor (mGluR)-dependent LTD. NMDAR-dependent LTD is usually induced by electrical low frequency stimulation (LFS) and requires activation of NMDARs, whereas mGluR-dependent LTD requires activation of group 1 mGluRs (includes mGluR1 and 5) and is often induced by DHPG [(RS)-3,5-dihydroxyphenylglycine], a selective agonist for group 1 mGluRs (Malenka and Bear, 2004, Lüscher and Huber, 2010). Of interest, recent studies indicate that mGluR5 may function as a receptor/co-receptor for A β , and an mGluR5 antagonist rescued cognitive defects in AD model mice (Um et al., 2013, Hu et al., 2014). However, the downstream signaling mechanisms mediating the above effects remain unclear.

Long-lasting forms of both LTP and LTD depend on intact mechanisms of *de novo* protein synthesis (Klann and Dever, 2004, Costa-Mattioli et al., 2009, Richter and Klann, 2009, Rosenberg et al., 2014). Protein synthesis is a highly regulated process, particularly at the initiation phase, and involves various translational factors including eukaryotic initiation factor 2 (eIF2), which plays a key role in synaptic plasticity and memory formation (Klann et al., 2004, Costa-Mattioli et al., 2007, Trinh and Klann, 2013). It is generally considered that in response to specific cellular stress, one (or more) of the four eIF2 kinases -- PKR, HRI, GCN2, or PERK, is activated to phosphorylate eIF2 on the α subunit, leading to inhibition of general protein synthesis and potentially memory impairments if the decreases in protein synthesis are long-lasting (Wek et al., 2006, Wek and Cavener, 2007, Trinh and Klann, 2013, Ma and Klann, 2014). Notably, recent studies have suggested a link between neurodegenerative diseases, including prion disease and AD, and disruption of translational homeostasis due to abnormal PERK/eIF2 α signaling. In particular, hyper-phosphorylation of eIF2 α via PERK activation is associated with AD and prion disease, and repression of

PERK activity rescues cognitive deficits in mouse models of AD, prion disease, and frontotemporal dementia (Moreno et al., 2012, Ma et al., 2013, Moreno et al., 2013, Ma and Klann, 2014, Radford et al., 2015).

Herein, we studied mGluR-LTD at CA3-CA1 synapses in hippocampal slices from aged APP/PS1 AD model mice. Our findings demonstrate that mGluR-LTD is blocked in AD mice, and importantly, the mGluR-LTD blockade is reversed by either genetically or pharmacologically suppressing activity of eIF2 α kinase PERK. These data are congruent with evidence that inhibition of eIF2 α phosphorylation via PERK suppression and consequently improved *de novo* protein synthesis can mitigate cognitive deficits in neurodegenerative diseases (Ma and Klann, 2014). Together with reports indicating that mGluR5 may mediate synaptotoxicity of A β (Um et al., 2013, Hu et al., 2014), our findings offer insights into novel therapeutic targets for AD and other cognitive syndromes.

2. Materials & Methods

2.1. Mice

All mice were housed in a barrier facility dedicated to transgenic mice at Wake Forest University School of Medicine. The facility operates in accordance with standards and policies of the *US Department of Agriculture's Animal Welfare Information Center* (AWIC), and the *NIH Guide for Care and Use of Laboratory Animals*. The facility is kept on a 12 h light/dark cycle, with a regular feeding and cage-cleaning schedule. Mice of either sex were used. APP/PS1 transgenic mice (APP^{swe} + PSEN1⁻ E9) were purchased from the Jackson Laboratory (Jankowsky et al., 2001). PERK forebrain conditional knockout mice (PERK cKO) were generated as described previously (Trinh et al., 2012). Creation of APP/PS1/PERK cKO double mutant mice was done as described previously (Ma et al., 2013). All genotypes were verified by polymerase chain reaction (PCR). Mice were used at the age of 12–15 months, except for the exogenous A β application experiments, for which wild type mice were used at the age of 3–6 months.

2.2. Hippocampal slices preparation and electrophysiology

Hippocampal slices were prepared as described previously (Ma et al., 2011). For electrophysiology experiments, slices were transferred to recording chambers (preheated to 32 °C) where they were superfused with oxygenated ACSF. Monophasic, constant-current stimuli (100 μ sec) were delivered with a concentric bipolar microelectrode (FHC Inc., Bowdoin, ME) placed in the stratum radiatum of area CA3, and the field excitatory postsynaptic potentials (fEPSPs) were recorded in the stratum radiatum of area CA1. fEPSPs were acquired, and amplitudes and maximum initial slopes measured, using pClamp 10 (Axon Instruments, Foster City, CA). To induce mGluR-LTD, slices were perfused with DHPG (100 μ M in ACSF) for 10 min.

2.3. Drug treatment

DHPG (Abcam, Cambridge, MA) was prepared as stock solution in distilled water and was diluted into a final concentration 100 μ M immediately preceding the experiments. Only DHPG stock prepared within one week was used in the experiments. PERK inhibitor I

(GSK2606414, Calbiochem/Millipore) was prepared as stock solution in DMSO and was diluted to its final concentration of 1 μ M before conducting experiments. A β (1–42) stock (100 μ M, Bachem) was prepared as described previously (Ma et al., 2010).

2.4. Western blot

As previously described (Ma et al., 2013), mouse hippocampal slices were flash-frozen on dry ice after drug treatment, followed by standard procedure for Western blot. All primary and secondary antibodies were diluted in blocking buffer. Blots were probed with primary antibodies for phospho-eIF2 α (1:1000; Cell Signaling), eIF2 α (1:1000; Cell Signaling), PERK (1:1000; Santa Cruz), and GAPDH (1:10,000, Cell Signaling). Protein bands were visualized using chemiluminescence (ClarityTM ECL; Biorad) and the Biorad ChemiDocTM MP Imaging System. Densitometric analysis was performed using ImageJ.

2.5. Data analysis

Data are presented as mean $\pm$ SEM. Summary data are presented as group means with standard error bars. For comparison between two groups, a two-tailed independent Student's *t*-test was used. For comparisons between multiple groups, ANOVA was used followed by individual *post hoc* tests when applicable. Error probabilities of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Hippocampal mGluR-LTD is impaired in APP/PS1 mice but unaltered with application of exogenous A β

We first asked whether mGluR-LTD was altered in AD model mice by using APP/PS1 mutant mice, a well-established AD model (Jankowsky et al., 2001). mGluR-LTD was induced at CA3-CA1 synapses (Schaffer collateral pathway) in hippocampal slices with application of group 1 mGluR agonist DHPG (100 μ M for 10 minutes). For slices derived from wild type (WT) mice, DHPG incubation reliably induced LTD lasting over 1 hour (Fig. 1A–C). In comparison, DHPG induced only transient LTD in slices from APP/PS1 mice (Fig. 1A–C).

Previous studies demonstrated that exogenous A β application results in enhancement of NMDAR-dependent LTD (Li et al., 2009, Ma et al., 2012). To examine the effects of A β on mGluR LTD, we applied DHPG to hippocampal slices pre-treated with A β (1–42) (A β , 500 nM). To our surprise, we found no significant difference in mGluR-LTD between slices treated with vehicle (Fig. 1D–F) and A β (Fig. 1D–F). Taken together, these findings indicate that mGluR-LTD is blocked in the aged, chronic AD model mice.

3.2. Conditional PERK gene deletion reverses mGluR-LTD failure in APP/PS1 mice

mGluR-LTD requires *de novo* protein synthesis (Huber et al., 2000, Hou et al., 2006). In addition, it was demonstrated recently that abnormal hyperphosphorylation of eIF2 α due to elevated PERK activity resulted in decreased *de novo* protein synthesis in the hippocampus of AD model mice (Ma et al., 2013). Therefore we investigated whether mGluR-LTD failure in APP/PS1 mice could be alleviated by restoring translational homeostasis via the genetic

suppression of PERK. We bred mice harboring a floxed *PERK* gene with mice expressing a brain-specific *Cre* recombinase to generate mice in which PERK was conditionally removed in excitatory neurons in the forebrain late in development (PERK cKO). We further generated a mutant mouse line that expressed both *APP^{swe}/PS1^{E9}* and homozygous *Cre PERK^{-/-}* flox transgenes (APP/PS1/PERK cKO), in which both eIF2 α hyperphosphorylation and *de novo* protein synthesis defects in AD mice were corrected (Ma et al., 2013). We found that hippocampal mGluR-LTD was not altered significantly in PERK cKO mice (Fig. 1G–I), compared to WT control mice (Fig. 1G–I). Notably, in slices from APP/PS1/PERK cKO mutant mice, DHPG successfully induced mGluR-LTD that was indistinguishable from WT control mice (Fig. 1G–I). These findings suggested that suppression of eIF2 α phosphorylation via the genetic removal of PERK in excitatory neurons improves AD-associated mGluR-LTD deficits.

3.3. Hippocampal mGluR-LTD impairments in APP/PS1 mice are rescued by a selective inhibitor of PERK

In addition to the genetic deletion of PERK, we used a pharmacological approach to examine the effects of repressing PERK/eIF2 α signaling on the mGluR-LTD impairments in AD mice. Recently it was reported that a newly characterized and specific inhibitor of PERK (GSK2606414) rescued memory deficits and brain pathology in a mouse model of prion disease (Moreno et al., 2013), and brain pathology in a mouse model of frontotemporal dementia (Radford et al., 2015). Notably, in both of these studies, the rescuing effects of the PERK inhibitor were associated with inhibition of PERK/eIF2 α signaling and restored levels of protein synthesis (Moreno et al., 2013, Radford et al., 2015). We first demonstrated that treatment of slices with GSK2606414 caused reduction of eIF2 α phosphorylation, but did not affect levels of total eIF2 α or PERK (Fig. 2A). Moreover, in slices from APP/PS1 mice treated with GSK2606414, DHPG-induced mGluR-LTD (Fig. 2B–D) was indistinguishable from WT control slices treated with the inhibitor (Fig. 2B–D). We further demonstrated that GSK2606414 by itself did not alter mGluR-LTD in WT slices (Fig. 2E–G) when compared to vehicle-treated WT slices (Fig. 2E–G). Additionally, the PERK inhibitor did not alter basal synaptic function in either WT or APP/PS1 mice, as indicated by the findings that neither fEPSP baseline nor synaptic input-output relationships was affected by GSK2606414 (Fig. 2H–J, and data not shown). Taken together, mGluR-LTD failure in APP/PS1 AD model mice can be rescued by PERK inhibitor GSK2606414.

4. Discussion

Long-lasting forms of synaptic plasticity including LTP and LTD remain the prime molecular mechanisms associated with learning and memory (Malenka and Bear, 2004). This is consistent with the notion that AD, the most common form of dementia in elderly, has been called a disease of “synaptic failure” (Selkoe, 2002). Numerous studies have indicated that deficiencies in neuronal plasticity characterize an early and sustaining key pathophysiology in AD (Ma and Klann, 2012). Consequently, elucidation of the detailed molecular mechanisms by which the neuronal plasticity impairments occur may present important opportunities for therapeutic discoveries. Here we describe for the first time that mGluR-LTD, a form of synaptic plasticity implicated in cognitive function mechanisms

(Lüscher and Huber, 2010), is inhibited in an aged AD mouse model. Importantly, AD-associated inhibition of mGluR-LTD was reversed by repressing the eIF2 α kinase PERK with either genetic or pharmacological approaches.

Many studies have demonstrated that A β impacts both LTP (inhibition) and LTD (enhancement) that are NMDAR-dependent (Shankar et al., 2008, Li et al., 2009, Ma et al., 2014). In contrast, we found that exogenous A β application does not affect mGluR-LTD (Fig. 1D–F). Besides factors that might be attributed to A β sources (synthetic vs. human extracts) and preparation (different oligomer species), our findings suggest that acute A β application targets mainly at signaling pathways connected to NMDAR-dependent neuronal plasticity. In contrast, hippocampal mGluR-LTD was impaired in aged APP/PS1 AD model mice (Fig. 1A–C), indicating that the chronic accumulation of A β with aging impairs cellular mechanisms underlying mGluR-LTD. One such mechanism could be *de novo* protein synthesis, which is essential for long-lasting synaptic plasticity, including both LTP and LTD, regardless of whether they are NMDAR- or mGluR-dependent (Richter and Klann, 2009). In agreement with this concept, repression of eIF2 α kinase PERK in APP/PS1 mice, which improves *de novo* protein synthesis (Ma et al., 2013), can alleviate AD-associated mGluR-LTD defects (Fig. 1G–I and Fig. 2). Of interest, it was reported that hippocampal mGluR-LTD was enhanced in PERK cKO mice at young ages (4–5 weeks) (Trinh et al., 2014). In contrast, here we observed normal mGluR-LTD in old (12–15 month) PERK cKO mice (Fig. 1G–I), suggesting aging-related alterations in the synaptic response to DHPG when PERK/eIF2 α signaling is repressed. It also should be noted that to induce mGluR-LTD in aged mice, DHPG must be applied to slices at a concentration of 100 μ M, compared to 50 μ M DHPG used in young mice (Trinh et al., 2014). Therefore, the relatively high dose DHPG might cause a “ceiling” effect at synapses so that the activation of mGluR is either maximized or saturated, which is associated with the blunting of mGluR-LTD enhancement in PERK cKO mice (Fig. 1G–I) and WT mice treated with the PERK inhibitor (Fig. 2). The four kinases for eIF2 α (PERK, GCN2, PKR, and HRI) were initially categorized and named for their response to a different cellular stress associated with activation of a single kinase (Wek et al., 2006). However, accumulating evidence indicates that in situations such as oxidative stress, which is linked to AD pathogenesis, multiple eIF2 α kinases (especially PERK and GCN2) are recruited either simultaneously or sequentially to help maintain cellular homeostasis (Zhan et al., 2004, Hamanaka et al., 2005). In agreement with this idea, genetically reducing the expression of either PERK or GCN2 was shown to improve NMDAR-LTP impairments in AD mice (Ma et al., 2013). Meanwhile, PERK appears to play a dominant role in controlling eIF2 α activity in brain based on the findings that basal levels of eIF2 α phosphorylation are decreased by PERK deletion but not by removal of genes for other eIF2 α kinases such as GCN2 or PKR (Ma et al., 2013). Although future investigation of aging-dependent alterations in translational control via each of the eIF2 α kinases is still required, therapeutics that normalize eIF2 α phosphorylation has potential for treatment of AD and other neurodegenerative disorders.

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Highlights

- Hippocampal mGluR-LTD is impaired in APP/PS1 AD model mice
- Exogenous synthetic A β application does not affect hippocampal mGluR-LTD.
- Brain-specific deletion of PERK reverses mGluR-LTD failure in APP/PS1 mice.
- Hippocampal mGluR-LTD impairments in APP/PS1 mice are rescued by PERK inhibitor.

Fig. 1.

Hippocampal mGluR-LTD is impaired in APP/PS1 mice. (A) DHPG-induced mGluR-LTD was inhibited in slices from APP/PS1 mice (filled circles), compared to WT mice (open squares). (B) Representative fEPSP traces before and after DHPG treatment to induce mGluR-LTD for the experiments shown in A. (C) Cumulative data showing mean fEPSP slopes 60 minutes after DHPG application for the mGluR-LTD experiments in A. $n=9$ for WT, $n=10$ for APP/PS1, $*p<0.05$. Independent unpaired two-tailed t test. (D) DHPG induced similar mGluR-LTD in WT slices treated with either vehicle (open squares) or 500 nM A β 1–42 (filled circles). (E) Representative fEPSP traces before and after DHPG treatment to induce mGluR-LTD for the experiments shown in D. (F) Cumulative data showing mean fEPSP slopes 60 minutes after DHPG application for the mGluR-LTD experiments in D. $n=10$ for WT, $n=12$ for WT+A β . $p=0.51$. Independent unpaired two-tailed t test. (G) DHPG induced normal mGluR-LTD in slices from APP/PS1/PERK cKO mice (open circles), compared to WT control mice (open squares). For comparison, mGluR-LTD data for APP/PS1 mice is shown as a horizontal gray bar. In addition, mGluR-LTD in slices from PERK cKO mice (filled triangles) was indistinct from WT control group. (H) Representative fEPSP traces before and after DHPG treatment for the mGluR-LTD experiments shown in G. (I) Cumulative data showing mean fEPSP slopes 60 minutes after DHPG application for the mGluR-LTD experiments in G. For comparison, mGluR-LTD data for APP/PS1 mice is shown as a horizontal gray bar. $n=9$ for WT, $n=8$ for PERK cKO, $n=10$ for APP/PS1/PERK cKO. $p=0.96$. one-way ANOVA.

Fig. 2.

Hippocampal mGluR-LTD impairments in APP/PS1 AD model mice are rescued by the PERK inhibitor GSK2606414. (A) Levels of phospho-eIF2 α were reduced in hippocampal slices treated with GSK2606414. Levels of total eIF2 α and PERK were not affected by GSK2606414. $n=6$. $*p<0.05$. Independent unpaired two-tailed t test. (B) In the presence of PERK inhibitor GSK2606414, DHPG induced similar LTD in slices from WT (open squares) and APP/PS1 mice (filled circles). For comparison, mGluR-LTD data for APP/PS1 mice is shown as a horizontal gray bar. (C) Representative fEPSP traces before and after DHPG treatment for the mGluR-LTD experiments shown in A. (D) Cumulative data showing mean fEPSP slopes 60 minutes after DHPG application for the mGluR-LTD experiments in A. $n=10$ for WT, $n=7$ for APP/PS1. $p=0.52$. Independent unpaired two-tailed t test. For comparison, mGluR-LTD data for APP/PS1 mice is shown as a horizontal gray bar. (E) DHPG-induced LTD was not affected in WT slices treated with GSK2606414 (filled circles), compared to vehicle-treated slices (open squares). (F) Representative fEPSP traces before and after DHPG treatment for the mGluR-LTD experiments shown in D. (G) Cumulative data showing mean fEPSP slopes 60 minutes after DHPG application for the mGluR-LTD experiments in D. $n=6$ for vehicle, $n=9$ for GSK2606414, $p=0.87$, Independent unpaired two-tailed t test. (H) GSK2606414 did not affect baseline fEPSPs. (I) Representative fEPSP traces before and after GSK2606414 treatment for experiments shown in G. (J) Cumulative data showing mean fEPSP slopes 60 minutes after GSK2606414

application for the mGluR-LTD experiments in G. n=12 for WT, n=9 for APP/PS1. $p=0.35$. Independent unpaired two-tailed t test.

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