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Presenilin-1 Mutation Impairs Cholinergic Modulation of Synaptic Plasticity and Suppresses NMDA Currents in Hippocampus slices

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Abstract

Presenilin-1 (PS1) mutations cause many cases of early-onset inherited Alzheimer's disease, in part, by increasing the production of neurotoxic forms of amyloid β -peptide ($A\beta$). However, $A\beta$ - independent effects of mutant PS1 on neuronal Ca^{2+} homeostasis and sensitivity to excitatory neurotransmitters have been reported. Here we show that cholinergic modulation of hippocampal synaptic plasticity is impaired in PS1 mutant knockin (PS1KI) mice. Whereas activation of muscarinic receptors enhances LTP at CA1 synapses of normal mice, it impairs LTP in PS1KI mice. Similarly, mutant PS1 impairs the ability of the cholinesterase inhibitor phenserine to enhance LTP. The NMDA current is decreased in CA1 neurons of PS1KI mice and is restored by intracellular Ca^{2+} chelation. Similar alterations in acetylcholine and NMDA receptor-mediated components of synaptic plasticity are evident in 3 $\times$ TgAD mice with PS1, amyloid precursor protein and tau mutations, suggesting that the adverse effects of mutant PS1 on synaptic plasticity can occur in the absence or presence of amyloid and tau pathologies.

Keywords

NMDA; PS1KI mice; LTP; Butyrylcholinesterase; Alzheimer disease

1. Introduction

The deficits in short-term memory that typify the early stages of Alzheimer's disease (AD) are believed to result from impaired plasticity of synapses without overt structural damage to neurons (Selkoe, 2002; Coleman et al., 2004; Mattson, 2004). While the cause(s) of the most common late-onset form of AD is unknown, mutations in presenilin-1 (PS1) cause many cases of early-onset familial AD (FAD; Rademakers et al., 2003). PS1 is an integral membrane protein and the enzymatic component of the γ -secretase enzyme complex that generates $A\beta$; PS1 mutations increase production of the neurotoxic 42 amino acid form of $A\beta$ (Hardy, 1997). However, FAD PS1 mutations have also been shown to perturb neuronal Ca^{2+} homeostasis by increasing the amount of Ca^{2+} in the endoplasmic reticulum resulting in enhanced Ca^{2+} release in response to activation of ryanodine and IP_3 receptors (Guo et al., 1997; Chan et al., 2000). Wild-type PS1 functions as a Ca^{2+} leak channel and FAD mutations

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compromise this biological activity of PS1 (Tu et al., 2006). The consequences of perturbed neuronal Ca^{2+} homeostasis caused by PS1 mutations for synaptic plasticity are unknown.

An increase in synaptic strength called long-term potentiation (LTP) can be experimentally induced at hippocampal CA1 synapses by high frequency stimulation of afferent axons (Dineley et al., 2001). As with learning and memory, LTP involves activation of glutamate receptors of the AMPA and NMDA subtypes, and is modulated by acetylcholine acting at muscarinic receptors (Muller et al., 1988; Blitzer et al., 1990; Collingridge, 2003). LTP is mediated by Ca^{2+} influx through NMDA receptors and voltage-dependent Ca^{2+} channels and is enhanced by acetylcholine-induced Ca^{2+} release from IP_3 -sensitive ER stores (Nagase et al., 2003; Malenka and Baer, 2004; Shinoue et al., 2005; Li et al., 2007). Impaired cholinergic signaling is implicated in the early memory loss in AD and, indeed, acetylcholinesterase inhibitors improve cognition in some AD patients (Lleo et al., 2006). Interestingly, whereas LTP is impaired by $\text{A}\beta$ oligomers in APP mutant transgenic mice (Chapman et al., 1999; Walsh et al., 2002; Klyubin et al., 2005). LTP is enhanced in PS1 mutant mice that lack amyloid pathology (Parent et al., 1999; Oddo et al., 2003), possibly as the result of enhanced Ca^{2+} release. Here we employ PS1 mutant knockin mice (PS1KI) and 3 $\times$ TgAD mice to reveal an adverse effect of mutant PS1 on cholinergic modification of LTP, and suppression of NMDA currents which can be restored by intracellular Ca^{2+} chelation.

2. Materials and Methods

2.1. Animals

Male (9-12 month-old) presenilin-1 mutant (M146V) knockin mice (Guo et al., 1999) and 3 $\times$ TgAD mice (Oddo et al., 2003), and wild-type mice of the same genetic background (C57BL/6) were used in this study. Mice were housed 4 per cage with continuous access to food and water, and were maintained on a 12 h light/12 h dark cycle. All procedures were approved by the National Institute on Aging Animal Care and Use Committee.

2.2. Slice preparation and electrophysiology

Hippocampal slices were prepared using procedures described previously (Wang et al., 1998, 2004b). Briefly, transverse slices of whole brain were cut at a thickness of 350 μm , and were allowed to recover for at least 1 h in a holding chamber in artificial cerebral spinal fluid (ACSF), bubbled with 95/5% (O_2/CO_2) at room temperature up to 6 h. Field potentials were recorded from CA1 stratum radiatum using pipettes (1-3 $\text{M}\Omega$) filled with bubbled ACSF, placed in stratum radiatum in response to stimulation of Schaffer collateral/commissural afferents. The stimuli (30 μs duration at 0.033 Hz) were delivered through fine bipolar tungsten electrodes; a stimulation intensity was used that evoked a response that was approximately 30-40% of the maximum fEPSP, and LTP was induced by high frequency stimulation (HFS, 100Hz 1s). Whole-cell excitatory postsynaptic currents (EPSC) were recorded from CA1 pyramidal neurons. The neurons were visualized by differential interference contrast microscopy using a 40 $\times$ water immersion lens. Series resistance was 10 to 15 $\text{M}\Omega$. The patch electrode (3-5 $\text{M}\Omega$) contained (in mM): potassium gluconate, 130; KCl, 10; EGTA, 10; CaCl_2 , 1; MgCl_2 , 3; HEPES, 20; MgATP, 5; Na-GTP, 0.5; QX 314 10; pH 7.2 (in some studies 20 mM BAPTA was included in the intracellular solution); the osmolality was 280 mmol/kg. AMPA and NMDAR currents were generated simultaneously by series voltage steps (20 mV and 150 ms) from -60 mV to +60 mV. To avoid capacitance transients generated by the step effect on the current, there was a delay of 50 ms between the start of the step and pre-synaptic stimulation. Data were collected using an Axopatch 200B (EPSCs) or an Axopatch 1D (fEPSPs, Molecular Devices, Sunnyvale, CA) amplifier. Signals were filtered at 2 kHz, digitized at 10 kHz, and analyzed using pCLAMP 8 software (Molecular Devices). All recording solutions for LTP studies contained 50 μM picrotoxin to block GABA_A activity. All slices were pre-selected;

only those with a steep input-output curve were included in the study. During recording, slices were maintained at 30–32°C.

2.3. Reagents

(-) Phenserine(+) tartrate (PT) was synthesized as described previously (Yu et al., 1997), and prepared as a concentrated stock in ethanol:Tween 80 (1:3) which was then diluted to the final concentration in ACSF. The vehicle had no significant effect on the fEPSP or EPSC. Carbachol was prepared as a 200 mM stock in water and was diluted in ACSF before perfusion. All other reagents were obtained from Sigma Chemical Co.

3. Results

3.1. Cholinergic agonist-induced enhancement of LTP is reversed in PS1 mutant knockin mice

The M1 muscarinic agonist carbachol (50 μ M) was bath applied to slices from wild-type and PS1KI mice while recording basal synaptic transmission. Carbachol induced a similar response in slices from WT and PS1KI mice characterized by a rapid depression of baseline fEPSP which was maintained for at least 30 minutes (Fig. 1A). The fEPSP amplitude rapidly returned to the baseline level upon washout of the carbachol. These findings are in agreement with previous data showing that although muscarinic agonists suppress basal synaptic transmission or induce LTD through a presynaptic M1 receptor, they do not affect LTP induction (McCutchen et al., 2006). It is possible that an elevated postsynaptic intracellular Ca^{2+} in response to carbachol may partially compensate for the presynaptic depression.

As expected from previous studies (Blitzer et al., 1990), the amplitude of LTP at CA1 synapses in hippocampal slices from wild-type mice was increased by treatment with the M1 muscarinic agonist carbachol (Fig. 1B). The normalized field excitatory postsynaptic potential (fEPSP) slope was $175 \pm 29\%$ of baseline in carbachol-treated slices compared to $151 \pm 17\%$ in vehicle-treated control slices ($P < 0.05$). The amplitude of LTP was significantly greater in slices from PS1KI mice compared to wild-type mice (Fig. 1B, C) ($245 \pm 11\%$), consistent with previous reports (Parent et al., 1999; Oddo et al., 2003). However, in contrast to wild-type slices carbachol treatment caused a large and highly significant suppression of LTP in slices from PS1KI mice (Fig. 1C) ($157 \pm 12\%$) ($P < 0.01$).

Because NMDA and AMPA receptors are critically involved in the LTP process, we recorded NMDA and AMPA currents simultaneously using whole-cell patch clamp methods. In slices from wild-type mice, carbachol treatment enhanced the NMDA current ($159 \pm 16\%$) compared to vehicle ($146 \pm 17\%$) (Fig. 1D). In contrast, carbachol treatment decreased the NMDA current in slices from PS1KI mice ($72 \pm 10\%$) compared to vehicle ($88 \pm 13\%$). Carbachol had no significant effects on the AMPA current in slices from wild-type mice (carbachol, $179 \pm 18\%$; vehicle, $178 \pm 17\%$) or PS1KI mice (carbachol, $178 \pm 19\%$; vehicle, $172 \pm 16\%$).

3.2. Cholinesterase inhibitor impairs LTP in PS1KI mice

To determine whether mutant PS1 affects the modulation of hippocampal synaptic plasticity by endogenous acetylcholine, we evaluated the effects of the cholinesterase inhibitor phenserine tartrate (PT) on LTP in slices from wild-type and PS1KI mice. In slices from wild-type mice, PT treatment significantly increased the normalized fEPSP slope compared to vehicle-treated slices. In slices from PS1KI mice, the magnitude of LTP was significantly decreased in response to PT treatment (Fig. 2A). We also measured paired pulse facilitation (PPF), an indicator of release of neurotransmitter from presynaptic terminals, in order to elucidate whether mutant PS1 alters the presynaptic component of LTP. The S2/S1 ratio (with a 50 ms interpulse interval) was significantly lower at CA1 synapses in PS1KI mice compared

wild-type mice (Fig. 2B, 1.4 ± 0.18 vs. 1.75 ± 0.03 , $n = 6$ and 8), suggesting that the PS1 mutation results in a decrease in neurotransmitter release. In the presence of carbachol ($50 \mu\text{M}$), the PPF ratio was not altered in slices from either wild-type or PS1KI mice (Fig. 2C, 1.42 ± 0.07 vs. 1.72 ± 0.07 , $n = 6$ and 12), suggesting that the effects of carbachol on synaptic plasticity impairments are likely mediated by postsynaptic mechanisms.

3.3. PS1 mutation suppresses NMDA current in an intracellular Ca^{2+} -dependent manner

We next recorded whole-cell NMDA and AMPA currents from wild-type and PS1KI mice (Fig. 3A, B). In wild-type CA1 neurons the NMDA receptor current amplitude (measured at $+40 \text{ mV}$ using 60% of the maximum stimulation intensity) was $154 \text{ pA} \pm 7 \text{ pA}$. The AMPA receptor current amplitude (recorded at -60 mV) was $328 \pm 6 \text{ pA}$. Perfusion of wild-type neurons with the intracellular Ca^{2+} chelator BAPTA had no significant effects on NMDA or AMPA current amplitudes (Fig. 3C). The NMDA current amplitude was significantly lower in neurons from PS1KI mice compared to wild-type mice. Intracellular Ca^{2+} chelation increased the NMDA current in neurons expressing mutant PS1 to a level as great, or greater than, that of wild-type mice. In contrast, AMPA currents were unaffected by mutant PS1 or BAPTA (Fig. 3D). Because PS1KI mice do not exhibit amyloid or tau pathologies (Guo et al., 1999), our data suggested that the abnormalities of NMDA currents and muscarinic modulation of LTP were independent of $\text{A}\beta$ and tau. To confirm the latter conclusion we therefore measured NMDA and AMPA currents in slices from 3×TgAD mice which exhibit $\text{A}\beta$ and tau pathology in CA1 neurons and impaired cognitive function (Oddo et al., 2003; Billings et al., 2005; Halagappa et al., 2007). Similar to neurons in PS1KI mice, neurons in 3×TgAD mice exhibited a significantly lower NMDA current compared to wild-type neurons (Fig. 3E). The AMPA current in CA1 neurons from 3×TgAD mice was not different than the AMPA current in CA1 neurons from wild-type or PS1KI mice. As in neurons of PS1KI mice, perfusion of neurons from 3×TgAD mice with BAPTA restored the NMDA current to a level similar to that of wild-type neurons (Fig. 3F).

3.4. Cholinesterase inhibitor decreases LTP at hippocampal synapses in 3×TgAD mice

We also found that LTP was inhibited when slices from 3×TgAD mice were treated with the cholinesterase inhibitor PT (Fig. 4), an abnormality similar to that found in PS1KI mice. The amplitude of LTP was reduced by more than 50% at CA1 synapses in slices from 3×TgAD mice compared to non-transgenic control mice. The latter findings are consistent with previous data showing that neurons from PS1KI mice and 3×TgAD mice exhibit late-onset elevated input resistance and exaggerated ER Ca^{2+} signals (IP_3 - and spike-evoked) relative to wild type mice (Guo et al., 1997; Chan et al., 2000; Stutzmann et al., 2006).

4. Discussion

Our findings identify novel adverse effects on hippocampal synaptic plasticity of a PS1 mutation that causes early-onset FAD. Treatment of slices from wild-type mice with the muscarinic agonist carbachol or the cholinesterase inhibitor PT resulted in enhanced LTP. In contrast, carbachol and PT impaired LTP at CA1 synapses in slices from PS1KI mice. In addition, the NMDA current amplitude was significantly lower in CA1 neurons of PS1KI mice compared to wild-type mice. There is no evidence of degeneration of hippocampal neurons or basal forebrain cholinergic neurons or synapses in mice expressing PS1 mutations alone or in combination with an APP mutation (Guo et al., 1999; Oddo et al., 2003), making it unlikely that a major structural abnormality is responsible for the perturbed synaptic function caused by mutant PS1. Instead, the abnormalities in hippocampal synaptic plasticity are likely the result of the perturbed endoplasmic reticulum Ca^{2+} regulation previously documented in studies of cultured neurons expressing FAD PS1 mutations. Those studies showed that PS1 mutations cause Ca^{2+} to accumulate at higher levels in the endoplasmic reticulum resulting in

an enhanced release of Ca^{2+} in response to muscarinic agonists (Guo et al., 1997; 1999; Begley et al., 1999; Leissring et al., 2001), as well as enhanced Ca^{2+} -induced Ca^{2+} release through ryanodine receptors (Chan et al., 2000; Pack-Chung et al., 2000). Apparently, wild-type PS1 functions as a Ca^{2+} leak channel in the endoplasmic reticulum membrane, and FAD-linked PS1 mutations impair this Ca^{2+} leak function (Tu et al., 2006). Consistent with a loss-of-function mechanism, cells lacking PS1 exhibit impaired Ca^{2+} responses to glutamate (Yang and Cook, 2004). Considerable evidence suggests that there is an optimal level of postsynaptic Ca^{2+} elevation required for LTP and memory, and that a further increase in the Ca^{2+} level can impair LTP and memory ((Jouveneau et al., 1999; Price et al., 1999; Tonkikh et al., 2006). Excessive Ca^{2+} release in response to muscarinic receptor activation could explain the impairment of LTP caused by carbachol and PT in neurons expressing mutant PS1. Excessive elevation of intracellular Ca^{2+} could also be responsible for reduced NMDA currents which are known to be inactivated by Ca^{2+} (Legendre et al., 1993; Furukawa et al., 1997). Indeed, we found that the intracellular Ca^{2+} chelator BAPTA restored NMDA currents in CA1 neurons of PS1KI mice.

Increasing evidence suggests that FAD PS1 mutations exert adverse effects on neuronal function that are independent of, or in addition to, altered γ -secretase activity and increased A β production (Guo et al., 1997, 1999; Herms et al., 2003; Shen and Kelleher, 2007). Alterations in neuronal Ca^{2+} regulation similar to those in PS1KI mice have been documented in neurons of 3 $\times$ TgAD mice (Smith et al., 2005). We found that, as in PS1KI mice, CA1 neurons in 3 $\times$ TgAD mice exhibited reductions in NMDA current that were reversed by intracellular Ca^{2+} chelation, as well as LTP impairment in response to cholinergic stimulation. Because CA1 neurons in 3 $\times$ TgAD mice exhibit accumulation of A β oligomers and hyperphosphorylated tau, whereas CA1 neurons in PS1KI mice do not (Guo et al., 1999; Oddo et al., 2003, 2006; Billings et al., 2005), our findings suggest that the adverse effects of PS1 mutations on cholinergic and NMDA-mediated synaptic plasticity are independent of A β and tau pathologies. Indeed, families have recently been identified in which PS1 mutations cause frontotemporal dementia [48] (Zekanowski et al., 2006) or cardiomyopathy and heart failure (Li et al., 2006), disorders that lack amyloid pathology. The mechanisms identified in the present study may explain the increased Ca^{2+} responses and increased afterhyperpolarization of hippocampal neurons (Barrow et al., 2000) and the defective associative learning (Wang et al., 2004a) previously documented in studies of presenilin mutant mice.

It has previously been reported that neurons expressing mutant presenilin-1 exhibit enhanced Ca^{2+} release from ryanodine- and IP_3 -sensitive ER stores, apparently as the result of an increase in the ER Ca^{2+} content (Guo et al., 1997; Chan et al., 2000). While some data suggest that Ca^{2+} release from ER can enhance LTP (Raymond and Redmond, 2002), other findings suggest the opposite. For example, mice lacking the type 3 ryanodine receptor exhibit enhanced LTP at hippocampal CA1 synapses and improved spatial learning ability (Futatsugi et al., 1999). Similarly, pharmacological inhibition of IP_3 receptors enhanced LTP at CA1 synapses in guinea pig hippocampal slices (Taufiq et al., 2005). Our findings suggest that, whereas activation of muscarinic receptors enhances LTP in normal neurons, the enhanced ER Ca^{2+} release caused by mutant presenilin-1 may impair LTP. We found that the intracellular Ca^{2+} chelator BAPTA restored NMDA currents in hippocampal neurons in slices from PS1KI mice, again suggesting that the presenilin-1 mutation causes an elevation of the intracellular Ca^{2+} concentration above that which is optimal for LTP induction.

The first studies that revealed adverse effects of PS1 mutations on neuronal Ca^{2+} homeostasis did so in the context of neuronal vulnerability to excitotoxicity, and oxidative and metabolic stress in cell culture and in animal models of epilepsy and ischemic stroke (Guo et al., 1997, 1999; Mattson et al., 2000). It was shown that PS1 mutations cause excessive release of Ca^{2+} from endoplasmic reticulum stores when neurons are exposed to glutamate, ischemia or

oxidative stress, an alteration that renders neurons more vulnerable to death induced by those insults. While PS1 mutant mice do not exhibit neuronal degeneration under normal conditions, hippocampal and cortical neurons are more prone to Ca^{2+} -mediated degeneration when stressed. Enhanced elevations in synaptic Ca^{2+} responses to depolarization and oxidative stress have been documented in studies of PS1 mutant mice (Begley et al., 1999). When taken together with previous findings, the present data therefore suggest that an early and perhaps pivotal event in the pathogenic actions of PS1 mutations is a perturbation of synaptic Ca^{2+} regulation which first impairs synaptic function and, with advancing age, results in synaptic degeneration and neuronal death.

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Figure 1.

Cholinergic agonist-induced enhancement of LTP is blocked and reversed in PS1 mutant knockin mice. **A**. Slices were perfused for 30 min with 50 μ M carbachol (Carb) and wash out for 20 min. **B and C**. Plots of the normalized initial EPSP slope. Following a 10 minute period of baseline recording, high frequency stimulation (HFS; 100 Hz, 1 s) was applied at CA1 stratum radiatum. Slices were incubated for 60 minutes and perfused with 50 μ M carbachol or vehicle (control) during the recording. In slices from wild-type (WT) mice, HFS induced a normal LTP and the LTP was significantly enhanced by carbachol. In slices from PS1KI mice, LTP was reduced in the presence of carbachol. Values are the mean and SEM (6-10 slices from 5 or 6 mice). * $p < 0.05$, ** $p < 0.01$, compared to the control value. **D**. I-V curves of AMPA and NMDA currents generated from whole-cell patch clamp recordings in slices from WT and PS1KI mice treated with either vehicle or carbachol. Currents were generated by a series voltage steps from -60 mV to $+60$ mV. The NMDA current (induced at positive holding potentials) was decreased in neurons from PS1KI mice compared to WT mice, ** $p < 0.01$.

Figure 2.

The cholinesterase inhibitor phenserine tartrate (PT) enhances LTP in wild-type mice, but inhibits LTP in PS1KI mice. **A.** The normalized EPSP slopes in slices from wild-type (WT) and PS1KI mice that had been treated with 5 μ M PT or vehicle. Values are the mean and SEM (6-8 slices from 5-6 mice). * p < 0.05 compared to the PS1KI value, ** p < 0.01 compared to the WT value). **B.** Paired pulse facilitation (PPF) analysis showing the S1/S2 ratios for increasing stimulation interpulse intervals in slices from WT and PS1KI mice (8-10 slices from 5-6 mice). * p < 0.05 compared to the PS1KI value. **C.** PPF analysis in the presence of 50 μ M carbachol.

Figure 3.

PS1 mutation suppresses NMDA current, in an intracellular Ca^{2+} -dependent manner. **A and B.** Representative recordings of NMDA (A) and AMPA (B) currents in wild-type neurons in the absence or presence of the NMDA receptor antagonist AP5. **C and D.** Representative recordings of NMDA (C) and AMPA (D) currents in neurons perfused with a patch pipette solution lacking (Control) or containing the Ca^{2+} chelator BAPTA (20 mM). **E and F.** Graphs showing summary data for peak NMDA (E) and AMPA (F) currents, recorded without (Con) or with 20 mM BAPTA in the patch pipette in neurons from WT, PS1KI and 3×TgAD mice. Values are the mean and SEM (6-10 neurons in 5-8 slices). * $p < 0.05$ compared to the

corresponding control value for WT mice and to the BAPTA value for mice of the same genotype.

Figure 4.

Cholinesterase inhibition results in a decreased LTP amplitude at CA1 synapses in hippocampal slices from 3xTgAD mice. The normalized field EPSP slopes are shown for slices from 3xTgAD mice that had been treated with 5 μ M PT or vehicle. Values are the mean and SEM (6-8 slices from 5-6 mice). * $p < 0.01$ compared to the control value.